Metabolic and endocrine adaptations to intrauterine growth restriction in the juvenile guinea pig

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For Brett, Jasmine and my family

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STATEMENT OF ORIGINALITY AND AUTHENTICITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. The author consents to this thesis being made available for photocopying and loan, if applicable and if accepted for the award of the degree.

Natasha K Campbell
June 2006

Signed,

Date: 6/7/06

TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

α-aN	Plasma α-amino nitrogen concentration
β-HSD	β-hydroxysteroid dehydrogenase
AC	Abdominal circumference
AGA	Appropriate for gestational age
AGR	Absolute growth rate
ANOVA	Analysis of Variance (statistical test)
ANSA	8-anilion-1-sulphonic acid
BMI	Body mass index
BSA	Bovine serum albumin
CO_2	Carbon dioxide
CRL	Crown-rump length
CVD	Cardiovascular disease
DHEAS	Dehydroepiandrosterone sulphate
DXM	Dexamethasone
EDL	Extensor digitorum longus
EDTA	Ethylenediamine tetra-acetic acid
FFA	Free fatty acid concentration
$FFA_{60'-120'}$	Free fatty acid concentration during the second hour of the hyperinsulinemic euglycemic clamp
FGR	Fractional growth rate
GAR	Goat anti-rabbit IgG
GH	Growth hormone
GIR	Glucose infusion rate
$GIR_{60'-120'}$	Glucose infusion rate during the second hour of the hyperinsulinemic euglycemic clamp
$GIR_{70-130'}$	Glucose infusion rate during the second hour of the hyper-IGF-I euglycemic clamp
GR	Glucocorticoid receptor
HCl	Hydrochloric acid
HEC	Hyperinsulinemic euglycemic clamp
HIEC	Hyper-IGF-I euglycemic clamp

HPAA	Hypothalamo-pituitary adrenal axis
HPLC	High performance liquid chromatography
HPTA	Hypothalamo-pituitary thyroid axis
HW	Head width
ID	Internal diameter
i.m.	Intramuscular
IGFBP	Insulin-like growth factor binding protein
IGF-I	Insulin-like growth factor-I
IGF-II	Insulin-like growth factor-II
IGF-IR	Type 1 Insulin-like growth factor receptor
IGF-IIR	Type 2 Insulin-like growth factor receptor
IgG	Immunoglobulin G
i.p.	Intraperitoneal
IMVS	Institute of Medical and Veterinary Science
IR	Insulin receptor
IRS	Insulin receptor substrate
IUGR	Intrauterine growth restriction (or retardation)
IVGTT	Intravenous glucose tolerance test
Kg	Kilogram
L	Litre
LGA	Large-for-gestational age
M	Molar
MCi	Milli Curie
Meq	Milli Equivalent
Mg	Milligram
Ml	Milli Litre
MM	Millimolar
MR	Mineralocorticoid receptor
MRNA	Messenger ribonucleic acid
Ms	Millisecond
MU	Milli Unit
NIDDM	Non-insulin dependent diabetes mellitus
Nmol	Nanomole

NFGR	Neonatal fractional growth rate
O_2	Oxygen
°C	Degrees centigrade
OD	Outer diameter
OH	Hydroxyl
PEG	Polyethylene glycol
Pg	Picogram
PI	Ponderal index
pmol	Picomole
PR	Placental restriction or placentally restricted
rhIGF-I	Human recombinant insulin-like growth factor-I
RIA	Radioimmunoassay
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation
SEM	Standard error of the mean
SGA	Small for gestational age
SSGIR	Steady state glucose infusion rate
TBG	Thyroxine-binding globulin
TG	Triglyceride
TH	Thyroid hormone
TRH	Thyrotropin-releasing hormone
TSH	Thyroid (thyrotropin)-stimulating hormone
µCi	Micro Curie
µg	Microgram
µl	Microlitre
%CV	Coefficient of variance
L-thyroxine (T4)	L-3,5,3',5'-tetraiodothyronine
L-triiodothyronine (T_3)	L-3,5,3'-triiodothyronine

PAPERS ARISING FROM THIS THESIS

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Natasha K Lloyd, Sanita Grover, Prema Thavaneswaran, Melissa R Walker, Karen L Kind and Julie A Owens.

Catch-up growth of lean tissues after IUGR is associated with increased metabolic sensitivity to IGF-I in the young guinea pig. ENDO conference, New Orleans, USA, 2004.

ABSTRACT

Intrauterine growth restriction (IUGR), evident in infants as a reduced weight or length at birth, and/or increased thinness for gestational age, is common globally. IUGR is characterised by altered postnatal growth, termed 'catch-up growth', which is due to as yet unknown mechanisms. Both IUGR and its associated catch-up growth are associated with increased risks of morbidity and mortality in the perinatal period, and with an increased risk of adult onset diseases, including obesity-related disorders such as type-2 diabetes and cardiovascular disease. Understanding the mechanisms responsible for catch-up growth may reveal how it contributes to later metabolic disease. In this study we investigated the effect of spontaneous fetal growth restriction, resulting from large litter sizes, in the guinea pig, and its postnatal outcomes on growth and development of the major endocrine axes that regulate early postnatal growth. We found that spontaneous fetal growth restriction reduces size at birth, but increases postnatal growth rates in the first 36 days of life in the guinea pig. The continual assymetrical growth retardation characteristic in fetal life near term was in part reversed in the juvenile offspring, which display sparing of the brain, but enhanced size of other vital organs, including the GIT and liver. However, low birth weight offspring have decreased adipose mass at 36 days of age, while low birth weight females have more and males less muscle mass. Low birth weight was associated with increased postnatal catch-up growth in terms of weight and crown-rump length. Low birth weight offspring displayed decreased circulating concentrations of plasma IGFs, and unchanged plasma IGFBP concentrations. However, animals small at birth displayed an increased

IGF-I sensitivity of glucose, yet no changes in IGF-I sensitivity of FFA metabolism. The glucose disposition index was also increased in animals small at birth. Increased postnatal growth was associated with an increased IGF-I sensitivity of glucose metabolism, in the presence of reduced plasma IGF-I and IGF-II. Increased fractional growth rates of weight and length were associated with an increased disposition index of glucose, but not of FFA. Salivary cortisol concentrations were increased in the first 2 weeks of life in low birth weight offspring, and remained elevated in males but not females after this time. Salivary cortisol in early life was correlated with an increase in the T_3:T_4 ratio at 36 days of age in low birth weight animals. Therefore in summary, the juvenile guinea pig of low birth weight undergoes catch-up growth in the first month of life in terms of overall size, with enhanced size of key visceral organs and lean tissue evident, but not of adipose tissue. This catch-up growth is associated with enhanced IGF-I and insulin sensitivity of glucose metabolism, and disposition indices of glucose, and increased TH activation, and occurs despite reduced circulating IGFs. Early catch-up growth may be driven by enhanced action of insulin, IGFs and TH and appears to occur in lean and visceral tissues rather than adipose tissue. This suggests that any increased adiposity or insulin resistance following low birth weight or catch-up growth presents later in this species, possibly longer term following weaning. This study has helped to elucidate the factors involved in postnatal catch-up growth, and this knowledge may be used to design and improve therapies for the human IUGR infant.

Chapter 1

INTRODUCTION

1.1 Intrauterine growth restriction (IUGR)

1.1.1 Characteristics of IUGR

Intrauterine growth restriction (IUGR) results from growing slowly before birth and is usually diagnosed by reduced weight, length or increased thinness for gestational age following delivery (Karlberg et al., 1997; Robinson et al., 1994). The primary causes of small size at birth in humans are placental dysfunction, reduced uterus size, maternal under nutrition, malnutrition or hypoxia, and to a limited extent, genetic factors. In Australia, maternal eating disorders and placental insufficiency are responsible for much of the clinical IUGR observed (Abraham et al., 1994). IUGR in the human is characterised by fetal hypoxemia, hypoglycaemia, and reduced levels of total and some essential amino acids (e.g. branched chain amino acids) in fetal blood, consistent with reduced nutrient and oxygen supply to the fetus as causes (Pardi et al., 2002). Fetal neuro-endocrine systems respond to a reduced substrate supply and consequent hypoglycaemia and hypoxemia, with reductions in the production and levels of anabolic hormones essential for growth, such as insulin, insulin-like growth factors-I and -II (IGFs) and thyroid hormones and increases in catabolic hormones, such as cortisol and catecholamines. Several of these anabolic hormones, such as the IGFs and TH are also necessary for differentiation and maturation of key fetal tissues and organs, and despite the late increase in cortisol, many fetal tissues and organs are immature in IUGR. These endocrine responses to deprivation, which are seen in humans and experimental IUGR, slow and alter fetal metabolism and growth, enabling the

fetus to adapt to and survive a substrate-deprived environment (Robinson et al., 1994). However, they may have adverse consequences in the long term.

1.1.2 IUGR – underlying mechanisms

The placentally restricted small for gestational age (SGA) infant has a smaller placenta and decreased placental: fetal weight ratio compared to normal fetuses of the same birth weight (Heinonen et al., 2001). Impaired placental perfusion results in uneven circulation and inefficient oxygen and nutrient exchange between the maternal space and fetus (Pardi et al., 2002). SGA infants have an increased nonessential/essential amino acid ratio, and are also hypertriglyceridemic (Ergaz et al., 2005). Cordocentesis has shown that SGA fetuses are hypoxemic, hypercapnic, hyperlacticemic and acidotic (Nicolaides et al., 1989).

Umbilical and uterine blood flows are also reduced in the placentally restricted sheep (Owens et al., 1986), and oxygen delivery to the fetus is significantly reduced in these sheep (Owens et al., 1987). The majority of animals born after IUGR have experienced reduced delivery of essential substrates as a fetus, such as oxygen and nutrients, due to maternal or placental limitations (Owens et al., 1986; Owens et al., 1987).

1.1.3 IUGR – perinatal outcomes

Poor fetal growth contributes to perinatal mortality and morbidity (Barker et al., 1993), the risks of which appear to increase with increasing severity of IUGR

(Kramer, 1990). Infants born with IUGR have increased risks of stillbirth, fetal distress, in-hospital mortality, and adverse metabolic and asphyxic neonatal outcomes (Kramer, 1990).

In response to intrauterine under nutrition, the human fetus centralizes blood flow by preferentially shunting to the brain at the expense of the body (Barker, 2001a; Newnham, 1998). Preferential perfusion of the fetal brain during severe IUGR can be represented by an increase in the ratio between umbilical artery pulsatility index and middle cerebral artery pulsatility index (Scherjon et al., 2000). An increase in this ratio results in a lower IQ at the age of 5 years, as well as twice as many children functioning below the expected level at this age compared to those born after a normal ratio. This suggests that although brain sparing acts to preserve brain flow to the brain, it does not lead to normal cognitive development (Scherjon et al., 2000). In this manner, SGA infants display delayed neurodevelopment (Newnham, 1998) with 10% of low birth weight babies having a physical handicap (Gaffney et al., 1994), and a further 5% showing neuro-developmental delay at the age of 9 years (Kok et al., 1998). At 4 months of age, neuromotor development of SGA and control infants is similar, however SGA infants perform more poorly in hearing/speech and performance scales (Newman et al., 1997).

1.2 IUGR and adverse postnatal outcomes

Low birth weight or thinness at birth is an antecedent of adult disease, including insulin resistance, as well as hyperlipidemia, hypertension, coronary heart

disease and premature death (Barker et al., 1993; Newnham, 1998; Seckl, 1997). Birth weight is inversely related to adult blood pressure (Huxley et al., 2000), and to glucose and insulin profiles in later life, including fasting plasma glucose and insulin, insulin resistance and insulin secretion (Newsome et al., 2003). Birth weight is also inversely related to the risk of non-fatal coronary heart disease and stroke in middle-late aged women, even after adjusting for such lifestyle factors as alcohol consumption, saturated fat consumption, physical activity and waist-hip ratio (Rich-Edwards et al., 1997). In male subjects aged 49-51 years, birth weight is positively correlated with fasting insulin and waist-hip ratio, and negatively correlated to presence of the metabolic syndrome (Parker et al., 2003), which encompasses glucose intolerance, hypertension, and raised plasma triglyceride concentrations (Hales & Ozanne, 2003).

The Dutch famine studies have provided direct evidence that prenatal insult can cause adult disease in humans. In addition, the timing of the prenatal insult is important to the postnatal outcome, with the highest mortalities occurring in those exposed to famine in late gestation, or in those born immediately before the famine (Roseboom et al., 2001). Compared to babies not exposed to famine, those exposed in late gestation were smaller, thinner and lighter with smaller heads, whereas those exposed early in gestation were heavier and longer at birth. In addition, risk factors for cardiovascular disease, including impaired glucose tolerance, hypercholesterolemia, elevated blood pressure and obesity are all programmed at varying gestational time points (Roseboom et al., 2001).

1.3 Experimental studies of outcomes of IUGR

Numerous animal models of IUGR have been utilised to study the postnatal outcomes of intrauterine insults. Umbilico-placental embolization (UPE) in the sheep in late gestation restricts fetal growth and results in fetal ventricular hypertrophy. In IUGR fetuses, mean arterial pressure (MAP) does not exhibit the increase seen in matched controls between 121 days and term, however near term there is no significant difference in MAP. Postnatally, offspring small at birth have lower MAP and diastolic pressure at 4 days after birth, and these remain lower at 8 weeks (Louey et al., 2000). In the pig, average MAP in those of low birth weight is not different from high birth weight animals at 3 months of age, however basal MAP at this age is negatively correlated with birth weight, and to head length:weight ratio at birth (Poore et al., 2002). Similarly, MAP at 5 months of age decreases with increasing birth weight of offspring following maternal undernutrition for 10 or 20 days from 105 days of gestation in the sheep. However, this effect is not observed at 30 months of age (Oliver et al., 2002).

Bilateral ligation of the uterine arteries (UAL) at day 17 of pregnancy in the rat results in a reduction in birth weight accompanied by reduced glomerular density in the kidneys, resulting in adult hyperfiltration and hyperperfusion of the glomeruli (Sanders et al., 2004). However, IUGR induced by UAL is not linked to changes in blood pressure 3-4 months postnatally (Jansson & Lambert, 1999; Sanders et al., 2004). Food restriction during pregnancy also results in

fetal growth restriction in the rat (Woodall et al., 1996). Body weight gain is lower in food-restricted offspring until 90 days of age, suggesting prolonged growth retardation during the neonatal period (Woodall et al., 1996). Maternal under nutrition leads to raised systolic blood pressure in offspring at 100 days of age in the rat, and hypercaloric feeding in the postnatal period elevates systolic blood pressures even higher above those fed a control diet (Vickers et al., 2000). Several studies in rats have also shown that protein malnutrition, for at least some part of pregnancy, may reduce nephron number and cause hypertension in the adult offspring (Dodic et al., 2002), and maternal under nutrition leads to offspring with smaller kidneys than controls (Vickers et al., 2000).

Maternal feed restriction in guinea pigs increases systolic blood pressure by 9% in young adult male offspring, but diastolic blood pressure, mean arterial pressure and heart rate are not altered (Kind et al., 2002). Systolic blood pressure and mean arterial blood pressure are negatively associated with head width at birth (Kind et al., 2002).

In addition to having long-term effects on brain and kidney development, IUGR also causes developmental delays in gastrointestinal growth and development. In particular, developmental delays in fetal disaccharidase activity, which is indicative of intestinal function and maturity, have been reported in rat models of IUGR (Lebenthal 1981). The 2 disaccharidases lactase and maltase are also lower in growth-retarded rabbit fetuses during gestation, and this continues into the neonatal period, until catch-up growth occurs (Buchmiller-Crair et al., 2001).

Therefore, animal studies have shown that IUGR may exert a deleterious effect on development of the key organs, which may be related to its effects on blood pressure and adverse adult outcome.

1.4 IUGR and disproportionate growth restriction

Pregnant guinea pigs deprived of food for 48h on gestational day 50 spawn offspring with a significant reduction in fetal body weight (Lingas et al., 1999). Fetal IUGR is accompanied by a significant increase in brain weight when expressed as a ratio of body weight, indicating that 'brain sparing' is occurring. There is a decrease in kidney weight, but not when expressed as a ratio of body weight indicating proportional growth restriction of this organ, however there are significant increases in ratio of lung and heart to body weight (Lingas et al., 1999). When food restriction is extended for a longer period (4 weeks before mating until day 60 of pregnancy), disproportionate growth of the guinea pig fetus is apparent, with relative weights of liver, biceps, thymus and spleen all reduced following feed restriction in the guinea pig, and relative brain and lung weights increased, indicating sparing of these vital organs. Weights of fetal heart, kidneys, adrenals, and biceps brachii are also reduced at day 60 of gestation (Kind et al., 2005). Fetal biceps brachii and soleus weights may be reduced by up to ~45% following maternal food restriction in the guinea pig during the last semester (Dwyer et al., 1992).

In the sheep, following 10-day fetal placental embolization, although fetal body weight is not significantly altered, assymetrical growth is apparent, with increased fetal brain to liver weight ratios, adrenal hypertrophy and reductions in thymus weight (Gagnon, 2003). In addition, restriction of uteroplacental blood flow, which results in fetal hypoxemia, results in a redistribution of blood flow to vital organs, including the brain, myocardium and adrenals (Gagnon, 2003).

1.5 Intrauterine programming

The thrifty phenotype hypothesis, proposed by Hales and Barker in 1992, states that fetal development is sensitive to the nutritional environment. When this is poor, an adaptive response occurs to optimise growth of certain organs (i.e. the brain), as opposed to others (i.e. the viscera). This leads to an altered postnatal metabolism, which enhances postnatal survival under conditions of poor nutrition. These adaptations only become detrimental when postnatal nutrition is overabundant and obesity results (Hales & Ozanne, 2003). The concept of programming, proposed by Barker and colleagues in 1993, therefore states that at critical times in development, an adverse environment may permanently or transiently alter the programmed development of key systems regulating metabolic or cardiovascular control (Barker et al., 1993). More recently, the *predictive adaptive response* has been suggested by Gluckman and Hanson (2004), in which the response of an organism to its current situation is predicted in expectation of its future environment (Gluckman & Hanson, 2004).

Programming represents the idea that substrate deprivation during early life can have persistent or long-term effects, which differ depending on the stage of development at which the insult occurs. Gluckman and Hanson (2004) suggested that the three mechanisms underlying programming are epigenetic change, changes in cell-cycle regulation and changes in cellular or tissue differentiation, with epigenetic change predicted to be the major mechanism underlying programming (Gluckman & Hanson, 2004). Long-term permanent effects of deprivation may include reduced cell number, altered organ structure, resetting of hormonal axes (Dennison et al., 1997), and altered intrinsic cell capacity (McMillen & Robinson, 2005). Programming of tissues may therefore occur if the *in utero* environment restrains hyperplasia of tissues, alters the balance of cell types, or induces gene expression at an inappropriate time during gestation (Barker, 2001b).

1.6 Catch-up growth overview

IUGR is characterised by altered postnatal growth. Typically, postnatal growths consist of rapid but decelerating growth in infancy, steady growth during childhood, and a puberty-associated growth spurt (Tanaka, 1996). Following birth, the majority of IUGR infants grow at an accelerated rate for the first six months of life (Chatelain et al., 1996; Chatelain et al., 1994; Karlberg et al., 1997), a period termed "catch-up growth". Catch-up growth has been defined as: "a growth velocity in terms of height and/or length above the statistical limits of normality for age and/or maturity during a defined period of time following a transient period of growth inhibition" (Prader et al., 1963). Studies of populations

in Hong Kong and Sweden show that catch-up growth is evident as early as 2 weeks of age, with most catch-up of weight and height completed by 2 months of age (Hokken-Koelega et al., 1995; Karlberg et al., 1997). By 6 months, 92% of children born SGA had reached a final height within normal range. Although these infants catch-up, their final height averages 1SD below normal and most are below the 50th percentile (Hokken-Koelega et al., 1995; Karlberg et al., 1996; Karlberg et al., 1997). Even some of those IUGR infants that do not catch-up grow more rapidly than normal in fractional terms, since they usually maintain their height or weight in standard deviation scores (SDS) (Albertsson-Wikland & Karlberg, 1994; Albertsson-Wikland et al., 1993). The remainder undergo little if any catch-up growth and at least half of these children remain short in stature (Karlberg et al., 1996).

1.7 Catch-up growth and adverse postnatal outcomes

Recent evidence suggests that the extent of catch-up growth after IUGR may independently influence the risk of developing adult hypertension, cardiovascular disease and insulin resistance (Cianfarani et al., 1999; Eriksson et al., 1999; Parker et al., 2003). Catch-up growth which leads to childhood obesity may be a key component in the development of adult disease, as proposed by the thrifty phenotype hypothesis (Hales & Ozanne, 2003). However, the path followed by those children undergoing catch-up growth in the first few months of life is a different one to those children who experience excessive weight gain after birth, following cessation of fetal growth restriction.

For example, India and parts of Asia are currently experiencing an escalating epidemic of diabetes and cardiovascular disease. India has the highest number of diabetic patients of any country, and the prevalence of coronary heart disease has increased by over 8% (Gupta & Gupta, 1996). The situation seems to be more complex than low birth weight being the sole cause, as small size at birth has been present in these populations for many years. Urban Indian babies are heavier than rural babies, but have a 5x higher susceptibility to diabetes (Yajnik, 2004). In urban Indian children, insulin resistance and other cardiovascular risk factors such as blood triglycerides, cholesterol and central obesity are highest in children with the lowest birth weights who reach a larger size (Bavdekar et al., 1999). However, it is an increased growth velocity from 4 to 8 years of age that strongly predicts insulin resistance and CVD risk (Yajnik et al., 2002), not 'catch-up growth', which occurs in the first few months of life (Chatelain et al., 1996; Chatelain et al., 1994; Karlberg et al., 1997).

A combination of small size at birth and during infancy, followed by accelerated weight gain from 3- to 11-years, predicts a higher incidence of coronary heart disease, type-2 diabetes, and hypertension (Barker et al., 2002). Low weight at 1 year of age is also associated with an increased incidence of type-2 diabetes in adult life, and incidence of the disease is associated with high weight and high BMI in later childhood (Eriksson et al., 2003a). Children with birth weights lower than 3.5kg, and who display catch up growth, begin to gain weight at ~2 years of age and their BMI increases rapidly. These children go on to develop type-2 diabetes in late adulthood (~70 years of age) (Forsen et al., 2000). Changes in intrauterine growth also affect the risk of children developing

childhood onset insulin dependent diabetes. However, in contrast to the development of adult-onset diabetes, being SGA diminishes the risk, and being large-for-gestational age (LGA) infants increases the risk (Dahlquist et al., 1996).

However, catch-up growth may have beneficial consequences for the SGA infant, as suggested in a cohort of children in Brazil who were studied at birth, 20 and 42 months of age. Catch-up growth from 0 to 20 months was related to a reduced risk of hospital admissions and mortality (Victora et al., 2001). Slow growing SGA children had the highest death rates from birth to 5 years of age, whereas SGA children who displayed catch-up growth had similar mortality rates to AGA children (Victora et al., 2001).

Previous studies show that there is an increased coronary mortality for adult men who are thin at birth but who catch up in weight, and for women, catch up in height rather than weight is associated with higher coronary morbidity and mortality (Barker, 2004). In general, a low birth weight combined with high rates of postnatal growth leads to high blood pressure later in adult life (Huxley et al., 2000), and catch-up growth in early childhood is also significantly associated with a higher central metabolic syndrome score in males, even after adjustment for other early life factors and adult lifestyle (Parker et al., 2003).

1.7.1 IUGR, catch-up growth and altered body composition in the human

Obesity is fast becoming a major health concern worldwide. Infants born SGA have a reduced fat mass at birth, which is thought to reflect decreased fat accumulation in adipocytes (Levy-Marchal et al., 2004). Higher birth weight is associated with an increased prevalence of obesity in male and female 4- to 12-year old children (Maffeis et al., 1994). As babies, Indian children, who are born low birth weight, are very thin, but this is due to relative paucity of skeletal muscle, therefore in fact making Indian babies relatively fat (Yajnik et al., 2002). Similarly, in white, black and Mexican-American children, LGA infants show an increased muscularity throughout infancy and childhood, and although they have an increased fatness compared to SGA infants, it is less than the increase in muscularity (Hediger et al., 1998). This relationship between large size at birth and increased fat-free mass has also been shown in adolescents aged 13-16 years in the UK, and in a group of younger children from Cambridge (Singhal et al., 2003).

As adults, Indians, although generally being smaller at birth, have thinner limbs than their UK counterparts, indicative of reduced muscle mass, but they are centrally obese in terms of waist circumference, waist-hip ratio (WHR), visceral fat and posterior subcutaneous abdominal fat (Yajnik, 2001). In 25-year old Caucasian adults, body weight, BMI and WHR do not differ between IUGR and control groups, however percentage of body fat mass is significantly higher in subjects born IUGR (Jaquet et al., 2000). Birth weight, length at 15 days of age and at 2 years of age are also positively associated with fat-free mass at 21-27

years of age in subjects born in ladino Guatemalan villages (Li et al., 2003). This study does not show an increased fatness in prenatally growth retarded subjects, but rather increased thinness as adults reflecting a decreased lean muscle mass (Li et al., 2003). An increase in birth weight has been associated with increased BMI in adult life (Gale et al., 2001; Parsons et al., 1999; Sorensen et al., 1997), and being LGA has been associated with higher proportions of total body fat and relatively lower lean body mass than 'normal' infants (Oken & Gillman, 2003).

Obesity at one or two years of age is not associated with an increased risk of adult obesity, however, after six years of age, the probability of obesity in adulthood exceeds 50% for obese children, compared to 10% for non-obese children (Whitaker et al., 1997). This may be due to a phenomenon called 'adiposity rebound', in which after the age of 2 years, obesity (measured by BMI) decreases to a minimum around 6 years of age, and then increases again (Rolland-Cachera 1984). Children with a higher BMI later in life have the earliest adiposity rebound, and are characterised by having the lowest BMI up to the age of 2 years, low ponderal index at birth, low BMI at 1 year of age, and low postnatal growth rates. Early adiposity rebound is also associated with an increased risk of developing type-2 diabetes in adult life (Eriksson et al., 2003b).

Early postnatal catch-up growth may also independently predict risk of adult adiposity, as children who show catch-up growth for weight between 0 and 2 years are heavier and taller at 5 years of age, and have greater BMI, % body fat, and summed fat mass than all other children at 5 years. They also have a

larger waist circumference (Ong et al., 2000). SGA infants who undergo catch-up growth in the first two years of life have increased body fat mass with more central fat distribution (Levy-Marchal et al., 2004). As previously mentioned, Indian children of 8 years of age who have the fastest growth rates from 4 to 8 years of age, and lower birth weights, have more adiposity than their slower growing counterparts, and this increased adiposity explains an increased risk of insulin resistance and cardiovascular disease risk (Yajnik, 2001).

Therefore, SGA infants who catch-up in the first 2 years of life may have an increased risk of becoming obese later in life. It also appears that infant and adult lean body mass may increase with increasing birth weight. It is possible that being at two opposite ends of the birth weight spectrum can have influences on adult outcome, but these risk factors may operate through separate causal pathways.

1.7.2 IUGR, catch-up growth and altered body composition in animal models

Recently, the effects of postnatal catch-up growth in rats on adiposity have been underlined in a study by (Desai et al., 2005), in which they cross-fostered offspring from food-restricted (50% ad lib) rats, onto ad lib fed mothers. The offspring grew faster, being much heavier at 3 weeks of age, and 9 months of age, with excess percent body fat at 9 months of age, compared to offspring who suckled on food-restricted mothers. They also had higher leptin levels at 3 weeks and 9 months (Desai et al., 2005). Offspring of mothers undernourished during lactation show catch-up growth in body length by 120 days, but show

incomplete catch-up growth of body weight (Williams et al., 1974), and this failure to catch up in weight is proposed to be due to under development of the adipose tissue organ (Williams et al., 1974).

Weights of the fetal interscapular, perirenal and retroperitoneal fat depots are reduced following maternal food restriction in the guinea pig, but interscapular and retroperitoneal relative weights are actually increased, indicating that these fetal guinea pigs are relatively fat (Kind et al., 2005). IUGR induced by maternal feed restriction may also have long-term effects on body composition. In the guinea pig, although maternal food restriction does not alter the combined weights of fat depots, or relative fat weights in adult offspring, in the male offspring, moderate maternal feed restriction increases retroperitoneal fat as a % of body weight, and also reduces relative weight of the biceps brachii. Food restriction does not alter fat cell size in the perirenal fat depot, or interscapular fat depot, and fat cell size is not related to measures of size at birth. However, adult weight correlates positively with interscapular fat cell size (Kind et al., 2003).

Recent studies in the guinea pig show that birth weight, abdominal circumference, head width and birth weight/birth length are all reduced in guinea pig offspring of feed-restricted mothers (Kind et al., 2002). In addition, mild but not moderate feed restriction reduces absolute growth rate (AGR) from birth to weaning in male and female offspring, but it does not change FGR (therefore catch-up growth). AGR correlates positively with birth weight, while FGR correlates negatively (Kind et al., 2003). However, growth rates of other

measures such as length, abdominal circumference and head size have not been reported in the guinea pig, and size measures have not been recorded during the catch-up growth period.

1.8 IUGR and catch-up growth summary

In summary, catching up in the first couple of years of life, although resulting in improvements in long-term adult stature, could have long-term negative consequences on adult health and longevity. Understanding the nature and causes of catch-up growth after IUGR may therefore lead to better understanding of how it influences this range of adult outcomes and their possible prevention or amelioration. It is critical to understand the mechanisms responsible for postnatal catch-up growth following IUGR. There are numerous potential endocrine factors, which normally control early postnatal growth that may be responsible for driving catch-up growth, including the insulin-like growth factors, growth hormone, cortisol and thyroid hormones.

1.9 Control of early postnatal growth

1.9.1 The IGF axis

1.9.1.1 Introduction

The somatomedins, or insulin-like growth factors, include insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II). IGF-I is a ~7.6 kDa single chain polypeptide of 70 amino acids, which is highly conserved across species (Louveau & Gondret, 2004; Monzavi & Cohen, 2002). IGF-II is a 67

amino acid peptide (Monzavi & Cohen, 2002). mRNA and protein for both IGF-I and –II are present in early gestation in all fetal tissues, with IGF-II being more abundant and predominant in fibroblasts and mesenchymal tissues (Sara & Carlsson-Skwirut, 1986). IGF-II mRNA is detected in human trophoblasts as early as day 12-18 of gestation (Monzavi & Cohen, 2002). The IGFs are produced by and act locally and systemically on most tissues to promote growth, are modulated by up to six or more distinct binding proteins, the IGFBPs, which can inhibit or facilitate their actions (Louveau & Gondret, 2004; Tanaka, 1996). Most circulating IGF-I and –II are found in a ternary complex with IGFBP-3 and the acid-labile subunit (ALS) in a 1:1:1 molar ratio (Le Roith & Roberts, 2003; Monzavi & Cohen, 2002) (Figure 1.1).

1.9.1.2 The IGFs and normal growth

The IGFs stimulate cellular hypertrophy and hyperplasia and influence differentiation in a wide range of cell types, throughout most of prenatal and postnatal development. Russell and Spencer, in 1985, showed the extent of IGF-I action by injecting GH into the epiphyseal cartilage growth plate of hypophysectomised rats. An increase in unilateral tibial growth was observed, suggesting that GH-stimulated locally produced IGF-I could also exert an autocrine/paracrine action (Russell & Spencer, 1985). An IGF-I deletion in mice results in a reduction in long bone growth. This reduced growth is a result of reduced chondrocyte hypertrophy. Chondrocyte proliferation is not significantly altered, suggesting a role for IGF-II in stimulation of this process (Wang et al., 1999).

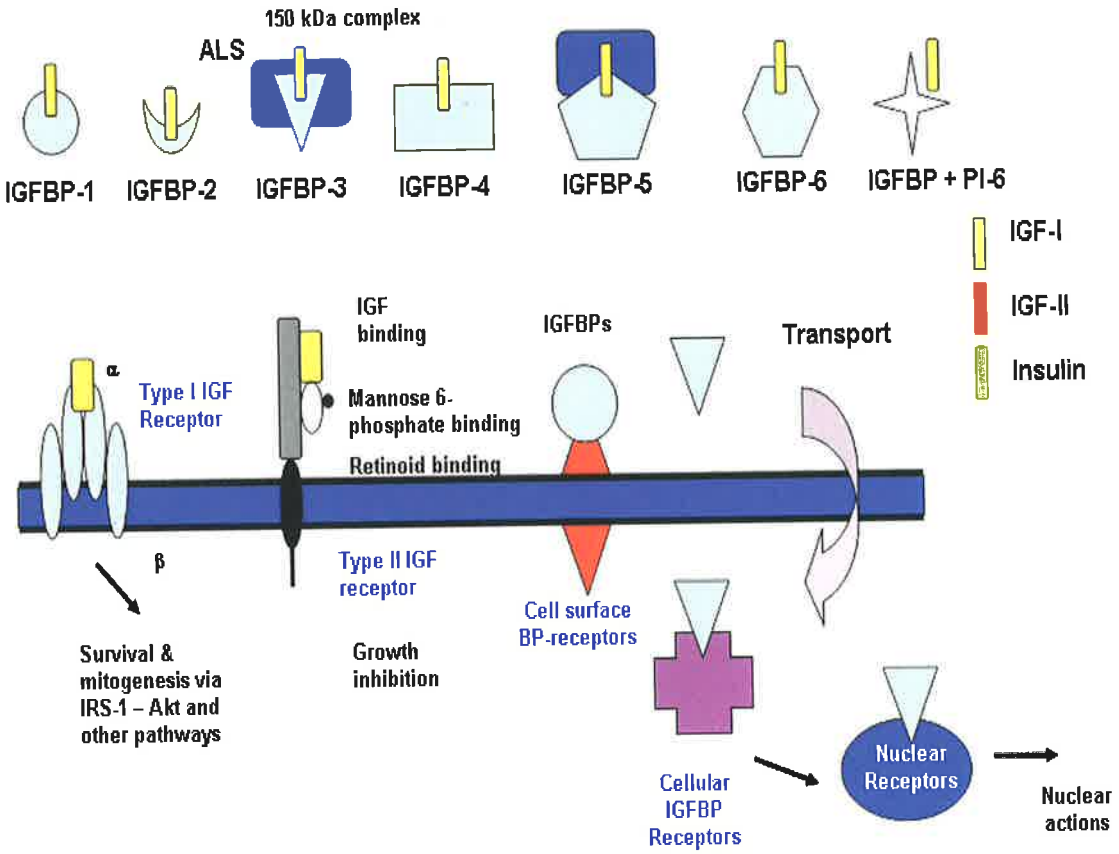

Figure 1.1: The IGF-IGFBP system

Figure adapted from (Monzavi & Cohen, 2002).

IGF-I and –II bind primarily to type I IGF receptor to induce cellular proliferation and/or differentiation through downstream pathways such as the phosphatidyl-inositol 3-kinase pathway (PI3K). Binding to the type 2 IGF receptor leads to growth inhibition.

IGF-I, with other local factors, is thought to mediate differentiation and proliferation of satellite cells, leading to rapid hyperplastic growth in skeletal muscle (Bell, 1992). The IGFs are the only known growth factors to stimulate both proliferation and differentiation in muscle cells. Addition of IGF-I to myotube cultures increases protein synthesis rates, and decreases protein degradation (Oksbjerg et al., 2004).

Growing adipocytes secrete growth factors, such as IGF-I, that induce proliferation of preadipocytes. Feeding a high-fat diet to rats for 85 days increases the total fat pad content of IGF-I in the inguinal and epididymal fat depots. Proliferation of preadipocyte and adipose tissue stromal-vascular (S-V) cell cultures treated with conditioned medium prepared from these rats is stimulated, whereas medium from low-fat fed rats does not cause this stimulation (Marques et al., 2000). In high fed lambs (200g milk powder Vs 100g milk powder for controls over the first month of life), a higher abundance of plasma IGF-I, and increased insulin secretion, is thought to promote adipose tissue growth (Gate et al., 2000). IGF-I mRNA abundance is increased in 3T3-L1 preadipocytes as they differentiate, and is also thought to down-regulate the IGF-I receptor as differentiation progresses (Zizola et al., 2002). S-V cells in culture also express the IGF-I receptor, and IGF-I increases preadipocyte replication and differentiation in these cells. IGF-I promotes differentiation of ovine preadipocytes (Soret et al., 1999). The increase in IGF-I transcripts in primary cultures of preadipocytes treated with exogenous IGF-I suggests a positive feedback mechanism for IGF-I in differentiation (Boney et al., 1994).

In the rat, IGF-I mRNA is highly expressed in the epididymal and retroperitoneal fat pads, and the lowest expression is in the subcutaneous inguinal region (Louveau & Gondret, 2004). In cold-exposed rats, IGF-I gene expression is upregulated in brown adipose tissue (BAT), suggesting an additional role in thermogenic differentiation of adipose tissue (Duchamp et al., 1997).

Although only measured in females, IGF-I mRNA expression has been reported in all tissues in adulthood in the guinea pig, with highest expression being in the uterus, liver and parametrial and interscapular adipose tissues (Olausson & Sohlstrom, 2003). IGF-II is also expressed in all guinea pig tissues, apart from the soleus muscle (Olausson & Sohlstrom 2003). Receptors for IGFs are also widespread, with IGF-IR mRNA being detected in porcine adipose tissue, adipocytes and preadipocytes, as well as insulin/IGF-I hybrid receptors being present in 3T3-L1 preadipocytes (Louveau & Gondret, 2004).

Although GH is the dominant regulator of IGF-I production, prenatally, factors other than GH stimulate fetal somatomedin production, as GH receptor levels are low in the fetal liver (Gluckman & Pinal, 2003). Fetal insulin appears to be the major regulator of circulating fetal IGF-I. IGF-II is the primary growth factor driving embryonic growth, however IGF-I produced by the fetal liver and other tissues is dominant in late gestation (Gluckman & Pinal, 2003). After birth, the production of IGF-I is dependent on GH and the presence of specific GHRs in the liver (Forhead et al., 2002). Induction of the hepatic GH receptor and a maturational switch from paracrine to hepatic IGF-I synthesis is initiated by a prepartum rise in cortisol in the fetal sheep, and is accompanied by increased

IGF-I mRNA production from the GH-sensitive promoter and down regulation of IGF-II in the fetal liver (Forhead et al., 2002).

1.9.2 The insulin axis

1.9.2.1 Introduction

Insulin consists of two unbranched peptide chains joined together by two disulfide bridges, which is formed as a single-chain proinsulin molecule. The insulin receptor (IR) is a tetramer with 2 α and 2 β glycoprotein subunits held together by disulfide bonds (Debuse & Horton-Szar, 1998). It is expressed as two isoforms, resulting from alternative splicing of exon 11. Splicing of exon 11 to yield isoform A may give the IR an increased ability to bind IGF-II (Kishimoto et al., 1994). However, splicing of this exon may exert a selective negative influence on IGF-I binding, as the isoform of IR lacking this exon has a marked reduced affinity for IGF-I, but not for insulin (Yamaguchi et al., 1993). The α unit is extracellular and contains the insulin-binding domain, whilst β spans the plasma membrane, containing tyrosine kinase activity (Debuse & Horton-Szar, 1998). Insulin binds to the α subunit, bringing the two α-subunits together. This allows ATP binding to the β-subunits intracellular domain, which then activates receptor auto-phosphorylation, and in turn activation of insulin receptor substrates 1 and 2 (IRS-1 and –2) (Kishimoto et al., 1994).

The primary function of insulin is to regulate glucose transport. Insulin catalyses the balance between phosphorylation and dephosphorylation of glucose, in part by increasing synthesis of glucokinase, thereby decreasing net output of

glucose and promoting uptake (Goodman, 1998b). The regulation of glucose transport occurs through glucose-transporter proteins, in particular GLUT-4, an insulin-sensitive glucose transporter. In muscle and adipocytes, insulin-stimulated glucose uptake is achieved by the translocation of GLUT-4 from the intracellular storage vesicles to the cell surface, in response to insulin binding to its receptor (Zorzano et al., 1996). Once blood levels of insulin decrease and insulin receptors are no longer occupied, the glucose transporters are recycled back into the cytoplasm (Zorzano et al., 1996).

1.9.2.2 The insulin axis and normal growth

Insulin serves as a growth-promoting hormone during the fetal period. Although insulin has little effect on differentiation and prepartum maturation of fetal tissues, infusion of insulin increases uptake, utilization and oxidation of glucose by fetal tissues (Fowden, 1995). Insulin may also alter concentrations of other growth-promoting factors such as the IGFs, and may stimulate cell proliferation in utero directly or indirectly through changes in fetal metabolism and IGF production (Fowden, 1995). Insulin's effects on postnatal growth may be mediated by regulating growth hormone receptor number, leading to growth hormone sensitivity, and then IGF-I secretion (Tanaka, 1996).

In muscle, insulin increases cellular uptake and metabolism of glucose by accelerating transmembrane transport of glucose and structurally related sugars (Debuse & Horton-Szar, 1998). Insulin also lowers the concentration of amino acids by stimulating uptake by muscle, and reducing their release (Goodman, 1998a). Therefore, in the absence of insulin, there is net loss of muscle protein.

Insulin also acts on the liver in a similar fashion to how it acts on muscle, by reducing outflow of glucose from the liver and promoting storage of glycogen (Goodman, 1998a).

In the fetus, insulin stimulates an increase in tissue accretion and accumulation of adipose tissue (Fowden, 1995). The storage of fat in adipose tissue depends on many insulin-sensitive reactions, such as the synthesis of long-chain fatty acids from glucose, synthesis of triglycerides from fatty acids and glycerol (esterification), breakdown of triglycerides to release glycerol and long chain fatty acids (lipolysis), and the uptake of fat from lipoproteins in the blood (Bajaj & DeFronzo, 2003). Insulin exerts an antilipolytic effect, lowering the concentration of plasma FFA's, which results in increased glucose uptake in muscle (Bajaj & DeFronzo, 2003).

1.9.3 The thyroid hormone axis

1.9.3.1 Introduction

Thyroid hormone exists in two main forms, T_3 and T_4; both are α-amino acid derivatives of tyrosine. The thyronine nucleus consists of two benzene rings in ether linkage, with both forms being rich in iodine; thyroxine, or T_4, contains 4 atoms of iodine, whereas triiodothyronine (T_3) only contains 3 (Shi et al., 2002). The synthesis and release of thyroid hormones requires the interaction of various hormones and organs. Thyrotropin releasing hormone (TRH), which is synthesised in the paraventricular nucleus of the hypothalamus, binds to TRH receptors in the pituitary thyrotropes, leading to synthesis and release of thyroid stimulating hormone (TSH), a 28-kDa protein (Yen, 2001). TSH is the principal

regulator of thyroid gland function, increasing blood flow and hormone biosynthesis and secretion. TSH binds to specific G-protein-linked receptors on the follicular cells (Debuse & Horton-Szar, 1998), resulting in activation of the thyroglobulin gene (Shi et al., 2002; Yen, 2001). Thyroglobulin then undergoes a series of post-translational processing steps, resulting in TH that is covalently bound to thyroglobulin. This covalent structure is stored in the colloid within the lumen of the follicle cells, and when needed endocytosed from these cells (Shi et al., 2002; Yen, 2001). The resulting circulating T_4 is secreted into plasma, and converted to T_3 by organs such as the liver and kidney, which contribute a large proportion of circulating plasma T_3. The conversion of T_4 to T_3 occurs through the action of 5'-deiodinase (Shi et al., 2002).

THs, along with glucocorticoids and catecholamines, cross the placenta transcellularly and are metabolised en route. Placental tissue contains an iodothyronine inner ring monodeiodinase which deiodinates T_4 to rT_3, and converts T_3 to inactive diiodothyronine (Fisher, 1998). The iodothyronine deiodinases are enzymes that contain selenocysteine. There are 3 main types of these enzymes: type 1 (5'-DI), which is mainly expressed in the kidney, liver, thyroid tissues and CNS, type 2 (5'-DII), which is expressed in the CNS, anterior pituitary and brown fat, and type 3 (5'-DIII), which is present in the placenta and the CNS. The 5'-DII enzyme acts to catalyse monodeiodination of T_4 and rT_3 to T_3 and 3,3'-diiodo-$_L$-thyronine (3,3'-T2), whereas 5'-DIII catalyses deiodination of T_4 to rT_3 and T_3 to 3-3'-T2 (Chan et al., 2002). Iodothyronine deiodinase enzymes are present in the human fetal cerebral cortex early in gestation,

suggesting that the fetus can generate T_3 from T_4 early in pre-natal life (Chan et al., 2002).

1.9.3.2 The thyroid hormone axis and normal growth

Thyroid hormones (TH) play an important role in growth and development of the infant both before and after birth (Fowden, 1995). T_4 is important in controlling stimulation of oxygen utilization by fetal tissues (Fowden 1995), and the TH axis is the major control in body thermoregulation postnatally (Fowden, 1995; Reed Larsen et al., 1998).

TH also increases glucose absorption from the digestive tract, glycogenolysis and gluconeogenesis in hepatocytes and glucose oxidation in liver, fat and muscle cells (Yen, 2001). TH alters the expression of various bone markers in serum, reflecting its actions on skeletal tissues (Yen, 2001). TH increases the expression of the bone growth marker osteocalcin in osteoblasts, up-regulates the serum osteoblast proteins alkaline phosphatase and collagen, and stimulates IGF-I and IGFBP-2 mRNA in rat primary cultures and osteoblastic cell lines (Yen, 2001). Thyroxine (T_4) causes cell hypertrophy, increasing chondrocyte size (Bohme et al., 1992). TH also potentiates effects of GH on long bone growth, and therefore may promote bone growth via stimulation of GH and IGF-I (Goodman, 1998c; Yen, 2001).

T_3 plays an important role in regulating basal oxygen consumption, fat stores, lipogenesis and lipolysis. It induces intracellular lipid accumulation, various adipocyte-specific markers such as malic enzymes, fatty acid synthase, ATP-

citrate lyase and glycerophosphate dehydrogenase, and adipocyte cell proliferation and fat cell cluster formation (Yen, 2001). TH also has major effects on the developing brain *in utero* and during the neonatal period (Yen, 2001), with maturation of the nervous system during the perinatal period depending absolutely on TH (Kilby et al., 2000).

1.9.4 The hypothalamo-pituitary-adrenal axis (HPAA)

1.9.4.1 Introduction

Glucocorticoids are produced in the adrenal cortex, in response to certain signals from the HPA axis (Bertram & Hanson, 2002). The HPA axis is controlled by a negative feedback system (Figure 1.2). Corticosteroid releasing hormone (CRH) is released from the hypothalamus, following signals such as stress, and act on the anterior pituitary to stimulate expression of pro-opiomelanocortin (POMC). POMC is post-translationally cleaved to release adreno-corticotrophic hormone (ACTH), which acts on the adrenal cortex to release cortisol (Figure 1.2) (Bertram & Hanson, 2002). The majority (90%) of cortisol is bound to corticosteroid-binding globulin (CBG), but the remaining free portion can enter cells and act on glucocorticoid receptors (GRs) to initiate gene transcription (Bertram & Hanson, 2002). The GR is a nuclear hormone receptor that binds to specific glucocorticoid response elements (GREs) to initiate down-stream pathways in target cells (Yudt & Cidlowski, 2002).

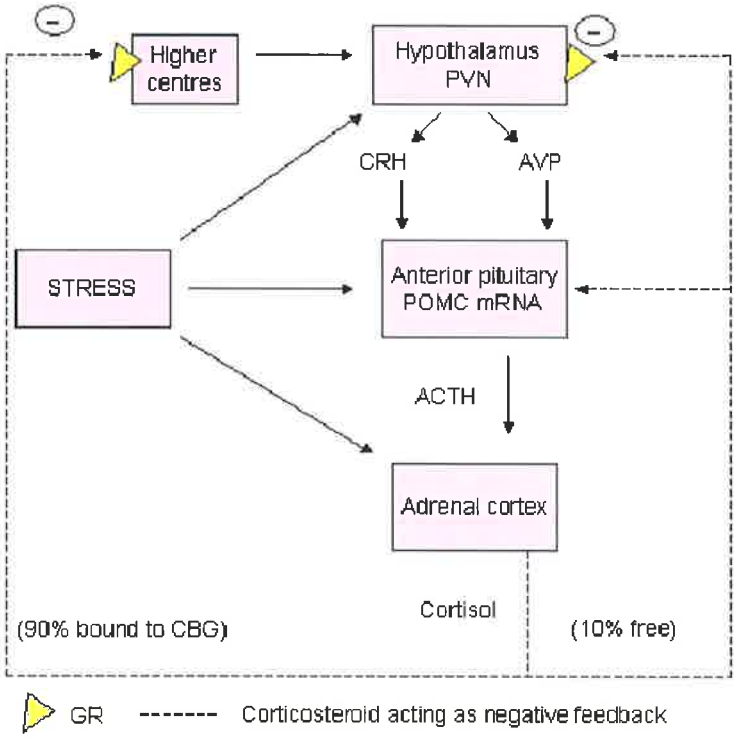

Figure 1.2: The hypothalamo-pituitary-adrenal axis (HPAA)

Figure is adapted from (Bertram & Hanson, 2002).

1.9.4.2 *The HPA axis and normal growth*

Cortisol maintains blood glucose during periods of fasting by stimulating gluconeogenesis, increasing breakdown of skeletal muscle protein, stimulating lipolysis in limbs and accumulation of fat in the trunk, and inhibiting antibody production and lymphoid-tissue growth (Fisher, 1998). In postnatal life, glucocorticoids inhibit growth, primarily through inhibiting the actions of insulin and IGFs (Fowden, 1995).

In the fetus, cortisol plays an important role in lung maturation, through increasing compliance and surfactant release to ensure breathing can occur at birth (Fowden, 1995). Cortisol also acts on the liver to up-regulate gluconeogenic enzyme activities in the fetus in late gestation (Liggins, 1994). As term approaches (in the last 10 weeks of gestation), fetal tissues including the liver and lung express 11-ketosteroid reductase activity that promotes local conversion of cortisone to cortisol (Fisher, 1998). The rise in cortisol helps to trigger birth, and has an important role in the maturation of several fetal systems critical to extrauterine survival (Challis et al., 2001; Fowden, 1995). Plasma ACTH and cortisol levels also rise towards the end of gestation in the fetal guinea pig. At this time, the fetus remains highly sensitive to glucocorticoid feedback (Owen et al., 2005).

1.10 IUGR and the endocrine system

1.10.1 The IGF axis

1.10.1.1 IUGR, catch-up growth and the IGF axis in the human

Small size at birth and its associated catch-up growth in infants and children is characterised by marked but somewhat variable alterations in the somatotropic and IGF axis. Most studies have focused on the consequences for circulating IGFs and to some extent the IGFBPs, with little known about the effects on IGF sensitivity. Hence the role of programmed changes in IGF action in postnatal catch-up growth after fetal growth restriction is unclear. In addition, both small size at birth and catch-up growth independently predict later risk of diabetes and cardiovascular disease, which could be due in part to persistence of early onset changes or deficiencies in IGF abundance and action.

Cord sera IGF-I levels are reduced in IUGR pregnancies (Cianfarani et al., 1998; Giudice et al., 1995; Gluckman et al., 1983), whereas cord sera IGF-II concentrations are not significantly correlated with birth size (Giudice et al., 1995; Gluckman et al., 1983). Reductions in concentrations of IGFBP-3 (Bazaes et al., 2003; Cianfarani et al., 1998; Giudice et al., 1995), and increases in IGFBP-1 are also seen in IUGR fetal cord sera (Bazaes et al., 2003; Cianfarani et al., 1998; Giudice et al., 1995).

In the IUGR infant, plasma IGF-I concentrations at 5 days of age postnatally are lower compared to controls, but increase to reach those of controls by 9 months of age (Thieriot-Prevost 1988). Catch-up growth following IUGR has been

consistently reported to occur in the presence of reduced, or at best, normal serum IGF-I concentrations in the first few months of life, up to one year of age (Bennett et al., 1983; Giudice et al., 1995; Leger et al., 1996; Ogilvy-Stuart et al., 1998; Ozkan et al., 1999; Toumba et al., 2005). Catch-up growth in the human IUGR infant has also been associated with an increase in plasma IGF-I concentrations to normal values, compared to persistent low IGFs in those infants who do not catch-up (Colle et al., 1976). Plasma IGF-II levels are also reduced in children with short stature born after IUGR, compared to controls (de Waal et al., 1994), but serum IGF-II levels are higher in IUGR infants undergoing catch-up growth compared to those infants not catching up, at 3 months of age (Garcia et al., 1996).

Although the relationship between IGFs and IUGR at birth is highly replicable, studies investigating the levels of IGFs in early childhood in children born IUGR report conflicting results. In 4- and 7-year old children from India and England, plasma IGF-I concentrations were higher in heavier and taller children of both sexes, and higher in those children who were shorter/lighter at birth, with smaller head circumferences and lower placental weight (Fall et al., 1995). Plasma IGF-I is also highest in children at 5 years of age with lowest birth weights and larger current size (Ong et al., 2002), and plasma IGF-I and IGF-II are higher in short IUGR children (age ~ 8 years) compared to short normal birth weight children (Cutfield et al., 2002). In contrast, in another study, childhood serum IGF-I levels were lower in those born SGA (Johnston et al., 1999), however these researchers did not report at what age the IGF-I measurements were made. Finally, total urinary IGF-I excretion in 9-year-old

children is strongly related to current weight, current height, BMI and height velocity, but not related to birth size (Fall et al., 2000).

Few assessments of circulating IGFBPs have been made following IUGR, however, plasma IGFBP-3 was higher in short IUGR children (age ~ 8 years) compared to short normal birth weight children in a study by (Cutfield et al., 2002). IGFBP-3 levels were also lower in children who were born SGA, when measured at 3.0-8.9 years of age, and this relationship was even stronger with those who had catch-up growth after the age of 6 months (Toumba et al., 2005). Changes in erythrocyte IGF-I receptor affinity and number have also been described in IUGR children aged 4-16 years. Receptor affinity is significantly reduced, whereas receptor number is increased, and in those children with these abnormalities, plasma IGF-I levels are significantly lower than in control children (Ducos et al., 2001).

Plasma IGF levels in adult life do not appear to be related to birth size. Serum IGF-I measured at 25 years of age is not associated with birth weight or birth length, and does not show any relation to catch-up growth (Ben-Shlomo et al., 2003). Plasma IGF levels in elderly subjects are also not related to birth size, as no relationship is seen between IGF-I concentrations in subjects aged 70-80 years and birth weight or other birth measurements, or height at 7 years of age (Kajantie et al., 2003).

In addition, two children with IGF-I receptor mutations that resulted in IUGR have recently been described (Abuzzahab et al., 2003). The first patient, who

reached a final height of 4.8 SD below average, had distinct single base pair substitutions in the codons for amino acid 108 and 115. These substitutions resulted in a decreased specific binding of IGF-I to IGF-I receptors on fibroblasts isolated from the patient, with IGF-I binding affinity being one third that of the control healthy children. The patient's fibroblasts also displayed a marked decrease in sensitivity of hormone binding on receptor signalling, as measured by decreased receptor phosphorylation. This patient also had increased basal serum IGF-I, IGFBP-3 and ALS concentrations, whereas IGFBP-2 was reduced and IGF-II levels were normal. The second patient reached a height of 2.6 SD below normal. His serum IGF-I concentrations ranged from 1.1 to 2.3 SD above the mean for age when measured on several occasions over a period of time. The patient was heterozygous for a point mutation in exon 2, of which a major outcome was a decrease in number of IGF-I receptors per fibroblast compared to control subjects. Although these two patients showed severe growth retardation, they were isolated cases (patient 1 from a study of 42 cases, and patient 2 from a study of 50 cases), therefore the authors suggest that IGF-IR mutations are uncommon causes of intrauterine and postnatal growth failure (Abuzzahab et al., 2003). However, the severe effects of mutations in the IGF-I receptor gene on intrauterine and postnatal growth suggests that changes in receptor expression may contribute to the adverse outcomes observed following IUGR.

Overall these findings suggest that plasma IGFs are initially low following birth, then increase over the first six months of life, especially in those undergoing catch-up growth, before increasing above normal in childhood and then

gradually normalising with increasing age in adult life. The impact if any on IGF sensitivity is unclear and whether the transient increase in plasma IGF-I in childhood represents IGF resistance or over production is unclear.

1.10.1.2 IUGR, catch-up growth and the IGF axis in animals models

Placental restriction in the sheep model is associated with asymmetric fetal growth retardation, reduced fetal oxygenation and fetal hypoglycaemia. Fetal plasma IGF-I is positively associated with fetal weight at 120 (Kind et al., 1995; Owens et al., 1994) and 127 days of gestation, and with crown-rump length (Owens et al., 1994). IGF-II also correlates positively with fetal weight at 127 days but not at 120 days of gestation (Owens et al., 1994), and IGFBP-3 levels are not significantly changed after placental restriction (Kind et al., 1995). Placental restriction reduces IGF-I mRNA in skeletal muscle, kidney and lung of the fetal sheep, however has no effect on IGF-II mRNA (Kind et al., 1995). Plasma IGF-I is lower in small sheep fetuses compared to normal size sheep fetuses, and decreases with advancing gestation in very small fetuses but not in normal or small fetuses. Plasma IGF-II is similar in normal and growth-retarded sheep however IGF-II decreases between 120 and 127 days in very small sheep. In the latter, reduced blood pO2 and glucose concentrations are also apparent (Owens et al., 1994). Fetal plasma IGF-II was not affected by maternal nutrient restriction imposed on sheep from day 83 to day 90 of gestation, nor was any effect on fetal plasma insulin or IGF-I concentrations seen in a study by McMullen et al (2004). However, fetal weight was also unaffected, therefore the severity of the nutrient restriction may not have been great enough to program endocrine changes (McMullen et al., 2004). Fetal plasma IGF-I levels were

reduced, but IGF-II levels unchanged following maternal feed restriction at 100-110 days of gestation in a study by Bauer et al, 1995. IGF-I levels were positively correlated with fetus weight (Bauer et al., 1995). Fetal placental embolization in the sheep elevates levels of fetal plasma IGFBP-1 and –2 (Gagnon, 2003).

The guinea pig IGF axis is similar to the human in that IGF-II is expressed in serum of adult guinea pigs, as it is in adult human tissues, and IGFBP-3 is expressed in fetal plasma of both species. In a recent study by Carter et al (2005), IUGR induced by maternal unilateral uterine artery ligation on day 30 of gestation increased IGFBP-2 and –4 in fetal plasma, and decreased IGFBP-3. Expression of IGFBP-2 mRNA was increased in the fetal liver, however no significant change was observed in expression of IGF-I and –II or IGFBP-3 and –5 mRNA in the liver or fetal skeletal muscle (Carter et al., 2005). In contrast, a decrease in plasma IGF-I has been observed in the fetal guinea pig following uterine artery ligation at day 30 of pregnancy (Jones et al., 1987; Jones et al., 1990), in association with a 2-4 fold increase in IGF-II (Jones et al., 1987).

Growth retardation induced by bilateral ligation of the maternal uterine artery in the rat results in lower fetal serum IGF-I, insulin and glucose concentrations. Placental mRNA for IGF-IR is also lower in the SGA group (Reid et al., 2002). Uterine ligation in the rat results in a significant reduction in plasma IGF-I only 24 hours after the operation, with a tendency for a reduction in plasma IGF-II. Significant correlations between plasma IGF-I levels and liver and brain weights are apparent in these fetuses. The availability of IGFBP is decreased,

suggesting an increased concentration of IGFBP in SGA fetuses (Unterman et al., 1993).

In addition to the effects of IUGR on the IGF axis pre-natally, there have been numerous studies on the postnatal expression of IGFs following fetal growth restriction. Chronic food restriction (~ 55% ad libitum) reduces IGF-I and IGF-II plasma levels in young adult guinea pigs (Sohlstrom et al., 1998), and virginal and pregnant female animals (Dwyer & Stickland, 1992; Olausson & Sohlstrom, 2003). Chronic food restriction (70% ad lib intake per kg body weight) also reduces the abundance of IGFBP-1, -3 and –4 in plasma, while increasing IGFBP-2 in young adult guinea pigs. IGF-I, IGF-II, IGFBP-1, -3 and –4 are all positively correlated to body weight and weight gain in these animals (Sohlstrom et al., 1998).

In response to undernourishment in ewes for 10 or 20 days from 105 days of gestation, offspring are smaller at birth, but do not differ from control fetuses from weaning onwards, showing complete catch-up growth. No effect of maternal food restriction on plasma IGF-I concentrations is observed in offspring at birth, or at 3 months, 5 months or 30 months of age. However, levels are associated with current weight at all ages (Oliver et al., 2002).

In rat offspring from mothers underfed (30% ad lib) during gestation, plasma IGF-I concentrations are not significantly changed (Vickers et al., 2000). Maternal food restriction also lowers plasma IGF-I and IGFBP-3 levels in rat offspring at day 22 of gestation, birth and day 9 of postnatal life, and increases

IGFBP-1 and –2 at these three ages (Woodall et al., 1996). However, uterine ligation on day 17 of pregnancy does not change plasma IGF-I levels in rat offspring at 100 days postnatally, nor does it significantly affect the adult GH secretory pattern (Houdijk et al., 2000).

Therefore, in animal studies of various IUGR models, IUGR consistently occurs in the presence of low or normal IGF levels. An increase in binding protein level often accompanies a decrease in IGF, suggesting the low IGF concentration is due to increased sequesteration by the IGFBPs, although this has yet to be investigated.

Recently in the sheep model of IUGR induced by placental restriction, it has been shown that sensitivity of FFA metabolism to IGF-I is increased in neonatal animals that were small at birth, and that circulating concentrations of IGF–II but not of IGF-I are decreased at 35 days of age (DeBlasio 2005). This suggests that prenatal restraint increases postnatal IGF sensitivity and helps cause catch-up growth, but whether the apparent decrease in plasma IGFs reflects increased clearance rather than decreased IGF production and the overall consequences of IGF action are unclear.

1.10.1.3 Insulin-like growth factors, adult disease and altered postnatal outcomes

Low serum IGF-I levels have been associated with the development of cardiovascular disease (Janssen et al., 1998). In contrast, higher IGF-concentrations correlate with higher systolic blood pressures (Fall et al., 1995;

Kajantie et al., 2003), increased fibrinogen levels, and insulin resistance (Kajantie et al., 2003). However, treatment with IGF-I in normal patients increases insulin sensitivity and is of beneficial consequence when used on patients with growth hormone deficiency, type 1 and type 2-diabetes mellitus (Froesch et al., 1996).

Abnormal IGF action has been implicated in the development of postnatal obesity due to its recognised role in adipocyte proliferation. In humans (and sheep), fetal adipose tissue is deposited primarily during the final third of gestation (Symonds & Lomax, 1992), which coincides with an increased abundance of circulating hormones involved in regulation of fetal adipose tissue development, including IGF-I (Lorenzo et al., 1993).

In 7- and 8-year old children, plasma IGF-I levels are correlated with an increased percentage of body fat, fat-free soft tissue, and total body bone mineral content (Garnett et al., 2004). However, in 12-13 year old pubertal girls, no association was found between serum IGF-I concentration and any measures of body composition, including BMI, triceps skin fold thickness, WHR, height or weight (Wilson et al., 1991).

In adults, negative associations between plasma IGF-I and BMI, WHR and abdominal fat content have been reported (Juul, 2003). However, women with a BMI below 30 kg/m^2 have higher total IGF-I and free IGF-I concentrations than do women with a BMI higher than 30 kg/m^2, yet in men, there is a negative correlation between BMI and free IGF-I in subjects that have a BMI lower than

30 kg/m^2 (Frystyk 2004). IGFBP-3 was also significantly correlated with BMI in a longitudinal study of multiple ages between 0 and 25 years. IGF-I, on the other hand was not related to BMI, however, these results were based on a single blood sample from nonfasting children (Juul et al., 1995).

Although various studies have investigated the long-term effects in terms of body composition following IUGR, very few researchers have looked at the factors linking IUGR to obesity later in life. Due to the proposed role of IGFs in postnatal catch-up growth following IUGR, and the role of the IGFs in stimulating growth and development of numerous tissue types, the question can be raised as to whether the link between IUGR, catch-up growth and adult disease is due in part to altered abundance and/or sensitivity of the IGFs. However, their action may be further complicated by the role of other endocrine systems in postnatal catch-up growth.

1.10.2 The insulin axis

1.10.2.1 IUGR, catch-up growth and the insulin axis in humans

The IUGR infant has low plasma insulin levels at birth, which remain low up to 6 months of age compared to normal sized infants (Colle et al., 1976). In the first 48 hours after birth, human SGA infants are more insulin sensitive compared to control subjects (Bazaes et al., 2003). Infants with the highest weight gain velocity (catch-up growth) and the lowest birth weights, have also been shown to be the most insulin sensitive in the first 2 months of life, as assessed by the homeostasis model assessment (HOMA) method, suggesting that increased insulin sensitivity is associated with catch-up growth (Gray et al., 2002).

Increased postnatal growth rate in the first 6 months of life is associated with increased insulin release in response to an injection of glucose (Colle et al., 1976). However, SGA children showing catch up growth and a BMI greater than 17 kg/m^2 have the lowest insulin sensitivity of glucose metabolism, as measured by hyperinsulinemic-euglycemic clamp, at 9 years of age compared to AGA or SGA children with catch-up growth but a lower BMI (Veening et al., 2002). Previous studies have also clearly demonstrated that subjects who are thin at birth, but develop obesity in childhood or adulthood, have the highest risk of cardiovascular disease and insulin resistance (Levy-Marchal et al., 2004).

1.10.2.2 IUGR, catch-up growth and the insulin axis in animal models

Increased insulin sensitivity is apparent in the fetal sheep following placental restriction (De Blasio, 2004). In the pig, there are no differences in fasting insulin levels between low and high-birth weight offspring at 3 or 12 months of age (Poore & Fowden, 2004). Insulin sensitivity, as measured by the glucose decrement during the first 10 minutes after an intravenous insulin bolus, was also not different between high and low birth weight pigs at 3 or 12 months. However, insulin sensitivity at 3 months was positively associated with fractional growth rate from birth to 1 month of age, and this effect was limited to the male offspring. At 12 months of age, insulin sensitivity was negatively associated with fractional growth rates from birth to 1 month, suggesting that insulin resistance developed in these animals between 3- and 12- months of age (Poore & Fowden, 2004).

Fractional growth rate correlates with fasting plasma insulin in male guinea pig offspring. In female guinea pigs, fasting glucose and insulin, glucose tolerance, and insulin response to glucose are not related to size at birth measures or FGR (Kind et al., 2003).

Therefore, in human and animal species, it appears that low birth weight and postnatal catch-up growth are associated with increased insulin sensitivity in the first few months of life. However, at a later postnatal age, the timing of which is yet to be established, there is a 'switch' from insulin sensitivity to insulin resistance, with IUGR being associated with insulin resistance later in life. The mechanisms by which low birth weight and postnatal catch-up growth are associated with postnatal insulin status has yet to be determined in detail. Moreover, the effect of spontaneous fetal growth restriction in the guinea pig on postnatal insulin homeostasis during the catch-up growth period has not been reported.

1.10.3 The thyroid hormone axis

1.10.3.1 IUGR, catch-up growth and the thyroid hormone axis in the human

In the human, plasma T_4 levels in cord serum increase with gestation, as do thyroxine-binding globulin (TBG) levels. Mean plasma T_3 levels in cord serum also increase significantly with increasing gestational age (Murphy et al., 2004). Thyroid status plays a critical role in the pathogenesis of low birth weight related morbidity, especially with respect to growth and development of the central nervous system (CNS) (Kilby et al., 2000). Cordocentesis has shown that plasma total T_4 and free T_4 are reduced in SGA fetuses compared to AGA

fetuses (Kilby et al., 1998; Thorpe-Beeston et al., 1991). Although the effect of IUGR on TH sensitivity in the fetus is unknown, a reduced immunochemical staining intensity of TRα1, α2, β1 and β2 expression is seen in the central nervous system (CNS) of IUGR fetuses in the first and second trimesters (Kilby et al., 2000), suggesting that there is down-regulation of TH receptor expression in IUGR.

In children (mean age 6.7 years) who have shown full catch-up growth, plasma TSH is significantly higher in SGA children compared to AGA children. Plasma free T_3 is also higher in these children, however plasma rT_3, free T_4, and free T_3:rT_3 ratio are similar between groups (Radetti et al., 2004).

1.10.3.2 IUGR, catch-up growth and the thyroid hormone axis in animal models

In sheep, surgical removal of the fetal thyroid at mid gestation leads to reduced bodyweight and CRL at term, and delayed maturation of the skin, skeleton and pulmonary and neuromuscular systems. Hypothyroidism produces an assymetrical type of growth retardation, as the reduced bodyweight is due primarily to a decrease in weight of the fetal carcass, which is mainly skin, bone and muscle. Growth retardation is caused by hypoplasia and hypotrophy in different fetal tissues (Fowden, 1995).

Chronic maternal food restriction (60% ad libitum) in the guinea pig does not affect plasma T_3 or T_4 concentrations in the fetus during late gestation (Dwyer & Stickland, 1992). However, following unilateral uterine artery ligation in the

guinea pig at day 30 of gestation, plasma thyroid hormone concentrations are reduced in the fetus in later gestation compared to normal (Jones et al., 1984). Similarly, 48 hours of maternal nutrient deprivation reduces plasma thyroxine levels in the guinea pig fetus at day 52 of gestation (Lingas et al., 1999). Following 48 hours of maternal nutrient deprivation, TRα1 and TRβ1 mRNA is upregulated in the brain of male fetuses at day 52 of gestation in the guinea pig, however TRβ1 mRNA is downregulated in female fetal brains, suggesting a sex-specific difference in the regulation of THR expression during gestation (Chan et al., 2005). In addition, maternal nutrient deprivation increases D2 deiodinase in the brain (Chan et al., 2005). The upregulation of deiodinase expression may be due to increased cortisol levels seen in these guinea pigs (Chan et al., 2005), as cortisol is a major stimulator of deiodination of T_4 to T_3 in the fetus (Forhead et al., 2000).

The neonatal pig, which has been specifically hypothyroid in utero in late gestation, has a greater postnatal surge in plasma total and free T_4 and T_3 than normal (Berthon et al., 1993). This suggests the hypothyroidism of the IUGR fetus could cause hyperthyroidism postnatally. The placentally restricted neonatal lamb, which is hypothyroid in utero, does have elevated plasma T_4 levels in the first 24h following birth (Mellor & Pearson, 1977). In the guinea pig, IUGR caused by uterine artery ligation at 28-30 days gestation does not result in any change to plasma T_3 or T_4 in offspring at 8 weeks of age (Briscoe et al., 2004). However, it is not known whether these reductions in TH levels persist into postnatal life.

Therefore, it appears that IUGR reduces TH levels in the first few weeks of life, but it is unclear whether these effects persist after this time. In addition, the role that THs play in postnatal catch-up growth is currently unknown. Due to the effect of cortisol on stimulation of TH synthesis, perturbations in the HPA axis through an adverse intrauterine environment may have long-term effects on the TH axis, and in addition cortisol may have direct effects on pre- and post-natal growth.

1.10.4 The HPA axis

1.10.4.1 IUGR, catch-up growth and the HPA axis in the human

There is considerable evidence that the HPA axis can be programmed by the early environment an animal or human experiences, however the mechanisms underlying programming of this axis are currently unknown (Matthews, 2002). Premature increases in fetal adrenocortical secretion caused by hypoxia and under nutrition may contribute to IUGR (Fowden, 1995). Glucocorticoids inhibit postnatal growth, having major effects on the differentiation and prepartum maturation of many fetal tissues in the period immediately before birth (Fowden, 1995). Glucocorticoid excess is associated with retarded postnatal growth, and hypertension and hyperglycemia in adulthood (Seckl, 1997).

Glucocorticoids are lipophilic, and can readily cross biological membranes such as the placenta. The majority of maternal cortisol, however, does not reach the fetus through the action of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts fetal cortisol is converted to cortisone (Fowden,

1995; Seckl, 1997). This protects the fetus as cortisone is relatively inactive, and high cortisol can retard placental and fetal growth (Fowden, 1995). Fetal glucocorticoid levels are usually determined by de novo metabolism, through adrenal maturation, in response to fetal stress, from the mother through transplacental transfer, or through production by the placental tissues (Challis et al., 2001). Increased glucocorticoid exposure in utero may permanently program HPAA function, leading to persistent elevations in glucocorticoids postnatally (Challis et al., 2001). Fetal cortisol levels are increased in human fetuses displaying IUGR (Goland et al., 1993). Cortisol/creatinine ratios are also higher in IUGR infants than controls when measured from 5-20 weeks postnatally. These infants have an increased excretion of cortisol in their urine (Jackson et al., 2004). Cortisol levels are inversely associated with birth weight in children who were born IUGR, and currently aged ~ 9 years. Cortisol is lowest in those children exhibiting catch-up growth (corrected height > 0 z-score), compared to those children without catch-up growth (corrected height < 0 z-score). This suggests that catch-up growth following IUGR may be affected by programming of the HPAA axis, and those children with highest cortisol levels may be at a greater risk of growth failure (Cianfarani et al., 2002). In addition, a recent study has shown children with higher cortisol/cortisone ratios have slower growth rates between 0 and 12 years of age, compared to those children that have lower ratios (Tenhola et al., 2005).

The effects of prenatal growth restriction on basal cortisol levels may persist into adult life, however previous studies do not report consistent results in this regard. Fasting plasma cortisol levels in 3 different populations (women aged

either ~ 63 years, men and women aged ~ 51 years, or young adults aged ~ 20 years) fell with increasing birth weight consistently across populations. Although cortisol did not differ significantly by sex, a strong inverse relationship with waist-to-hip ratio was seen in men but not women. Shortness at birth was also associated with elevated plasma cortisol in adult life, however ponderal index and head circumference at birth were not (Phillips et al., 2000). However, in women aged ~ 65 years, salivary cortisol awakening response is not related to birth size, nor does it correlate with current serum triglycerides, cholesterol concentrations, blood pressure, OGTT or body fat content (Kajantie et al., 2003).

1.10.4.2 IUGR, catch-up growth and the HPAA axis in animal models

Under-nutrition from day 26 of gestation in sheep reduces fetal plasma cortisol and cortisone levels, an effect not replicated when the periods of under-nutrition are only transient throughout gestation (McMullen et al, 2004), suggesting that the timing of prenatal insult is important in development of the fetal HPA axis. Unilateral uterine artery ligation in the guinea pig at day 30 of gestation decreases cortisol concentrations in the offspring in late gestation, and also blunts the cortisol rise that occurs in late gestation (Jones et al., 1984).

Increased cortisol concentrations are also seen near term in the sheep following placental restriction (Robinson & Owens, 1996). Under-nutrition for 10 days in late gestation in the sheep reduces baseline plasma cortisol concentrations at 30 months of age compared to ad libitum fed controls, however 20-days of under nutrition does not significantly affect cortisol values. Plasma cortisol is

also not affected by birth weight or current weight at 30 months of age in the sheep. The authors suggest that a brief period of under nutrition alters regulation of the HPA axis in adult life, an effect independent of birth weight and current size (Bloomfield et al., 2003). Maternal protein deprivation in rats does not have any long-term effects on plasma cortisol levels in adult male offspring, with no differences in basal plasma corticosterone secretory patterns between IUGR and control groups (Nolan et al., 2001). Bilateral uterine artery ligation in the guinea pig at 28-30 days gestation causes a 100% increase in plasma cortisol in 8-week old offspring (Briscoe et al., 2004). There was no difference in basal plasma cortisol levels between high and low birth weight pigs at 3 months of age in a study by Poore et al (2002), however plasma cortisol at this age was positively correlated with head length:CRL and head length:abdominal circumference ratios at birth (Poore et al., 2002).

1.10.4.3 Increased glucocorticoids before birth and programming of growth and endocrine function postnatally

Corticosteroids are often administered to pregnant women at risk of preterm delivery. However, administration of corticosteroids to women more than 7 days before giving birth results in a significant increase in the risk of perinatal mortality (McLauglin et al., 2003), and multiple doses of antenatal corticosteroids may reduce neonatal birth weight (Mariotti et al., 2004).

Administration of betamethasone to pregnant rats in late gestation at doses similar to those given to pregnant women lowers the birth weight of offspring (McDonald et al., 2003). Repeated injections of betamethasone to pregnant

sheep also lowers fetal weight, and postnatal growth rates are reduced following repeated exposure compared to fetuses exposed to a single dose of betamethasone (Moss et al., 2002). When betamethasone is administered to baboons at the equivalent age of gestation that human fetuses would be exposed, fetal weight, although tending to be slightly lower, is not significantly different to vehicle-treated baboons. However, fetal exposure to betamethasone decreases immunoreactivity of neuronal cytoskeletal proteins and presynaptic terminals in the fetal brain, suggesting acute effects of exogenous glucocorticoid exposure on brain development (Antonow-Schlorke et al., 2003). DXM administration for 2 days at 27 days of gestation in the sheep does not affect insulin sensitivity of glucose or amino acid metabolism in offspring at 5 years of age, however insulin sensitivity of the inhibition of lipolysis is increased. This suggests that in utero exposure to increased glucocorticoids may increase the risk of developing obesity in later life (Gatford et al., 2000).

Dexamethasone treatment administered to pregnant guinea pigs during days 40 to 70 of gestation does not affect birth weight of offspring (Banjanin et al., 2004; Liu et al., 2001), nor are there differences in postnatal growth rates over the first 60 days of life (Liu et al., 2001). Similarly, repeated exposure of lower doses of dexamethasone or betamethasone from day 40-60 of gestation in the guinea pig does not affect fetal body weight at day 62 of gestation, however a 10x higher dose of dexamethasone reduces body weight in female offspring (McCabe et al., 2001). DXM also appears to have specific affects according to gender of the offspring. In a study by (Liu et al., 2001), following maternal DXM treatment, female guinea pig offspring at 80 days of age had increased relative

adrenal weights, whereas males had no change in adrenal weight but a decrease in brain weight relative to body weight. Male offspring exposed to dexamethasone also had reduced plasma cortisol levels in the morning and early afternoon, but not late afternoon, whereas females have elevated plasma cortisol levels at all time points (Liu et al., 2001). DXM treatment also appears to increase expression of mineralocorticoid receptor (MR) levels in the hippocampus in young adult offspring (Banjanin et al., 2004; Liu et al., 2001).

Metyrapone is a competitive inhibitor of the steroidogenic enzyme 11β-hydroxylase (Lye & Challis, 1984), the last enzyme in the biosynthetic pathway for cortisol which catalyses the formation of cortisol from 11-desoxycortisol. Therefore metyrapone acts to inhibit cortisol synthesis. Metyrapone infused from 125-140 days of gestation to the fetal sheep increases fetal plasma ACTH and plasma 11-desoxycortisol concentrations in the first 72 hours after infusion, and they remain higher at 137-139 days of gestation (Warnes et al., 2003). Metyrapone infusion does not significantly alter fetal body weight or crown-rump length in the sheep (Warnes et al., 2003; Warnes et al., 2004), however increases the relative weights of adrenals, kidneys and liver (Warnes et al., 2003). The relative expression of 11βHSD-2 mRNA in the brain is significantly increased in metyrapone-infused fetuses, suggesting reduced cortisol levels in the adrenocortical cells (Warnes et al., 2004).

Therefore prenatal insults can affect programming of the HPAA axis in utero, leading to long-term consequences that may be detrimental to species survival. Cortisol appears to inhibit postnatal growth, with higher concentrations present

in children with slower growth rates. It is clear that the IGFs, insulin and THs have independent roles in driving postnatal growth, but the interaction between these hormonal axes and cortisol postnatally has not been elucidated.

1.11 Interaction between the somatotropic axis, HPA axis, TH and insulin in the fetus

In AGA newborns, cortisol concentrations from cord blood are inversely related to IGF-I concentrations, and directly to IGFBP-1 (Cianfarani et al., 1998). Cortisol may act to initiate maturational changes in the somatotropic axis, as GHR mRNA in the fetal ovine liver during late gestation is cortisol dependent (Li et al., 1999). Cortisol down-regulates IGF-II mRNA in the sheep liver late in gestation (Li et al., 1998b). However, cortisol has also been shown to enhance IGF-I gene expression in the fetal sheep liver (Fowden, 1995). Cortisol infusion increases hepatic IGF-I and GH receptor gene expression in adrenalectomised fetuses and in intact fetuses (Li et al., 1996). Conversely, fetal adrenalectomy prevents the normal decline in IGF-I mRNA abundance in muscle that occurs in late gestation in the fetal sheep, and cortisol infusion lowers muscle IGF-I mRNA compared to saline infused controls (Li et al., 2002), suggesting a tissue-specific role for cortisol in regulating the somatotropic axis. This may explain why fetal cortisol infusion does not affect fetal plasma IGF-I concentrations (Li et al., 1996), as the muscle and hepatic sources may act equal and opposite to each other.

It may be possible that cortisol acts through initiating other endocrine changes, such as affecting the thyroid hormone axis. Cortisol acts to stimulate deiodination of T_4 to T_3, leading to a prepartum rise in T_3 that coincides with increased hepatic GHR and IGF-I gene expression towards term (Forhead et al., 2000). It is possible that T_3 (Forhead et al., 2000) or T_4 (Forhead et al., 2002) may be the direct stimulus to hepatic GHR gene up regulation near term. In contrast, cortisol and thyroid hormones may mediate IGF-I abundance by acting through changes in the GH receptor (Forhead et al., 2002). Serum cortisol correlates positively with free T_4 in preterm newborns born at <34 weeks gestation (Ng et al., 2001). Cortisol infusion into intact and thyroidectomised (TX) fetuses significantly increases plasma T_3 concentration, but does not affect T_4 (Forhead et al., 2002). Thyroidectomy (TX) in sheep at 105-110 days of gestation suppresses IGF-I gene expression in skeletal muscle at 127-130 days gestation, but does not affect IGF-II expression. Cortisol infusion further suppresses IGF-I gene expression in muscle of TX and of intact fetuses, but T_3 infusion does not significantly affect skeletal muscle gene expression of IGF-I or IGF-II (Forhead et al., 2002). Therefore an active thyroid gland appears to be essential to the normal expression of IGF-I in skeletal muscle.

Prenatal cortisol may play a role in programming of postnatal insulin action and glucose metabolism, as cortisol increases insulin sensitivity of skeletal muscle in the fetal sheep, through increasing expression of GLUT-4, an insulin-sensitive glucose transporter (Li et al., 1998a).

1.12 Interaction between the somatotrophic axis, HPA axis and TH and insulin in postnatal life

In the postnatal animal, cortisol inhibits growth partially by antagonising the actions of insulin and partially by down-regulating IGF gene expression (Li et al., 1999). Cortisol has been shown to inhibit transcription of IGF-I in animal and human osteoblasts (Delany & Canalis, 1995; Swolin et al., 1996). Dexamethasone (DXM) treatment increases IGF-II mRNA concentrations in placental tissue (Ain et al., 2005), and also increases IGF-II and IGF-IR mRNA in fetal rat liver and lung tissue at 20 days of gestation (Price et al., 1992).

This upregulation is suggested to compensate for the decreased IGF bioavailability during DXM-induced growth retardation (Price et al., 1992). Expression of IGFBP-1 is also increased in the liver and lung with a concomitant increase in serum IGFBP-1 concentration (Price et al., 1992). IGFBP-5 serum levels are also regulated by glucocorticoids (Juul, 2003). The mechanism of cortisol action on IGF-I is unclear. It is unlikely that cortisol directly acts on the IGF-I gene, as it does not contain any glucocorticoid response elements (GREs) (Dickson 1991).

TH abundance influences IGF-I production and circulating levels and is a major influence on skeletal muscle fibre type, maturation, energy metabolism and growth, in part through interactions with GH and IGF-I in their actions on skeletal muscle, skeletal and adipose tissue growth and development (White et al., 2001; Williams et al., 1998). Serum levels of IGF-I are reduced significantly by 21% in TR$\alpha^{-/-}\beta^{-/-}$mice. In the pituitary, GH protein and mRNA levels are

decreased, implicating GH deficiency as a proximal cause of retarded growth in TR double mutants (Gothe et al., 1999).

In healthy human adult blood donors, plasma T_4 and free T_4 are negatively correlated with total plasma IGF-I levels, but the ratio of plasma T_3:T_4 is positively correlated with plasma IGF-I (Peeters et al., 2005). Plasma T_3, rT_3 and TSH are not correlated with IGF-I. Free IGF-I and T_4 are negatively correlated, whereas T_3:T_4 ratio is positively correlated to free IGF-I in these healthy blood donors. In addition, a significant association of the haplotype allele 2 of type 1 iodothyronine deiodinase (D1) is seen with free IGF-I levels. This haplotype results in a decreased activity of D1 (Peeters et al., 2005).

Therefore in summary, prenatally cortisol acts to increase free T_3 levels by increasing deiodination of T_4 to T_3, and the prepartum rise in cortisol levels seen in numerous species immediately before birth is associated with an increase in T_3 levels, and may also coincide with an increase in activation of the somatotropic axis. It appears that a functional thyroid hormone axis is required for 'normal' activation of the IGF axis, however it is not clear whether thyroid hormones act to inhibit or enhance IGF action. To date, the interaction between cortisol, the IGFs, insulin and THs during postnatal catch-up growth is unknown. It is likely that catch-up growth is driven by all of the hormonal axes mentioned, but the contribution of each is yet to be elucidated. Due to the detrimental long-term consequences of IUGR and subsequent catch-up growth, it is essential to determine the endocrine changes that occur in early postnatal life following such intrauterine insults. This project was designed to investigate the factors

driving postnatal catch-up growth following spontaneous fetal growth restriction in the juvenile guinea pig. The guinea pig, like the human, is mature at birth. Fetal growth restriction occurs as a result of variable litter size (Horton, DH, personal communication), therefore the guinea pig is an appropriate model to study due to its similarities to the human.

1.13 General hypothesis

Spontaneous fetal growth restriction (FGR) will reduce size at birth and increase postnatal growth rates, through increased abundance and/or sensitivity to the insulin-like growth factors, insulin, thyroid hormones or cortisol.

1.14 Specific hypotheses

1. Spontaneous FGR will decrease size at birth, but increase fractional postnatal growth rates in the juvenile guinea pig.
2. Spontaneous FGR will increase metabolic sensitivity to IGF-I, which will in turn predict increased postnatal growth.
3. Spontaneous FGR will increase postnatal fractional growth rate, and alter body composition in the juvenile guinea pig, in part through altered abundance of IGFs, THs and cortisol.
4. Maternal metyrapone treatment will increase size at birth in the offspring, and reduce postnatal fractional growth rate through altered abundance of IGFs, THs and decreased metabolic sensitivity to insulin.

1.15 Aims and significance of the project

This project aims to determine whether fetal growth restriction in the guinea pig results in altered postnatal growth during the catch-up growth period, and whether these changes occur through altered action of the major endocrine regulators of growth, the IGFs, insulin and TH. In addition, we hope to elucidate how increasing prenatal cortisol exposure affects these postnatal outcomes by determining the effect of reducing prenatal glucocorticoid exposure. The functional significance of this project is to provide evidence for the development of subsequent alternate therapies in the IUGR infant, to improve long-term wellbeing.

Chapter 2

IUGR, postnatal growth and body composition

2.1 Introduction

Intrauterine growth restriction (IUGR) results from growing slowly before birth and is usually diagnosed by reduced weight, length or increased thinness for gestational age following delivery (Karlberg et al., 1997; Robinson et al., 1994). IUGR is characterised by altered postnatal growth. Following birth, most IUGR infants grow at an accelerated rate for the first six months of life (Chatelain et al., 1996; Chatelain et al., 1994; Karlberg et al., 1997), a period termed "catch-up growth".

Poor fetal growth contributes to perinatal mortality and morbidity (Barker et al., 1993), and low birth weight or thinness at birth is an antecedent of insulin resistance in adulthood, as well as hyperlipidemia, hypertension, coronary heart disease and premature death (Barker et al., 1993; Newnham, 1998; Seckl, 1997). Recent evidence suggests that the extent of catch-up growth after IUGR may independently influence the risk of developing adult hypertension, cardiovascular disease (Barker et al., 2002), diabetes (Barker et al., 2002; Eriksson et al., 2003a), and central metabolic syndrome in males (Parker et al., 2003).

This may be mediated in part by catch-up growth leading to increased adiposity and overt obesity (Rogers & Group., 2003). Increased birth weight is also associated with increased fat-free mass throughout infancy and childhood (Hediger et al., 1998; Li et al., 2003; Singhal et al., 2003). Although short-term

benefits of catch-up growth, including a reduced risk of hospital admissions and mortality have also been described (Victora et al., 2001), there are clearly long-term negative consequences from such catch-up growth. Understanding the nature and causes of catch-up growth after IUGR may therefore lead to better understanding of how it influences this range of adult outcomes and their possible prevention or amelioration.

Recently, it has been shown in the guinea pig that maternal feed restriction reduces absolute growth rate (AGR) from birth to weaning in male and female offspring, but it does not change fractional growth rate (FGR) (therefore catch-up growth) (Kind et al., 2003). However, AGR of weight correlates positively with birth weight, while FGR of weight correlates negatively (Kind et al., 2003). Growth rates of other measures such as length, abdominal circumference and head size have not been reported in the guinea pig, and postnatal growth of these measures have not been recorded during the catch-up growth period.

Spontaneous fetal growth restriction is achieved in the guinea pig through an increased litter size, in which some or all of the offspring have reduced oxygen and nutrient supply due to impaired placental development following implantation in less well vascularised areas of the uterus as embryos compete for space due to increased competition for maternal substrates. To date, there are no studies on how spontaneous fetal growth restriction affects growth of the offspring, in particular during the catch-up growth period (until ~60 days of age; JA Owens, personal communication) in the guinea pig. Therefore this study aims to determine the effect of spontaneous growth restriction on size at birth,

and growth rates of various body size parameters, in addition to the outcomes for body composition, especially adiposity, at 36 days of age, a time at which the guinea pigs are undergoing a rapid rate of postnatal growth.

2.2 Aims

General Aim:

To determine the effect of spontaneous fetal growth restriction on early postnatal growth and body composition in the juvenile guinea pig.

Specific Aim:

To determine the effect of spontaneous fetal growth restriction (FGR) in the neonatal guinea pig on size at birth and postnatal growth rates of weight, crown-rump length (CRL), abdominal circumference (AC), head length (HL) and head width (HW). In addition to examine the associations between FGR and body composition following weaning, including weights and relative weights of individual organs, skeletal muscles and adipose sites.

Specific Hypothesis:

Spontaneous fetal growth restriction reduces size at birth in terms of all size parameters, increases postnatal fractional growth rates, and changes body composition in the juvenile guinea pig.

2.3 Materials and Methods

2.3.1 Treatment of Animals

All surgical and experimental procedures performed in this project were approved by the Adelaide University Animal Ethics Committee.

2.3.2 Mating and housing of guinea pigs

All guinea pigs (IMVS tri coloured, Gilles Plains Resource Centre, SA) were caged individually in plastic tubs with wire lids, and maintained in a room with a 12:12h light:dark cycle at 25^{O}C, with *ad libitum* access to guinea pig/rabbit ration (Ridley Agriproducts, Australia). Males used for mating were fed lucerne in addition to the guinea pig chow. All guinea pigs also had *ad libitum* access to tap water, containing 400mg/L vitamin C. Females were weighed and checked for oestrus daily, as recognized by an opening of the vaginal orifice associated with a drop in body weight occurring every 15-17 days. They were put into a wire cage with a male for the duration of oestrus. Pregnancy was detected by the presence of a copulatory plug, and/or the absence of oestrus in the next cycle. Upon birth, guinea pigs were allocated into a low or high birth weight group according to the mean birth weight of the current cohort, which was determined to be 102.3g. This divided the animals into two groups of ≤102.3g (low birth weight) and >102.3 (high birth weight).

2.3.3 Size at birth and postnatal growth measurements

A description of the total number of animals used in each study can be found in Appendix A, and total numbers of litters used is summarised in Appendix C. Twenty-nine guinea pigs were weighed at birth. Postnatal growth was measured at 5-day intervals from 0-30 days in terms of weight in all animals, and postnatal growth of crown-rump length (CRL), abdominal circumference (AC), head width (HW), and head length (HL) in 21 animals (Table 2.1). Body mass index was calculated as weight/CRL^2 ($g.cm^{-2}$), and ponderal index as weight/CRL^3 ($g.cm^{-3}$). All pups were weaned at 25 days of age onto guinea pig/rabbit ration and fed *ad libitum*, with access to water containing 400mg/L vitamin C.

2.3.4 Post-mortem

Twenty-two juvenile guinea pigs were killed at 36 ± 2 days by an intraperitoneal overdose of sodium pentobarbitone (325 mg/ml, Lethabarb, Virbac, Australia), following an overnight fast. Blood samples were collected by cardiac puncture into heparinised tubes, and plasma was obtained after centrifugation at 4°C and stored frozen at –20°C until assay. All organs, muscles and adipose depots were dissected and weighed.

Table 2.1: Measurements of juvenile guinea pigs

Measurement	Description
Crown-rump length:	Measured from the tip of the nose along the back to the coccyx bone. Three measurements were taken with string, measured using a ruler, and then averaged to give an accurate measure
Abdominal circumference:	Measured around the abdominal region, immediately adjacent to the pelvic bone. Three measurements were taken with string, measured using a ruler and averaged to give an accurate measure.
Head length:	Measured using callipers, which spanned the length from the nose to the back of the skull. Two measurements were taken and averaged.
Head width:	Measured using callipers, which spanned the width of the head immediately in front of the ears. Two measurements were taken and averaged.

2.3.5 Calculations of postnatal growth

Absolute growth rates were calculated for each growth measurement for each guinea pig (AGR) by calculating the slope of the regression line of size versus postnatal age. Fractional growth rate was calculated by dividing each measure of absolute growth rate by the size at birth for that parameter, to give a measure of catch up growth (FGR).

2.3.6 Statistical Analysis

Low and high birth weight category analysis was carried out using univariate ANOVA analysis with the statistical analysis program, SPSS (SPSS Inc, Chicago), with birth weight and sex as between factors. All correlations were analysed using Pearson's 1-tailed correlations, using the statistical analysis program SPSS. Unless otherwise stated, significance was assumed at the level $p<0.05$. Results are presented as mean ± the standard error of the mean (SEM).

2.4 Results

2.4.1 *The effect of birth weight class and sex on size at birth and postnatal growth*

Size at birth and postnatal growth data are summarised in Table 2.2. The low birth weight (LBW) guinea pigs were smaller than those of high birth weight (HBW) in terms of crown-rump length (CRL) ($p=0.005$), abdominal circumference (AC) ($p=0.002$), and head length (HL) ($p=0.0001$) and width (HW) ($p=0.01$) at birth. In addition, weight:length ($p=0.0001$) and body mass index (BMI; $p=0.02$) were reduced in LBW compared to HBW animals (Table 2.2). Absolute growth rates (AGR) of weight ($p=0.046$) and CRL ($p=0.026$) were lower in LBW animals, whereas fractional growth rates (FGR) of weight were higher in LBW animals ($p=0.0001$) (Table 2.2). There were no sex differences for any size at birth measures, nor was there any interaction between birth weight and sex (Table 2.2). However, males had higher AGR_{weight} and FGR_{weight} compared to females, whereas females had higher AGR and FGR of AC, HL and HW than males ($p<0.05$). There were no significant interactions between birth weight and sex for any postnatal growth measures (Table 2.2).

Table 2.2: Effect of birth weight class and sex on size at birth and postnatal growth

Size/growth parameter	Low Birth Weight		High Birth Weight		ANOVA p-value		
Size at birth	***Male***	***Female***	***Male***	***Female***	***BWt***	***Sex***	***BWt x Sex***
Weight (g)	88.2 ± 3.2	90.7 ± 3.0	113.4 ± 3.3	111.0 ± 1.8			
Crown-rump length (mm)	152.3 ± 3.7	156.4 ± 5.4	164.4 ± 3.4	163.9 ± 2.2	0.005	ns	ns
Abdominal Circumference (mm)	80.1 ± 1.4	82.6 ± 2.7	88.2 ± 2.4	88.8 ± 1.5	0.002	ns	ns
Head Length (mm)	44.2 ± 0.5	44.2 ± 0.3	47.3 ± 0.5	46.2 ± 0.3	0.0001	ns	0.08
Head Width (mm)	23.8 ± 0.2	22.9 ± 0.2	24.5 ± 0.5	24.8 ± 0.4	0.01	ns	0.08
Weight:length ($g.mm^{-1}$)	5.8 ± 0.2	5.8 ± 0.2	6.9 ± 0.1	6.8 ± 0.1	0.0001	ns	ns
Ponderal Index ($g.mm^{-3}$) (*10)	0.25 ± 0.02	0.24 ± 0.02	0.26 ± 0.01	0.26 ± 0.01	ns	ns	ns
Body Mass Index ($g.mm^{-2}$)	0.38 ± 0.02	0.37 ± 0.02	0.42 ± 0.01	0.42 ± 0.01	0.02	ns	ns
Absolute Growth Rate							
Weight (g/day)	9.5 ± 0.2	9.0 ± 0.2	10.5 ± 0.4	9.4 ± 0.3	0.046	0.008	ns
CRL (cm/day)	0.32 ± 0.03	0.24 ± 0.10	0.35 ± 0.02	0.35 ± 0.01	0.026	ns	0.09
AC (mm/day)	0.35 ± 0.01	0.44 ± 0.06	0.40 ± 0.01	0.45 ± 0.02	ns	0.003	ns
Head length (mm/day)	1.58 ± 0.06	2.07 ± 0.29	1.65 ± 0.07	1.80 ± 0.13	ns	0.013	ns
Head Width (mm/day)	2.38 ± 0.12	3.12 ± 0.39	2.66 ± 0.25	3.03 ± 0.27	ns	0.032	ns
Fractional Growth Rate							
Weight (%/day)	0.108 ± 0.003	0.099 ± 0.004	0.093 ± 0.003	0.084 ± 0.002	0.0001	0.002	ns
CRL (%/day)	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.02	0.22 ± 0.01	ns	ns	ns
AC (%/day)	0.45 ± 0.01	0.54 ± 0.06	0.45 ± 0.02	0.51 ± 0.03	ns	0.012	ns
Head length (%/day)	0.29 ± 0.06	0.47 ± 0.06	0.35 ± 0.02	0.39 ± 0.03	ns	0.008	0.052
Head width (%/day)	0.10 ± 0.004	0.14 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	ns	0.04	ns

All values expressed as mean ± SEM.

2.4.2 Correlations of postnatal growth and size at birth

Birth weight was positively correlated with CRL (r=0.66, Figure 2.1(a)), abdominal circumference (r=0.80, Figure 2.1(b)), head length (r=0.87, Figure 2.1(c)), and head width (r=0.59) (Table 2.3). Birth weight was also positively correlated to weight:length (r=0.89) and BMI at birth (r=0.45). Birth CRL was positively correlated with AC (r=0.50) and HW (r=0.64), and negatively correlated with PI (r=-0.74) and BMI (r=-0.38) at birth.

An increase in birth weight predicted an increased AGR_{weight} (r=0.45, Fig. 2.2 (c)), whereas a *reduced* birth weight predicted an increased FGR_{weight} (r=–0.76, Figure 2.2 (b)). Similarly, a decreased birth weight correlated with an increasing FGR_{CRL} (r=-0.41) (Figure 2.2 (a), Table 2.3). CRL was negatively correlated with FGR_{weight} (r=-0.54) (Table 2.3). Birth AC was positively correlated to AGR_{weight} (r=0.40), and negatively correlated with FGR_{weight} (r=-0.57) and FGR_{CRL} (r=-0.47) (Table 2.3). HL was positively associated with AGR_{weight} (r=0.41), but negatively correlated with FGR_{weight} (r=-0.65) and FGR_{CRL} (r=-0.54) (Table 2.3). HW was positively correlated with AGR_{weight} (r=0.47), but negatively correlated with FGR_{CRL} (r=-0.63), AGR_{HL} (r=-0.48) and FGR_{HW} (r=-0.53) (Table 2.3).

Table 2.3: Size at birth and postnatal growth in the juvenile guinea pig

	SIZE AT BIRTH				
	Weight	***Crown-rump length***	***Abdominal Circumference***	***Head Length***	***Head Width***
Size/growth parameter	***r***	***r***	***r***	***r***	***r***
Size at birth					
Weight (g)	-	0.66**	0.80**	0.87**	0.59**
Crown-rump length (mm)	0.66**	-	0.50*	0.64**	0.05
Abdominal Circumference (mm)	0.80**	0.50*	-	0.72**	0.61**
Head Length (mm)	0.87**	0.05	0.61**	-	0.45*
Head Width (mm)	0.59**	0.64**	0.72**	0.45*	-
Weight:length	0.89**	0.24	0.73**	0.72**	0.73**
Ponderal Index ($g.cm^{-3}$)	0.02	-0.74**	0.05	-0.07	0.45*
Body Mass Index ($g.cm^{-2}$)	0.45*	-0.38*	0.39*	0.30#	0.66**
Absolute Growth Rate					
Weight (g/day)	0.45*	0.26#	0.40*	0.41*	0.47*
Crown-rump length (cm/day)	0.19	0.13	0.29#	0.08	0.03
Abdominal Circumference (mm/day)	0.34#	0.09	0.26	0.20	0.13
Head length (mm/day)	-0.01	0.10	-0.09	0.06	-0.48*
Head Width (mm/day)	0.13	0.10	0.04	0.04	-0.37#
Fractional Growth Rate					
Weight (%/day)	-0.76**	-0.54**	-0.57**	-0.65**	-0.27#
CRL (%/day)	-0.41*	-0.16	-0.47*	-0.54*	-0.63**
AC (%/day)	-0.07	-0.17	-0.25	-0.16	-0.20
Head length (%/day)	0.11	0.24	0.03	0.07	-0.36#
Head width (%/day)	0.01	0.06	-0.10	-0.05	-0.53*

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

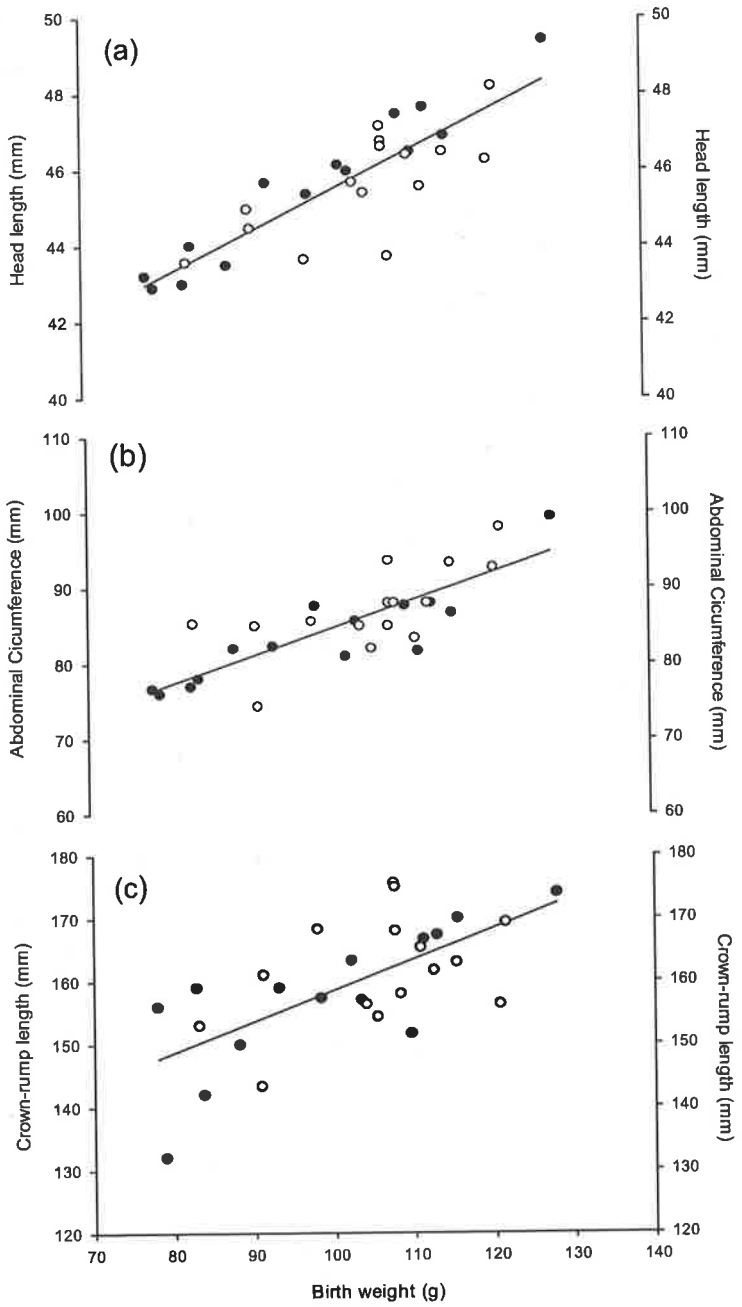

Figure 2.1: Correlations of birth weight with size at birth in the juvenile guinea pig

Males are represented by closed, and females by open circles. Birth weight was positively correlated with (a) HL (r=0.87), (b) AC (r=0.80) and (c) CRL (r=0.66) ($p<0.001$ for all).

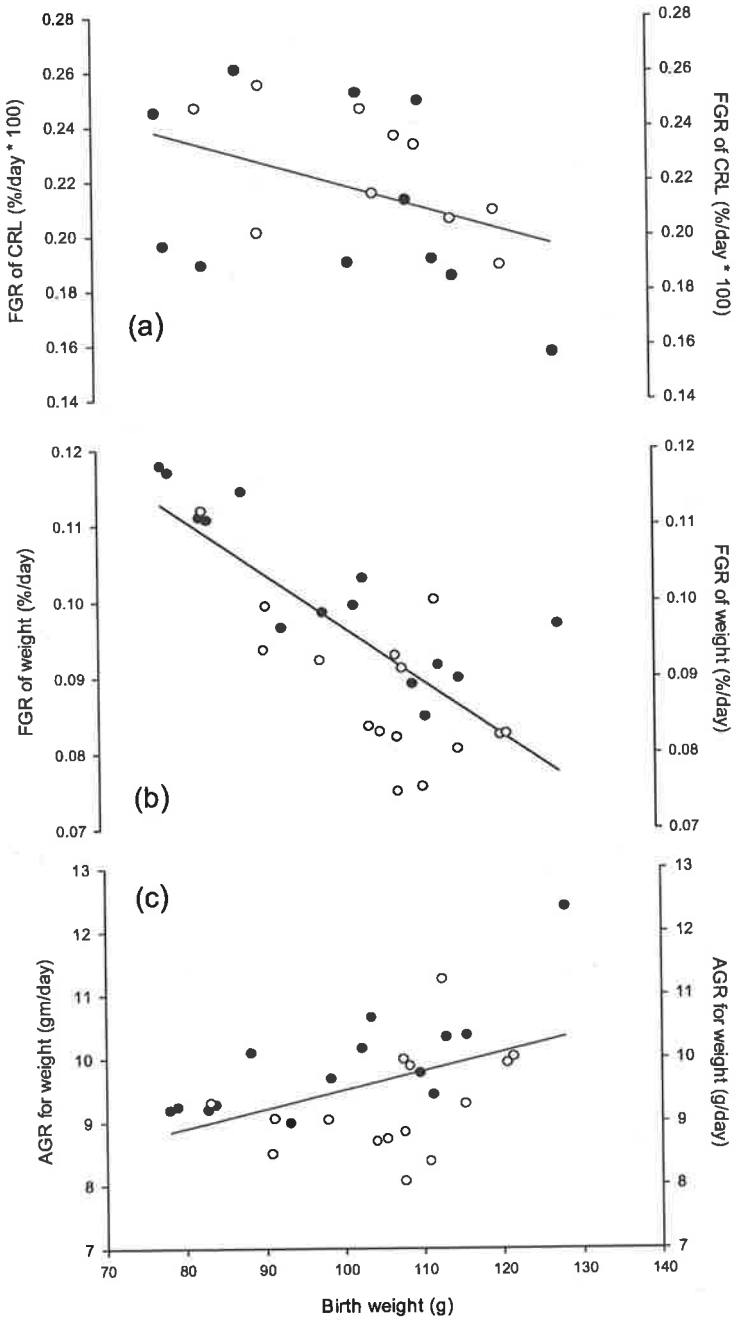

Figure 2.2: Correlations of birth weight with postnatal growth in the juvenile guinea pig

Birth weight was negatively correlated with (a) FGR of CRL ($r=-0.41$; $p<0.05$), and (b) FGR of weight ($r=-0.76$; $p<0.001$), and positively correlated with (c) AGR of weight ($r=0.45$; $p<0.05$). (a)

2.4.3 Birth weight class, sex and organ weights in the juvenile guinea pig

At 36 days of age, LBW animals weighed less than HBW animals (p=0.027), but were not different in length, weight:length or BMI to HBW animals (Table 2.4). LBW animals had greater relative weights of brain (p=0.005) and GIT (p=0.039) than those of HBW (Table 2.4). Absolute (p=0.018) and relative (p=0.032) weights of the spleen and heart were decreased in LBW animals (Table 2.4). LBW animals had greater summed visceral mass when expressed in terms of current weight (p=0.031) (Table 2.4).

Females had increased absolute (p=0.03) and relative weights of adrenals (p=0.008), as well as increased thyroid absolute (p=0.027) and relative (p=0.017) weights compared to males. However they had lighter heart weights (p=0.049), kidney weights (p=0.046) and absolute (p=0.029) and relative (p=0.027) weights of the spleen than males (Table 2.4). An interaction between birth weight and sex occurred for current CRL (p=0.017), and for brain weight (p=0.008) (Table 2.4).

2.4.4 Birth weight class, sex and skeletal and adipose tissue weights in the juvenile guinea pig

LBW animals had reduced vastus lateralis muscle weight (p=0.04) compared to those of HBW (Table 2.5). Males and females differed in weight of diaphragm muscle, with males having a greater weight of this muscle (p=0.048). An interaction between birth weight and sex occurred for summed muscle mass

(p=0.045), as well as for tibialis absolute weight (p=0.006) and relative weight (p=0.01), biceps femoris absolute weight (p=0.029) and EDL absolute and relative weights (p=0.012 for both) (Table 2.5). This was due to LBW females having increased absolute and relative muscle weights compared to those of HBW, but males having reduced muscle weights compared to HBW males (Table 2.5).

LBW animals had reduced summed adipose tissue (p=0.026; Figure 2.3), and tended to have less summed fat mass as a % of body weight (p=0.06) than those of HBW (Table 2.5). LBW animals had reduced absolute weights of shoulder fat (p=0.035), retroperitoneal fat (p=0.048), perirenal fat (p=0.012) and groin fat (p=0.049), and decreased relative weight of perirenal fat (p=0.016) compared to those of HBW. LBW animals had an increased summed muscle:fat ratio (p=0.018) (Table 2.5). Females had greater neck fat mass than males (p=0.041) (Table 2.5).

Table 2.4: Effect of birth weight class and sex on organ size in the juvenile guinea pig

	Low Birth Weight		High Birth Weight		ANOVA p-value		
Current size	***Male***	***Female***	***Male***	***Female***	***BWt***	***Sex***	***BwtxS***
Weight (g)	326 ± 13	333 ± 16	373 ± 13	342 ± 9	0.027	ns	0.08
CRL (mm)	254 ± 6	245 ± 5	261 ± 4	249 ± 3	ns	0.017	ns
Wt:CRL ($g.mm^{-1}$)	1.37 ± 0.07	1.36 ± 0.06	1.43 ± 0.04	1.37 ± 0.03	ns	ns	ns
BMI ($g.mm^{-3}$)	0.54 ± 0.03	0.55 ± 0.02	0.55 ± 0.02	0.55 ± 0.01	ns	ns	ns
Organ size							
Adrenals (g)	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.18 ± 0.008	0.052	0.03	ns
% BW	0.04 ± 0.001	0.05 ± 0.004	0.04 ± 0.004	0.05 ± 0.002	ns	0.008	ns
Kidney (g)	3.51 ± 0.07	3.26 ± 0.30	3.83 ± 0.17	3.38 ± 0.17	ns	0.046	ns
% BW	1.68 ± 0.05	0.98 ± 0.08	1.03 ± 0.06	0.99 ± 0.04	ns	ns	ns
Pancreas (g)	1.09 ± 0.10	1.09 ± 0.12	1.21 ± 0.10	1.04 ± 0.07	ns	ns	ns
% BW	0.33 ± 0.02	0.33 ± 0.03	0.33 ± 0.03	0.30 ± 0.02	ns	ns	ns
Spleen (g)	1.47 ± 0.18	0.76 ± 0.13	2.07 ± 0.24	1.54 ± 0.29	0.018	0.029	ns
% BW	0.45 ± 0.05	0.23 ± 0.04	0.56 ± 0.07	0.44 ± 0.08	0.032	0.027	ns
GIT (g)	60.1 ± 1.5	59.7 ± 0.2	60.9 ± 1.7	44.4 ± 6.6	ns	ns	ns
% BW	18.4 ± 0.2	19.1 ± 0.1	16.7 ± 0.2	12.2 ± 2.4	0.039	ns	ns
Brain (g)	3.28 ± 0.11	3.44 ± 0.14	3.52 ± 0.11	3.08 ± 0.08	ns	ns	0.008
% BW	1.01 ± 0.05	1.04 ± 0.06	0.95 ± 0.03	0.90 ± 0.02	0.005	ns	ns
Liver (g)	15.0 ± 0.9	14.0 ± 1.2	14.6 ± 1.0	14.1 ± 0.7	ns	ns	ns
% BW	4.61 ± 0.19	4.19 ± 0.40	3.94 ± 0.31	4.12 ± 0.16	0.08	ns	ns#
Lungs (g)	2.50 ± 0.29	2.36 ± 0.17	2.58 ± 0.11	2.52 ± 0.14	ns	ns	ns
% BW	0.79 ± 0.07	0.71 ± 0.04	0.69 ± 0.03	0.75 ± 0.06	ns	ns	ns
Heart (g)	1.57 ± 0.18	1.33 ± 0.24	1.76 ± 0.13	1.45 ± 0.05	ns	0.027	ns
% BW	0.48 ± 0.04	0.40 ± 0.06	0.47 ± 0.04	0.43 ± 0.02	ns	0.042	ns
Thymus (g)	0.39 ± 0.10	0.60 ± 0.10	0.55 ± 0.10	0.34 ± 0.06	ns	ns	ns
% BW	0.12 ± 0.01	0.18 ± 0.04	0.15 ± 0.03	0.10 ± 0.01	ns	ns	ns
Thyroid (g)	0.05 ± 0.005	0.08 ± 0.01	0.06 ± 0.003	0.08 ± 0.01	ns	0.027	ns
% BW	0.016 ± 0.002	0.024 ± 0.005	0.016 ± 0.001	0.023 ± 0.003	ns	0.017	ns
Sum visceral (g)	84.9 ± 8.5	79.9 ± 12.0	86.0 ± 7.0	69.5 ± 7.0	ns	ns	ns
% BW	0.260 ± 0.017	0.255 ± 0.024	0.236 ± 0.014	0.193 ± 0.014	0.031	ns	ns

All values expressed as mean ± SEM. GIT refers to gastro-intestinal tract; Sum visceral refers to summed weights of visceral organs, %BW refers to weight expressed as a percentage of body weight.

Table 2.5: Effect of birth weight class and sex on lean and adipose tissue mass in the juvenile guinea pig

Muscle/ adipose depot	Low Birth Weight		High Birth Weight		ANOVA p-value		
	Male	***Female***	***Male***	***Female***	***BWt***	***Sex***	***BwtxS***
Plantaris (g)	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0.02	0.12 ± 0.01	ns	ns	ns
% BW	0.03 ± 0.006	0.03 ± 0.003	0.03 ± 0.005	0.03 ± 0.003	ns	ns	ns
Gastrocnemius (g)	1.86 ± 0.18	1.93 ± 0.07	1.98 ± 0.11	1.91 ± 0.08	ns	ns	ns
% BW	0.57 ± 0.03	0.58 ± 0.03	0.53 ± 0.02	0.56 ± 0.01	ns	ns	ns
Tibialis (g)	0.34 ± 0.03	0.44 ± 0.08	0.51 ± 0.04	0.38 ± 0.03	0.09	ns	0.006
% BW	0.11 ± 0.01	0.13 ± 0.02	0.14 ± 0.01	0.11 ± 0.006	ns	ns	0.01
Semitendinosus (g)	1.49 ± 0.15	1.63 ± 0.03	1.77 ± 0.15	1.50 ± 0.08	0.07	ns	0.06
% BW	0.46 ± 0.03	0.49 ± 0.02	0.48 ± 0.04	0.44 ± 0.01	ns	ns	0.08
Biceps Femoris (g)	4.21 ± 0.34	4.76 ± 0.26	4.94 ± 0.30	4.32 ± 0.18	ns*	ns	0.029
% BW	1.29 ± 0.08	1.43 ± 0.04	1.32 ± 0.05	1.26 ± 0.04	ns	ns	0.055
Vastus Lateralis (g)	1.38 ± 0.15	1.47 ± 0.03	1.53 ± 0.12	1.44 ± 0.06	ns	ns	ns
% BW	0.42 ± 0.04	0.44 ± 0.006	0.41 ± 0.03	0.42 ± 0.01	ns	ns	ns
EDL (g)	0.12 ± 0.03	0.16 ± 0.02	0.16 ± 0.02	0.09 ± 0.02	ns	ns	0.012
% BW	0.04 ± 0.008	0.05 ± 0.006	0.04 ± 0.004	0.03 ± 0.004	ns	ns	0.012
Biceps brachii (g)	0.42 ± 0.04	0.45 ± 0.02	0.46 ± 0.03	0.44 ± 0.02	ns	ns	ns
% BW	0.13 ± 0.008	0.14 ± 0.009	0.12 ± 0.007	0.13 ± 0.004	ns	ns	ns
Diaphragm (g)	1.22 ± 0.06	1.12 ± 0.05	1.39 ± 0.11	1.18 ± 0.07	0.09	0.04	ns
% BW	0.38 ± 0.02	0.34 ± 0.12	0.37 ± 0.03	0.34 ± 0.01	ns	0.06	ns
Summed muscle (g)	11.34 ± 0.61	12.06 ± 0.36	12.12 ± 0.68	11.38 ± 0.50	ns	ns	0.048
% BW	3.51 ± 0.12	3.63 ± 0.09	3.39 ± 0.13	3.32 ± 0.08	ns	ns	0.07
Dorsal Fat (g)	2.41 ± 0.37	2.40 ± 0.48	2.52 ± 0.22	2.90 ± 0.28	ns	ns	ns
% BW	0.73 ± 0.09	0.73 ± 0.11	0.67 ± 0.05	0.85 ± 0.07	ns	ns	ns
Shoulder Fat (g)	1.74 ± 0.20	1.91 ± 0.32	2.24 ± 0.17	2.42 ± 0.23	0.035	ns	ns
% BW	0.54 ± 0.05	0.57 ± 0.08	0.60 ± 0.03	0.70 ± 0.05	0.072	ns	ns
Neck Fat (g)	0.31 ± 0.09	0.35 ± 0.01	0.33 ± 0.03	0.51 ± 0.05	0.083	0.04	ns
% BW	0.09 ± 0.02	0.10 ± 0.006	0.09 ± 0.006	0.15 ± 0.02	ns	0.06	ns
Retro Fat (g)	0.96 ± 0.16	1.16 ± 0.15	1.55 ± 0.18	1.51 ± 0.25	0.048	ns	ns
% BW	0.29 ± 0.04	0.35 ± 0.04	0.41 ± 0.04	0.43 ± 0.07	0.07	ns	ns
Perirenal Fat (g)	0.92 ± 0.13	0.68 ± 0.02	1.18 ± 0.15	1.27 ± 0.15	0.012	ns	ns
% BW	0.28 ± 0.03	0.21 ± 0.01	0.31 ± 0.04	0.38 ± 0.04	0.016	ns	0.07
Groin Fat (g)	1.33 ± 0.14	2.00 ± 0.41	2.40 ± 0.47	2.24 ± 0.30	0.049	ns	ns
% BW	0.41 ± 0.05	0.59 ± 0.11	0.64 ± 0.11	0.66 ± 0.08	0.085	ns	ns
Summed Fat (g)	7.37 ± 0.81	8.35 ± 1.25	9.89 ± 0.98	10.70 ± 1.06	0.025	ns	ns
% BW	2.25 ± 0.17	2.51 ± 0.31	2.63 ± 0.19	3.04 ± 0.28	0.06	ns	ns
Summed muscle:fat	1.54 ± 0.12	1.51 ± 0.15	1.33 ± 0.12	1.17 ± 0.09	0.018	ns	ns

All values expressed as mean ± SEM.

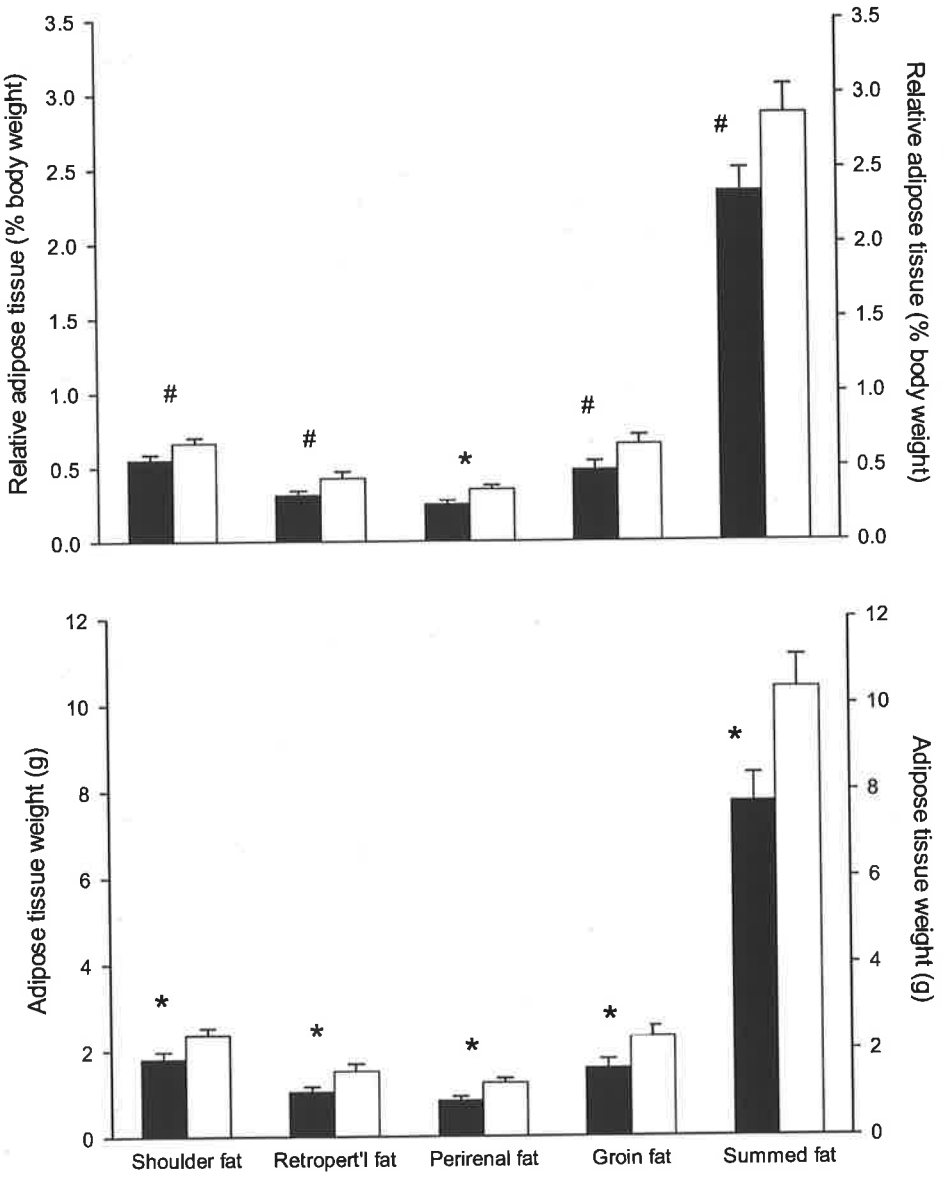

Figure 2.3: Effect of size at birth on adiposity in the juvenile guinea pig

Low birth weight category (n=8) is represented by black bars, and the high birth weight category (n=13) by white bars. Males and females are combined. Data are mean ± SEM. * Denotes significance at $p<0.05$; # $p<0.1$.

2.4.5 *Effect of size at birth and postnatal growth on body composition in the juvenile guinea pig*

Juvenile body weight (r=0.62) and body length (r=0.57) at 36 days of age were positively correlated birth weight. Juvenile body weight was positively correlated with BMI at birth (r=0.37). Juvenile weight:length correlated positively with PI at birth (r=0.52), and BMI at birth (r=0.59). Juvenile BMI correlated negatively with birth CRL (r=-0.46), but positively with PI (r=0.53) and BMI (r=0.48) at birth (Table 2.6).

Weights of the adrenals, spleen (both r=0.41) and heart (r=0.38) were positively correlated with birth weight, but the relative weights of the GIT (r=-0.59) and brain (r=-0.68) were negatively correlated with birth weight (Figure 2.4(a)) (Table 2.6). Adrenal weight was positively correlated with CRL at birth (r=0.38). The relative weight of the brain was negatively correlated with birth AC (r=-0.49; Figure 2.4(b)), HL (r=-0.54, Figure 2.4(c)) and HW (r=-0.55, Figure 2.4(d)), as well as BMI at birth (r=0.39) (Table 2.6). Spleen weight was positively correlated with BMI at birth (r=0.39). Liver weight was negatively correlated with birth AC (r=-0.57), and the relative weight of the liver was negatively correlated with birth HL (r=-0.37). The absolute weight (r=0.49) and relative weight (r=0.44) of the spleen, and weight of the kidney (r=0.50) were positively correlated with birth HW (Table 2.6). Summed visceral organ relative weight correlated negatively with birth weight (r=-0.62; Table 2.6).

Juvenile body weight and length at 36 days of age were positively correlated with AGR_{weight} (r=0.66 for both), and juvenile weight:length correlated positively

with AGR_{weight} (r=0.52) (Table 2.7). Absolute GIT weight at 36 days of age was positively correlated with AGR_{weight} (r=0.75) and FGR_{weight} (r=0.64), as was the relative GIT weight (r=0.58 and r=0.82 respectively) (Table 2.7). Adrenal weight was negatively correlated with FGR_{weight} (r=-0.45), as was its relative weight (r=-0.42) (Table 2.7; Figure 2.5). Kidney weight (r=0.67), spleen weight (r=0.55) and heart weight (r=0.37) were positively correlated with AGR_{weight} (Table 2.7). Summed visceral organ weight correlated positively with AGR_{weight} (r=0.79), and summed visceral organ relative weight correlated positively with FGR_{weight} (r=0.84; Table 2.7).

Table 2.6: Correlations of size at birth and organ weight in the juvenile guinea pig

	SIZE AT BIRTH						
	Weight	*CRL*	*AC*	*HL*	*HW*	*BMI*	*PI*
	r	*r*	*r*	*r*	*r*	*r*	*r*
Current size							
Weight (g)	0.62**	0.28	0.45*	0.44*	0.41*	0.37*	0.08
CRL (mm)	0.57*	0.33#	0.21	0.53*	0.59*	0.21	-0.10
Wt:CRL ($g.mm^{-1}$)	0.30	-0.27	0.21	-0.01	0.30	0.59*	0.52*
BMI ($g.mm^{-3}$)	-0.04	-0.46*	0.08	-0.31#	-0.05	0.48*	0.53*
Organ size							
Adrenals (g)	0.41*	0.38*	0.18	0.06	0.12	0.03	-0.19
(% BW)	0.15	0.32#	0.02	-0.09	-0.08	-0.20	-0.28
Kidney (g)	0.32#	0.12	0.08	0.18	0.50*	0.23	0.08
(% BW)	-0.16	-0.12	-0.29#	-0.17	0.21	-0.03	0.05
Pancreas (g)	0.09	0.02	-0.17	0.13	-0.17	0.10	0.03
(% BW)	-0.15	-0.07	-0.32#	-0.04	-0.36#	-0.09	-0.04
Spleen (g)	0.41*	0.09	0.31#	0.15	0.49*	0.39*	0.20
(% BW)	0.32#	0.04	0.23	0.08	0.44*	0.34#	0.19
GIT (g)	-0.31	-0.43	0.14	-0.20	0.37	-0.08	0.06
(% BW)	-0.59*	-0.57#	-0.10	-0.39	0.18	-0.33	-0.14
Brain (g)	0.09	-0.07	-0.08	-0.12	-0.21	-0.07	-0.06
(% BW)	-0.68**	-0.34#	-0.49*	-0.54*	-0.55*	-0.41*	-0.11
Liver (g)	0.09	0.12	-0.26	-0.07	0.16	-0.06	-0.13
(% BW)	-0.33#	-0.08	-0.57*	-0.37*	-0.11	-0.30	-0.17
Lungs (g)	0.25	0.36#	0.03	0.16	-0.11	-0.16	-0.28
(% BW)	-0.06	0.20	-0.19	-0.06	-0.30#	-0.34#	-0.30#
Heart (g)	0.38*	0.35#	-0.08	0.33#	0.26	0.01	-0.18
(% BW)	0.12	0.25	-0.31#	0.15	0.09	-0.18	-0.24
Thymus (g)	-0.03	-0.21	0.03	0.11	-0.03	0.22	0.30
(% BW)	-0.12	-0.22	-0.03	0.05	-0.12	0.12	0.22
Thyroid (g)	0.12	-0.10	0.07	0.04	0.16	0.29	0.23
(% BW)	-0.32#	-0.16	-0.01	-0.28	-0.19	0.20	0.20
Sum visceral (g)	-0.22	-0.37	0.18	-0.16	0.48#	-0.01	0.11
(% BW)	-0.62*	-0.57#	-0.18	-0.42	0.21	-0.35	-0.16

CRL refers to crown-rump length, AC to abdominal circumference, HL to head length, HW to head width, BMI to body mass index and PI to ponderal index. GIT refers to gastrointestinal tract; %BW refers to weight expressed as a percentage of body weight. ** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

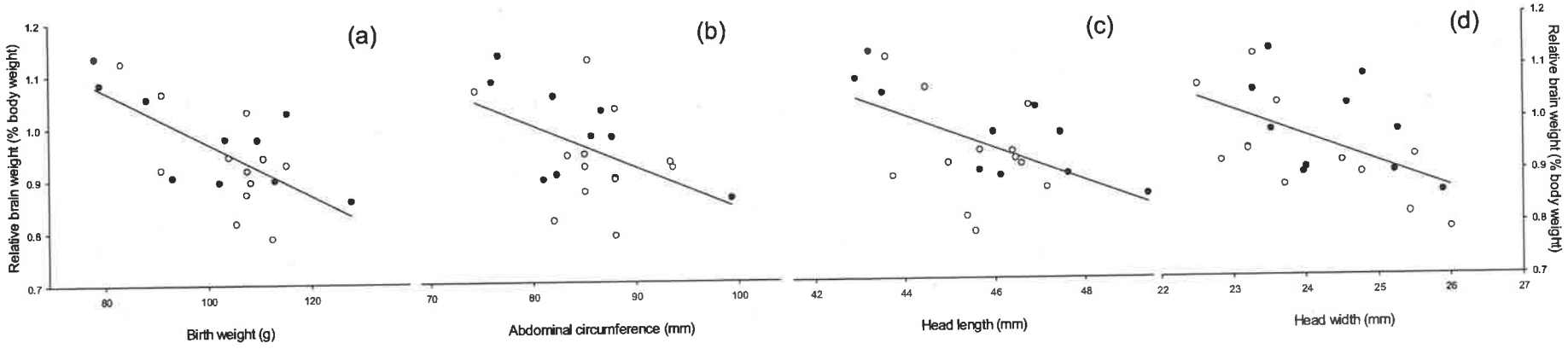

Figure 2.4: Associations between relative brain weight in the juvenile guinea pig and size at birth

Males are represented by closed, and females by open circles. The following size at birth measures were negatively correlated with relative brain weight: (a) birth weight ($r=-0.68$; $p<0.001$), (b) AC ($r=-0.49$), (c) HL ($r=-0.54$) and (d) HW ($r=-0.55$) ($p<0.05$ for all).

Table 2.7: Organ size and postnatal growth in the juvenile guinea pig

	Absolute Growth Rate		Fractional Growth Rate	
	Weight (g)	*(%BW)*	*Weight (g)*	*(%BW)*
	r	*r*	*r*	*r*
Current size				
Weight (g)	0.66**	N/A	-0.15	N/A
CRL (mm)	0.66**	N/A	0.13	N/A
Wt:CRL ($g.mm^{-1}$)	0.52*	N/A	0.24	N/A
BMI ($g.mm^{-3}$)	0.14	N/A	0.16	N/A
Organ size				
Adrenals (g)	-0.02	0.34#	-0.45*	-0.42*
Kidney (g)	0.67**	0.20	0.19	0.34#
Pancreas (g)	0.07	-0.19	-0.07	0.01
Spleen (g)	0.55*	0.46*	0.02	0.06
GIT (g)	0.75*	0.58*	0.64*	0.82*
Brain (g)	0.35#	-0.27	0.39*	0.53*
Liver (g)	0.25	-0.18	0.08	0.20
Lungs (g)	0.32#	-0.06	-0.02	0.04
Heart (g)	0.37*	0.08	-0.11	-0.06
Thymus (g)	-0.01	-0.13	-0.01	-0.01
Thyroid (g)	-0.18	-0.10	-0.30#	0.26
Sum visceral (g)	0.79*	0.56#	0.57#	0.84*

CRL refers to crown-rump length, BMI to body mass index. GIT refers to gastrointestinal tract; %BW refers to weight expressed as a percentage of body weight. ** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

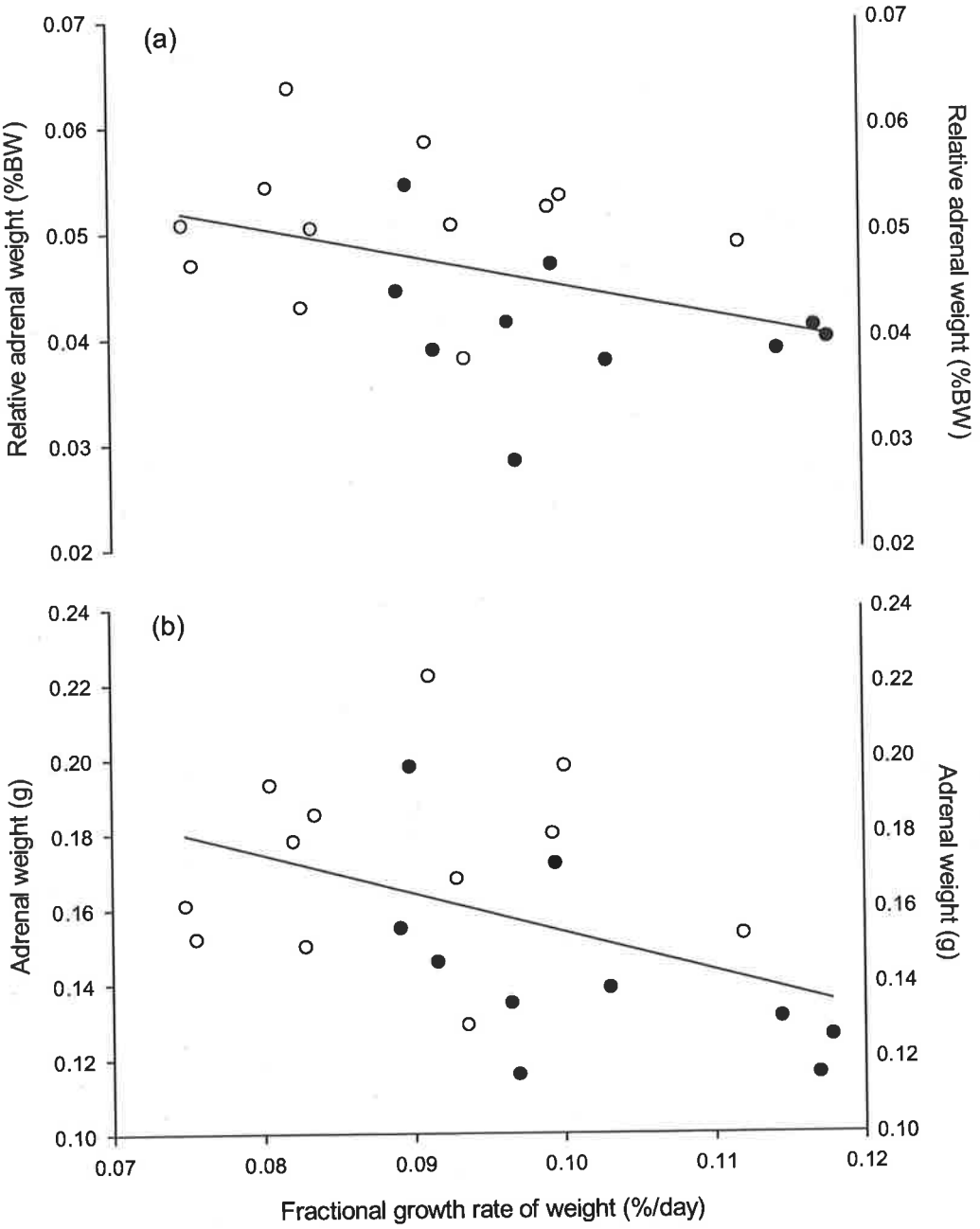

Figure 2.5: Associations between adrenal weight and postnatal growth in the juvenile guinea pig

Males are represented by closed, and females by open circles. Fractional growth rate of weight was negatively correlated with (a) relative adrenal weight (r=-0.42) and (b) adrenal weight (r=-0.45).

2.4.6 Skeletal muscle mass and adipose tissue mass and size at birth in the juvenile guinea pig

Summed muscle mass at 36 days of age correlated positively with birth weight (r=0.42; Figure 2.7 (a); Table 2.8). The biceps femoris muscle weight was positively correlated with birth weight (r=0.49), and birth HL (r=0.39). Diaphragm weight correlated positively with BMI at birth (r=0.37). Relative diaphragm weight was negatively correlated with birth CRL (r=-0.40), and relative vastus lateralis weight was negatively correlated with birth HW (r=-0.38) (Table 2.8).

Summed fat mass in absolute (r=0.61) and relative terms (r=0.48) correlated positively with birth weight (Figure 2.6(a) and (b)). In fact, weights of every adipose depot measured correlated positively with birth weight, excluding parametrial fat (see Table 2.8). Similarly, summed fat mass (r=0.47), and summed relative fat mass (r=0.39) were positively correlated with birth CRL (Figure 2.6(c) and (d)), as were the absolute and relative weights of dorsal (r=0.41 and r=0.37 respectively) and groin fat weight (r=0.38) (Table 2.9). Epididymal fat weight (r=0.81) and groin fat weight (r=0.39) were positively correlated with birth AC. Summed fat mass was positively correlated to birth HW (r=0.39) and HL (r=0.38). The absolute weights and relative weights of parametrial fat, epididymal fat and groin fat were all positively correlated to birth HW ($p<0.05$) (Table 2.9), whereas the absolute weights and relative weights of epididymal fat and groin fat were positively correlated with birth HL ($p<0.05$; Table 2.9). The absolute (r=0.57) and relative (r=0.73) weights of epididymal fat were positively correlated with BMI at birth. Summed muscle:fat correlated

negatively with weight (r=-0.57), CRL (r=-0.37) and HW (r=-0.38) at birth (Table 2.9).

Table 2.8: Size at birth and skeletal muscle mass in the juvenile guinea pig

	Weight	*CRL*	*AC*	*HL*	*HW*	*BMI*	*PI*
Muscle	*r*	*r*	*r*	*r*	*r*		
Plantaris (g)	-0.01	0.01	0.14	-0.23	-0.08	-0.02	-0.06
(% BW)	-0.15	-0.03	0.06	-0.33#	-0.20	-0.16	-0.12
Gastrocnemius (g)	0.34#	0.22	0.24	0.13	0.12	0.10	-0.09
(% BW)	-0.10	0.07	-0.07	-0.24	-0.25	-0.24	-0.23
Tibialis (g)	0.24	0.11	0.14	0.07	0.13	0.13	0.01
(% BW)	0.01	-0.01	-0.02	-0.12	-0.02	0.01	-0.01
Semitendinosus (g)	0.22	0.01	0.13	0.10	0.15	0.24	0.11
(% BW)	-0.13	-0.18	-0.12	-0.17	-0.09	0.07	0.10
Biceps Femoris (g)	0.49*	0.23	0.28	0.39*	0.17	0.29#	0.08
(% BW)	0.12	0.02	-0.04	0.11	-0.13	0.12	0.10
Vastus Lateralis (g)	0.34#	0.25	0.29#	0.16	-0.05	0.06	-0.12
(% BW)	-0.01	0.14	0.05	-0.10	-0.38*	-0.21	-0.23
EDL (g)	0.03	-0.06	0.02	-0.01	-0.04	0.07	0.04
(% BW)	-0.14	-0.17	-0.11	-0.15	-0.14	0.01	0.05
Biceps brachii (g)	0.32#	0.18	0.23	0.04	0.09	0.13	-0.04
(% BW)	-0.06	0.02	-0.03	-0.30#	-0.23	-0.13	-0.13
Diaphragm (g)	0.20	-0.12	0.16	0.07	0.28	0.37*	0.27
(% BW)	-0.23	-0.40*	-0.16	-0.26	0.06	0.21	0.31#
Summed muscle (g)	0.42*	0.18	0.29	0.24	0.17	0.26	0.05
(% BW)	-0.05	-0.07	-0.07	-0.15	-0.23	0.01	0.02

CRL refers to crown-rump length, AC to abdominal circumference, HL to head length, HW to head width, BMI to body mass index and PI to ponderal index. EDL refers to extensor digitorum longus, %BW refers to weight expressed as a percentage of body weight. ** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

Table 2.9: Size at birth and adipose tissue mass in the juvenile guinea pig

	SIZE AT BIRTH						
	Weight	*CRL*	*AC*	*HL*	*HW*	*BMI*	*PI*
Adipose tissue	*r*	*r*	*r*	*r*	*r*		
Dorsal Fat (g)	0.44*	0.41*	0.13	0.29#	0.13	0.05	-0.17
(% BW)	0.29#	0.37*	-0.02	0.19	-0.02	-0.09	-0.23
Shoulder Fat (g)	0.53*	0.23	0.30#	0.17	0.40*	0.36#	0.12
(% BW)	0.40*	0.17	0.17	0.05	0.31#	0.29#	0.12
Neck Fat (g)	0.42*	0.39#	0.20	0.21	-0.08	0.04	-0.16
(% BW)	0.27	0.29	0.07	0.11	-0.17	-0.01	-0.13
Parametrial Fat (g)	0.36	-0.11	0.07	-0.09	0.64*	0.49#	0.39
(% BW)	0.34	-0.06	0.11	0.08	0.57*	0.29	0.20
Retro Fat (g)	0.43*	0.22	0.14	0.21	0.28	0.26	0.07
(% BW)	0.35#	0.19	0.05	0.15	0.21	0.21	0.06
Perirenal Fat (g)	0.47*	0.32#	0.18	0.17	0.34#	0.17	-0.06
(% BW)	0.39*	0.32#	0.09	0.10	0.26	0.07	-0.11
Epididymal Fat (g)	0.83*	0.47#	0.81*	0.87**	0.75*	0.57*	0.14
(% BW)	0.51#	0.04	0.50#	0.58*	0.74*	0.73*	0.48#
Groin Fat (g)	0.58*	0.38*	0.39*	0.52*	0.54*	0.24	0.01
(% BW)	0.48*	0.35#	0.29#	0.45*	0.47*	0.17	-0.02
Summed fat (g)	0.61*	0.47*	0.29	0.38*	0.39*	0.21	-0.08
(% BW)	0.48*	0.39*	0.14	0.27	0.30#	0.15	-0.06
Sum muscle:fat (g)	-0.57*	-0.37*	-0.17	-0.37#	-0.38*	-0.29#	-0.04

%BW refers to weight expressed as a percentage of body weight. ** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

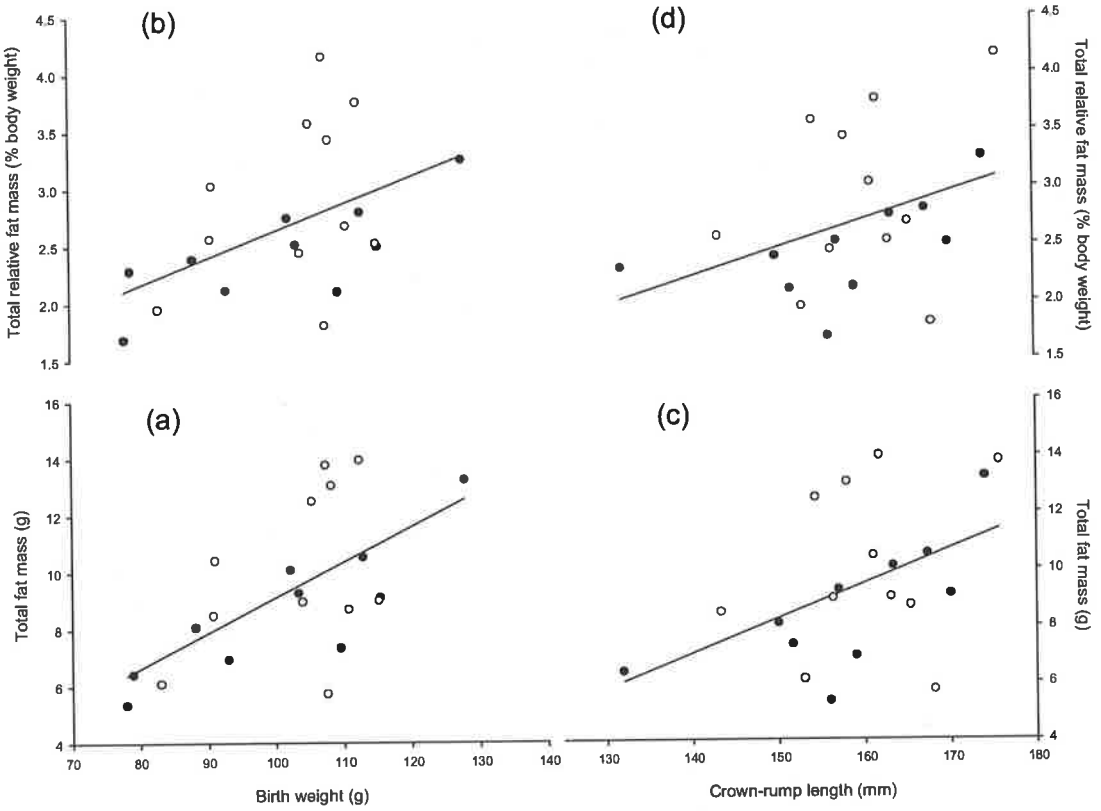

Figure 2.6: Adiposity in the juvenile guinea pig and size at birth

Males are represented by closed, and females by open circles. Correlations are significant at $p<0.05$. (a) Summed fat mass was positively correlated with birth weight ($r=0.61$), as was (b) summed relative fat mass ($r=-0.48$). (c) summed fat mass was positively correlated with birth CRL ($r=0.47$), as was (d) summed relative fat mass ($r=0.39$) ($p<0.05$ for all).

2.4.7 Skeletal muscle mass and adipose tissue mass and postnatal growth in the juvenile guinea pig

Summed muscle mass was positively correlated with AGR_{weight} (r=0.63; Figure 2.7 (b)). Weights of all muscles were positively correlated with AGR_{weight} ($p<0.05$ for all; Table 2.10), with the exception of plantaris. The relative weights of EDL (r=0.37) and diaphragm (r=0.44) muscles were positively correlated with FGR_{weight} (Table 2.10).

Summed fat mass (r=0.56) and relative fat mass (r=0.42) were positively correlated to AGR_{weight}. All fat depots, excluding neck fat and parametrial fat, were positively correlated with AGR_{weight} ($p<0.05$ for all; Table 2.10). Neck fat weight (r=-0.52; % BW r=-0.45), and epididymal fat weight (r=-0.74; % BW r=-0.58) were negatively correlated with FGR_{weight} (Table 2.10).

Table 2.10: Skeletal muscle mass and adipose tissue mass and postnatal growth in the juvenile guinea pig

	Absolute growth rate		Fractional growth rate	
Muscle/adipose tissue	***Weight (g)*** *r*	***(% BW)*** *r*	***Weight (g)*** *r*	***(% BW)*** *r*
Plantaris (g)	0.07	-0.08	0.04	0.08
Gastrocnemius (g)	0.47*	0.05	0.01	0.14
Tibialis (g)	0.40*	0.21	0.06	0.15
Semitendinosus (g)	0.51*	0.27	0.14	0.31#
Biceps Femoris (g)	0.61*	0.25	-0.05	0.07
Vastus Lateralis (g)	0.41*	0.04	-0.04	0.03
EDL (g)	0.39*	0.28	0.28#	0.37*
Biceps brachii (g)	0.41*	0.03	-0.03	0.09
Diaphragm (g)	0.54*	0.20	0.23	0.44*
Summed muscle (g)	0.63*	0.30#	0.04	0.27
Dorsal Fat (g)	0.41*	0.21	-0.18	-0.17
Shoulder Fat (g)	0.49*	0.34#	-0.17	-0.16
Neck Fat (g)	-0.04	-0.17	-0.52*	-0.45*
Parametrial Fat (g)	0.47#	0.32	0.01	-0.08
Retro Fat (g)	0.49*	0.40*	-0.09	-0.08
Perirenal Fat (g)	0.50*	0.39*	-0.11	-0.10
Epididymal Fat (g)	0.60*	0.23	-0.74*	-0.58*
Groin Fat (g)	0.51*	0.36#	-0.22	-0.23
Summed fat (g)	0.56*	0.42*	-0.23	-0.20
Sum muscle:fat (g)	-0.37#	N/A	0.34#	N/A

EDL refers to extensor digitorum longus; %BW refers to weight expressed as a percentage of body weight. ** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$.

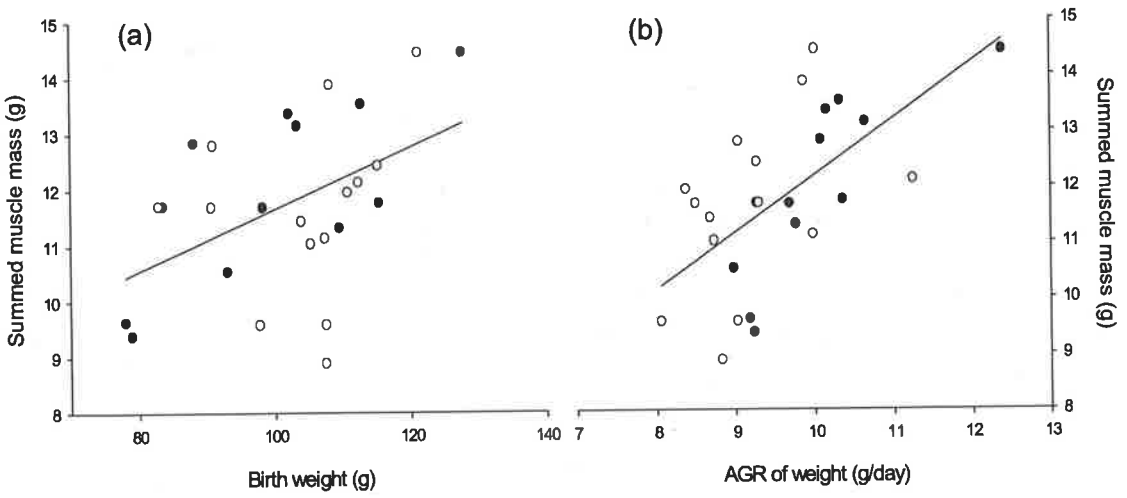

Figure 2.7: Summed muscle mass and birth weight and postnatal growth in the juvenile guinea pig

Males are represented by closed, and females by open circles. Summed muscle mass was positively correlated with (a) birth weight ($r=0.42$) and (b) AGR of weight ($r=0.63$) ($p<0.05$ for each).

2.5 Discussion

This study has shown that, in the guinea pig, spontaneous fetal growth restriction in terms of weight, reduces crown-rump length, abdominal circumference, head width and head length at birth. Animals small at birth in terms of weight have decreased absolute growth rates, but increased fractional growth rates of weight, from birth to 36 days of age, a period at which guinea pigs are pre-pubertal and ~ 10 days post-weaning. Small animals in terms of weight, crown-rump length, abdominal circumference and head measures all have increased fractional growth rates of weight and/or CRL, AC and head measures. Therefore, in all aspects of growth these smaller animals are growing faster in fractional terms, undergoing 'catch-up growth'. LBW animals remain smaller as juveniles, and have less fat mass, while LBW females have more and LBW males less skeletal muscle in absolute and relative terms. However, LBW animals show enhanced size of or relatively larger vital organs, including GIT, brain and liver. This indicates that catch-up has involved restoration of hepatic and GIT growth, both disproportionately reduced in the near term under-nourished fetal guinea pig (Kind et al., 2005). Furthermore, the catch-up phenomenon has increased skeletal muscle mass, at least in females. Despite the links between catch-up growth and later obesity in the human, the LBW animals remained lean after a month of catch-up growth.

The newborn guinea pig that is growth restricted resembles the human IUGR infant, which is commonly characterised by reduced weight, length or increased thinness for gestational age, and is characterised by placental insufficiency

(Karlberg et al., 1996; Robinson et al., 1994), and the neonatal lamb which has been placentally restricted (De Blasio, 2004). Like these species, fetal growth restriction in the guinea pig is followed by substantial catch-up growth in the first month of life (Chatelain et al., 1996; Chatelain et al., 1994; Karlberg et al., 1997). Maternal food restriction in the guinea pig also restricts fetal growth and size at birth and induces catch-up growth postnatally (Kind et al., 2003). Low birth weight pigs resulting from spontaneous fetal growth restriction have increased fractional growth rates in the first month of postnatal life, however, have slower fractional growth rates in the post-weaning period until 3 months of age compared to high birth weight pigs (Poore et al., 2002). Therefore, unlike rodents, in larger mammalian species placental or similar restriction of substrate supply appears to 'program' or induce postnatal catch-up growth.

Small size at birth due to spontaneous fetal growth restriction in the current study did not lead to increased fatness in the juvenile guinea pig. We had predicted that LBW animals would have increased fat mass, due to previous studies in our laboratory which indicate that, in the sheep, IUGR induced by placental restriction results in smaller animals at birth that have increased fat mass by 40 days of age postnatally (De Blasio, 2004). However, these sheep show complete catch up growth by day 35 postnatally in terms of weight, CRL and abdominal circumference (De Blasio, 2004), with the remaining growth parameters showing complete catch-up before the onset of puberty (120-150 days postnatally). According to previous studies in our laboratory, we estimated the catch-up growth period in the low birth weight guinea pig to span the first 6-7 weeks of life (results unpublished), and it appears that juvenile animals

studied here do exhibit signs of being in the midst of their catch-up growth phase, as indicated by increased fractional growth rates. However, they have failed to complete catch-up growth at this time, as they were smaller in weight and length at 36 days of age compared to high birth weight animals. Therefore it is possible that the discrepancy between the two species is due to the guinea pigs exhibiting incomplete catch-up growth at the time of the study. In addition, the placentally restricted lamb is a different experimental model, with IUGR being induced by the removal of all but a few of the endometrial caruncles (Owens et al., 1986). The lambs arising from such a surgical technique are severely growth restricted, with birth weight being reduced by 25%, whereas LBW animals in the present study have birth weights 21% reduced from that of HBW animals. Although the guinea pigs are small at birth, the deprivation from nutrients and oxygen may be more pronounced in the sheep model.

The positive correlation between size at birth and muscle mass may add to growing evidence that being born small for gestational age may lead to decreased lean muscle mass, as has been shown in the human (Hediger et al., 1998; Li et al., 2003; Rogers & Group., 2003; Singhal et al., 2003), as opposed to evidence that being born small leads to an increased fat mass (Ong et al., 2002). However, an increased summed muscle:fat ratio in the LBW animals indicates that these animals probably have relatively normal muscle mass, but less fat. In the present study, LBW females showed no deficit in muscle mass as a proportion of body weight as juvenile animals, suggesting any fetal deficit has been ameliorated by catch-up growth in muscle. In contrast, the LBW males

still have a deficit in skeletal muscle in absolute and relative terms, consistent with the findings to date in humans.

In addition to having long-term effects on brain development, IUGR has also been shown to cause reductions in gastrointestinal growth and delays in development in the fetus. In the present study, guinea pigs in the present study display catch-up growth in size of the GIT. Developmental delays in fetal disaccharidase activity, which is indicative of intestinal function and maturity, have been reported in rat models of IUGR (Lebenthal et al., 1981). The two disaccharidases, lactase and maltase are also lower in the jejenum of growth-retarded rabbit fetuses during gestation, and this continues into the neonatal period, until catch-up growth occurs at two weeks of age, when levels normalise (Buchmiller-Crair et al., 2001). The increased relative weight of the GI tract in the juvenile guinea pigs may therefore be due to increased growth as compensation for earlier developmental delays whilst in utero. Although we have no data regarding digestive function of the GIT, catch-up growth of this organ suggests adequacy at least. At any rate, it seems that, in general terms, being growth retarded may have effects on growth of vital organs, which may persist long-term.

In conclusion, spontaneous fetal growth restriction in terms of weight in the juvenile guinea pig results in offspring of small size at birth in terms of crown-rump length, abdominal circumference and head length and width and increased thinness at birth. The animals smallest at birth, although showing signs of catch-up growth through increased fractional growth rates, do not

complete catch-up in the first 36 days of life. They do show normalisation of absolute or relative size of vital organs, in particular the brain, GIT and liver, but remain smaller compared to HBW animals. LBW males also have reduced skeletal muscle mass. The LBW juvenile guinea pig does not exhibit increased adiposity however, despite accelerated growth after birth. This suggest catch-up growth after IUGR can occur and at least restore gross size of some key organs involved in nutrition and metabolism, without onset of obesity.

Chapter 3

Spontaneous fetal growth restriction increases sensitivity to IGF-I but reduces abundance of IGFs postnatally

3.1 Introduction

Small size at birth and its associated catch-up growth in infants and children is characterised by marked but somewhat variable alterations in the somatotropic and IGF axis. In addition, most studies have focused on the consequences for circulating IGFs and to some extent the IGFBPs, with little known about the effects on IGF sensitivity. Hence the role of prenatally programmed changes in IGF action in postnatal catch-up growth after fetal growth restriction is unclear. Furthermore, catch-up growth independently predicts later risk of diabetes and cardiovascular disease, which could be due in part to persistence of early onset changes or deficiencies in IGF abundance and action (Monzavi & Cohen, 2002).

In human IUGR pregnancies, fetal cord sera IGF-I levels are reduced (Cianfarani et al., 1998; Giudice et al., 1995; Gluckman et al., 1983). Plasma IGFBP-3 is reduced (Bazaes et al., 2003; Cianfarani et al., 1998), and IGFBP-1 increased (Bazaes et al., 2003; Cianfarani et al., 1998; Giudice et al., 1995) in IUGR fetal cord sera. Plasma IGF-I levels are consistently reported as being reduced, or at best, normal, in the first few months of life and up to 1 year of age in the presence of catch-up growth in the human IUGR infant (Bennett et al., 1983; Giudice et al., 1995; Leger et al., 1996; Ogilvy-Stuart et al., 1998; Ozkan et al., 1999). Serum IGF-II levels appear higher in IUGR infants undergoing catch-up growth, compared to those infants not catching up at 3 months of age (Garcia et al., 1996). Plasma IGFBP-1 and IGFBP-3 levels are

not different between IUGR neonates who undergo catch-up growth to 6-9 months of age, and those who don't (Ozkan et al., 1999).

At 5 days of age postnatally, plasma IGF-I is lower in IUGR infants compared to controls (Thieriot-Prevost et al., 1988), and plasma IGFBP-3 levels are lower in SGA neonates at this age (Cance-Rouzaud et al., 1998). Overall these findings suggest that plasma IGFs are initially low following birth, then increase over the first six months of life, especially in those undergoing catch-up growth, before increasing above normal in childhood and then gradually normalising with increasing age in adult life. The impact if any on IGF sensitivity is unclear and whether the transient increase in plasma IGF-I in childhood represents IGF resistance or over production is unclear.

In the sheep, fetal plasma IGF-I and IGF-II are positively associated with fetal weight at 127 days of gestation (Owens et al., 1994). Circulating concentrations of IGF–II but not of IGF-I are decreased at 35 days of age postnatally following placental restriction in the sheep, and in addition, sensitivity of FFA metabolism to IGF-I is increased in neonatal animals that were small at birth (De Blasio, 2004). This suggests that prenatal restraint increases postnatal IGF sensitivity and helps cause catch-up growth, but whether the apparent decrease in plasma IGFs reflects increased clearance rather than decreased IGF production and the overall consequences of IGF action are unclear.

The guinea pig IGF axis is similar to the human in that IGF-II is expressed in serum of adult guinea pigs, as it is in adult human tissues, and IGFBP-3 is

expressed in fetal plasma of both species. IUGR induced by maternal unilateral uterine artery ligation in the pregnant guinea pig on day 30 of gestation increases IGFBP-2 and –4 in fetal plasma, and decreases IGFBP-3 (Carter et al., 2005). IGFBP-2 mRNA expression is increased in the fetal liver, however expression of IGF-I and –II or IGFBP-3 and –5 mRNA in fetal liver and skeletal muscle is unchanged (Carter et al., 2005). In contrast, a decrease in plasma IGF-I has been observed in the fetal guinea pig following uterine artery ligation at day 30 of pregnancy (Jones et al., 1987; Jones et al., 1990), in association with a 2-4 fold increase in IGF-II (Jones et al., 1987). Postnatally, plasma IGF-I and IGF-II are reduced in young adult guinea pigs following chronic maternal feed restriction (Dwyer & Stickland, 1992; Olausson & Sohlstrom, 2003; Sohlstrom et al., 1998). Plasma IGFBP-1, -3 and –4 are also reduced, but IGFBP-2 are increased (Sohlstrom et al., 1998).

Therefore the fetal changes observed in the IUGR guinea pig are similar to that observed in the human fetus. However, circulating concentrations of these growth factors are to date unknown in the growth-restricted guinea pig undergoing catch-up growth. In addition, the effect of placental restriction on the IGF system postnatally and its relationship to catch up growth in the sheep as previously mentioned (De Blasio, 2004) was carried out after the period of maximal catch-up growth. Therefore it was hypothesised that restriction of fetal supply and growth in the guinea pig would increase metabolic sensitivity to IGF-I, in the presence of reduced or unchanged plasma IGF-I and IGF-II in the very young guinea pig undergoing catch-up growth.

3.2 General Aim

To determine the effect of spontaneous fetal growth restriction in the juvenile guinea pig on the insulin-like growth factor (IGF) axis during postnatal catch-up growth.

Specific Aim 1:

To determine the effect of fetal growth restriction on IGF-I sensitivity of whole body glucose metabolism as indicated by that of circulating glucose and free fatty acids respectively in the juvenile guinea pig during catch-up growth.

Specific Aim 2:

To determine the effect of fetal growth restriction on circulating IGF-I, IGF-II and IGFBP concentrations and their relationship to size at birth and postnatal growth in the juvenile guinea pig during catch-up growth.

Specific Aim 3:

To determine the relationship between metabolic sensitivity to IGF-I and circulating IGFs and IGFBPs with size at birth and postnatal growth in the juvenile guinea pig.

3.3 Materials and methods

3.3.1 *Mating and housing of guinea pigs*

Guinea pigs (IMVS tri coloured, Gilles Plains Resource Centre, SA) were caged individually in plastic tubs with wire lids, and maintained in a room with a 12:12h light:dark cycle at 25°C, with *ad libitum* access to guinea pig/rabbit ration (Ridley Agriproducts, Australia). Males used for mating were fed lucerne in addition to the guinea pig chow. All guinea pigs also had *ad libitum* access to tap water, containing 400mg/L vitamin C. Females and males were mated as described in Chapter 2. Upon birth, guinea pigs were classed as being low or high birth weight according to the mean birth weight of the cohort (102.3g).

3.3.2 *Size at birth and postnatal growth measurements*

A description of the total number of animals used in each experiment can be found in Appendix A, and total numbers of litters used is summarised in Appendix C. Guinea pigs were weighed at birth, and size in terms of weight, crown-rump length (CRL), abdominal circumference (AC), head width (HW), and head length (HL) measured at birth and subsequently at 5-day intervals in (see Table 2.1). All pups were weaned at 25 days of age onto guinea pig/rabbit ration and fed *ad libitum*, with access to water containing 400mg/L vitamin C. Absolute growth rates were recorded for each growth measurement for each guinea pig (AGR) by calculating the slope of the regression line of postnatal age

and size, and then expressed in terms of individual birth size to give a measure of catch up growth (fractional growth rate: FGR).

3.3.3 Insertion of vascular catheters and maintenance

At 28 ± 1 days of age, guinea pigs were weighed, and anaesthetised (Atropine 0.05mg/kg s.c., Xylazine 6mg/kg i.m., ketamine 75mg/kg i.p., Lignocaine locally as required). Catheters were inserted into the external jugular vein (23G 1¼ TW needle hub in 1 mm OD x 0.5 mm ID tubing), and the right common carotid artery (22G 1½ TW needle hub in 0.96 mm OD x 0.58 mm ID tubing). The catheters were then fixed at the back on the neck. Catheterised pups were weighed daily. Catheters were flushed daily with 500 µl heparinised saline (50 µU/ml), and sealed with a clean plug.

3.3.4 Hyper-IGF-I-euglycaemic clamp (HIEC)

At 32 ± 1 days of age, IGF-I sensitivity of glucose metabolism was measured by HIEC to give IGF-I sensitivity of whole body glucose metabolism. Animals were fasted overnight (6:00 pm to 9:00 am) before the clamp, but were allowed water *ad libitum*. The animals were weighed, and subsequently glucose, recombinant human (rh) IGF-I and saline infusion syringes were prepared. IGF-I was infused intravenously for 2 hours at a rate of 12 µg/kg/min, and a variable glucose infusion delivered during the IGF-I infusion to maintain euglycaemia, using a programmable infusion pump (World Precision Instruments SP 220i).

Blood was sampled from the arterial catheter at –10, -5 and 0 minutes prior to infusion, and mean fasting blood glucose concentration. Blood was sampled at 5-minute intervals from t=0 to t=120 using a Hemocue glucometer (Hemocue AG, Sweden) for rapid blood glucose measurement to allow adjustment of the glucose infusion rate (GIR) to restore the mean fasting blood glucose level. The GIR was calculated using a specific algorithm adapted from a previously published version (DeFronzo et al, 1979). The uncorrected value for IGF-I sensitivity of glucose metabolism was determined as the GIR from 70-120 mins during the hyper-IGF-I euglycaemia clamp, and the corrected value calculated as the GIR divided by the plateau plasma IGF-I measured in pooled samples from t=100 and t=120 for each animal. The average GIR during the clamp for all animals can be seen in Figure 1.2.

3.3.5 HPLC separation of plasma IGF-I and –II from IGFBPs

Ninety µl of plasma from guinea pigs was acidified by adding 150 µl of 4x acidic mobile phase (800 mM acetic acid, 200 mM trimethylamine, 400mM HCl, 5ml/L Tween-20, pH 2.8), and 360 µl of water to a final volume of 600 µl. After an incubation of 30 mins at room temperature, the diluted plasma samples were mixed with an equal volume of freon (1,1,2-trichloro-1,2,2-trifluoroethane) and centrifuged for 10 mins at 10,000 rpm. The upper aqueous phase was recovered and the defatted plasma filtered by centrifugation through a microfilter containing a 0.45 µm cellulose acetate filter membrane (Alltech Associates Inc.). Three hundred µl of filtered sample was loaded into HPLC injection vials, with 150 µl being injected onto a Protein-Pak 125 HPLC column

(Waters/Millipore) using an automatic injector (ICI AS 2000, ICI Instruments) (Owens et al, 1994). Samples were eluted at 1 ml/min in 1 x acidic chromatography mobile phase (200 mM acetic acid, 50 mM trimethylamine, 100 mM HCl, 0.5 ml/L Tween -20, pH 2.8).

Initially, 36 0.5ml fractions were collected to determine elution times for free IGFs during the clamp (pooled samples from the last hour of the HIEC from 2 animals), low birth weight animals (pooled fasted samples from 2 animals) and high birth weight animals (pooled fasted samples from 2 animals) (Figure 3.1). IGF-I and –II were measured in each fraction by specific RIAs. The IGFBPs eluted at between 0.5 and 2.5 mins, and free IGFs eluted at between 3 and 5.5 mins (Figure 3.1). Eluent was therefore collected for these times and subjected to RIA. Fraction 1 contained IGFBPs, and fraction 3 contained purified IGF-I and IGF-II.

3.3.6 Measurement of plasma IGF-I

Recombinant human IGF-I (GroPep Pty Ltd, Adelaide, SA) was used to prepare the standard and radiolabelled ligand for the radioimmunoassay (RIA). Ten standards of 200 µl of the following concentrations were prepared: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.91 pg/tube. Each sample was assayed in triplicate by addition of 100 µl to a polystyrene tube, followed by 200 µl of RIA buffer (30 mM Na_2PO_4; 0.02% (w/v) protamine sulphate; 10 mM disodium EDTA; 0.05% (w/v) Tween-20; 0.02% (w/v) NaN_3; pH 7.5). Mobile phase was added to standard tubes (100 µl), and 60 µl of 0.4 M Tris base to

every tube to neutralise to pH 7.4. Rabbit anti-human IGF-I antiserum (MAC Ab 89/1) (50 µl) and radio-iodinated h-IGF-I (20,000 cpm) (50 µl) were also added to each tube. Three tubes contained only radio-iodinated h-IGF-I to provide a measure of total radioactivity (totals), as well as three tubes containing RIA buffer and h-IGF-I, which served as blank tubes. All tubes were briefly vortexed, and incubated for 18-20 h at 4^{O}C. Following this incubation, 10 µl of rabbit IgG (cat no. P0448, DAKO, Australia Pty. Ltd) diluted 1:20 in RIA buffer, and 50 µl of sheep anti-rabbit IgG (Silenus, Victoria, Australia) (1:20 dilution) were added. Tubes were vortexed and incubated at room temperature for 30 min. One ml of ice-cold polyethylene glycol (PEG) (6% w/v; 150 mM NaCl) was added to all tubes except totals, vortexed, and then spun for 20 mins, 4000 rpm, at 4^{O}C (J-6B Beckman Instruments, USA). The supernatant was carefully aspirated, and the pellet counted for ^{125}I using a gamma scintillation spectrometer (1261 Multigamma, LKB Pharmacia and Wallace Oy). The inter-assay and intra-assay variations for IGF-I were 6.4% and 4.7% respectively.

3.3.7 Measurement of IGFBP fraction

The apparent plasma IGFBP concentration was measured by interference in and apparent activity in the IGF-I RIA, as stated in section 3.3.6. The concentration revealed by RIA is a measure of the IGFBP interference in the RIA. A lower counts per minute (cpm) count is indicative of increased apparent IGF present in the plasma, therefore the higher the cpm, the greater amount of IGFBP is present in the sample.

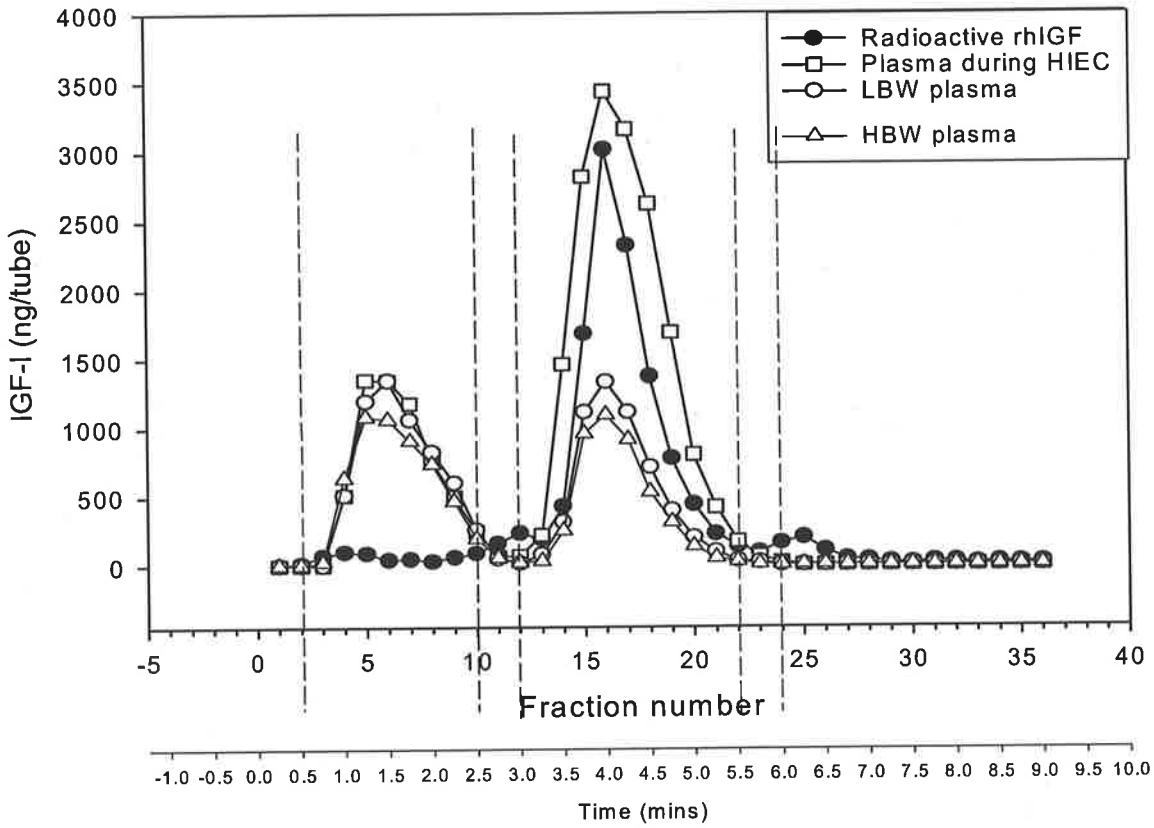

Figure 3.1: Elution profiles for HPLC

Fractions were eluted from 4 different samples to determine the collection times for the IGF peak and IGFBP interference peak. The radioactive tracer, represented by closed circles, eluted at fraction 16. Plasma pooled from two animals during the last hour of the clamp (t=100 and 120) (open squares), from two LBW animals (open circles) or two HBW animals (open triangles) had very similar elution profiles, suggesting no difference existed in elution times between these samples. The times of collection of four samples (IGFBP interference peak 0.5 to 2.5 min, wait 2.5 to 3.0 min, IGF peak 3.0 to 5.5 min, and wait 5.5 to 6.0 min) are represented by dashed lines.

3.3.8 Measurement of plasma IGF-II

Recombinant human IGF-II (GroPep Pty Ltd, Adelaide, SA) was used to prepare the standard and radiolabelled ligand for the radioimmunoassay (RIA). Ten standards of 200 µl of the following concentrations were prepared: 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.91pg/tube. Each sample was assayed in triplicate by addition of 50 µl to a polystyrene tube, followed by 200 µl of RIA buffer (30 mM Na_2PO_4; 0.02% (w/v) protamine sulphate; 10 mM disodium EDTA; 0.05% (w/v) Tween-20; 0.02% (w/v) NaN_3; pH 7.5). Mobile phase was added to standard tubes (100 µl), and 60 µl of 0.4M Tris base to every tube to neutralise to pH 7.4. Amano mouse anti-rat IGF-II monoclonal antibody (50 µl) and radio-iodinated h-IGF-II (20,000 cpm) (50 µl) were also added to each tube. Three tubes contained only radio-iodinated h-IGF-II to provide a measure of total radioactivity (totals), as well as three tubes containing RIA buffer and h-IGF-II, which served as blank tubes. All tubes were briefly vortexed, and incubated for 18-20 h at 4^{O}C. Following this incubation, 10 µl of mouse IgG (cat no. P0448, DAKO, Australia Pty. Ltd) diluted 1:20 in RIA buffer, and 50 µl of sheep anti-mouse IgG (Silenus, Victoria, Australia) (1:20 dilution) were added. Tubes were vortexed and incubated at room temperature for 30 min. One ml of ice-cold polyethylene glycol (PEG) (6% w/v; 150 mM NaCl) was added to all tubes except totals, vortexed, and then spun for 20 mins, 4000 rpm, at 4^{O}C (J-6B Beckman Instruments, USA). The supernatant was carefully aspirated, and the pellet counted using a gamma scintillation spectrometer (1261 Multigamma, LKB Pharmacia and Wallace Oy). The inter-assay and intra-assay variations for IGF-II were 3.4% and 8.7% respectively.

3.3.9 Measurement of blood glucose

Blood glucose in the arterial blood samples taken during the hyper-IGF-I-euglycaemic clamp was measured using a glucometer (Hemocue AB, Sweden). The glucometer quantifies the formation of a coloured formazan using two wavelengths of 660 and 840nm.

3.3.10 IGF-I sensitivity of plasma glucose

Plasma glucose concentrations were measured prior to and during the final 60 mins of the HIEC (t=-20, -10, 0, 70, 85, 100, 115, 120 minutes), using the COBAS MIRA automated sample system. The Roche Glucose HK assay kit and Cfas calibrator, as well as the quality controls Precinorm U and Precipath U (Roche Diagnostics, Australia) were used. The average plasma glucose values during the clamp can be seen in Figure 3.2. The mean co-efficient of variation was less than 1%.

3.3.11 IGF-I sensitivity of circulating free fatty acids

Plasma free fatty acid (FFA) concentrations were measured prior to and during the final 60 mins of the HIEC (t=-20, -10, 0, 70, 85, 100, 115, 120 minutes). These measurements were performed using the COBAS MIRA automated sample system, using the NEFA-C Free Fatty Acid kit (NovoChem), and quality controls QCS1 and 2 (Bio-Rad, Australia). The mean co-efficient of variation was 4.29%. The means of the plasma FFA values of all animals studied at each time point in the HIEC can be seen in Figure 3.2. The change in plasma FFA

was determined as the difference between fasting plasma FFA levels, and plasma FFA averaged over the last hour of the HIEC. IGF-I sensitivity of circulating FFA was determined as the percentage change in plasma FFA from fasting levels to those during the last hour of the HIEC (%Δ FFA, %Δmeq.ng^{-1}.ml^{-1}). IGF-I sensitivity of plasma FFA was also adjusted by dividing by the plateau plasma IGF-I during the last hour of the HIEC (adjusted %Δ FFA, %Δmeq.ng^{-2}).

3.3.12 Disposition Indices for glucose and FFA

Basal disposition indices for IGF-I action of glucose and FFA metabolism were calculated by multiplying the IGF-I sensitivity of glucose or FFA metabolism (unadjusted or adjusted) by the fasting plasma IGF-I.

3.3.13 Calculation of IGF-I clearance and secretion rates

The clearance rate of IGF-I during the clamp was determined by dividing the rate of IGF-I infusion by the plateau IGF-I concentration achieved during the last hour of the clamp.

$$\text{IGF-I clearance } (\mu g.ml.ng^{-1}.kg^{-1}.min^{-1}) = \frac{\text{IGF-I infusion rate } (12\mu g/kg/min)}{\text{Plateau IGF-I } (ng/ml)}$$

The IGF-I secretion rate was calculated by multiplying the fasting plasma IGF-I concentration by the IGF-I clearance rate calculated above.

$$\text{IGF-I secretion } (\mu g.kg^{-1}.min^{-1}) = \text{Fasting plasma IGF-I } (ng/ml) \times \text{IGF-I clearance rate } (\mu g.ml.ng^{-1}.kg^{-1}.min^{-1})$$

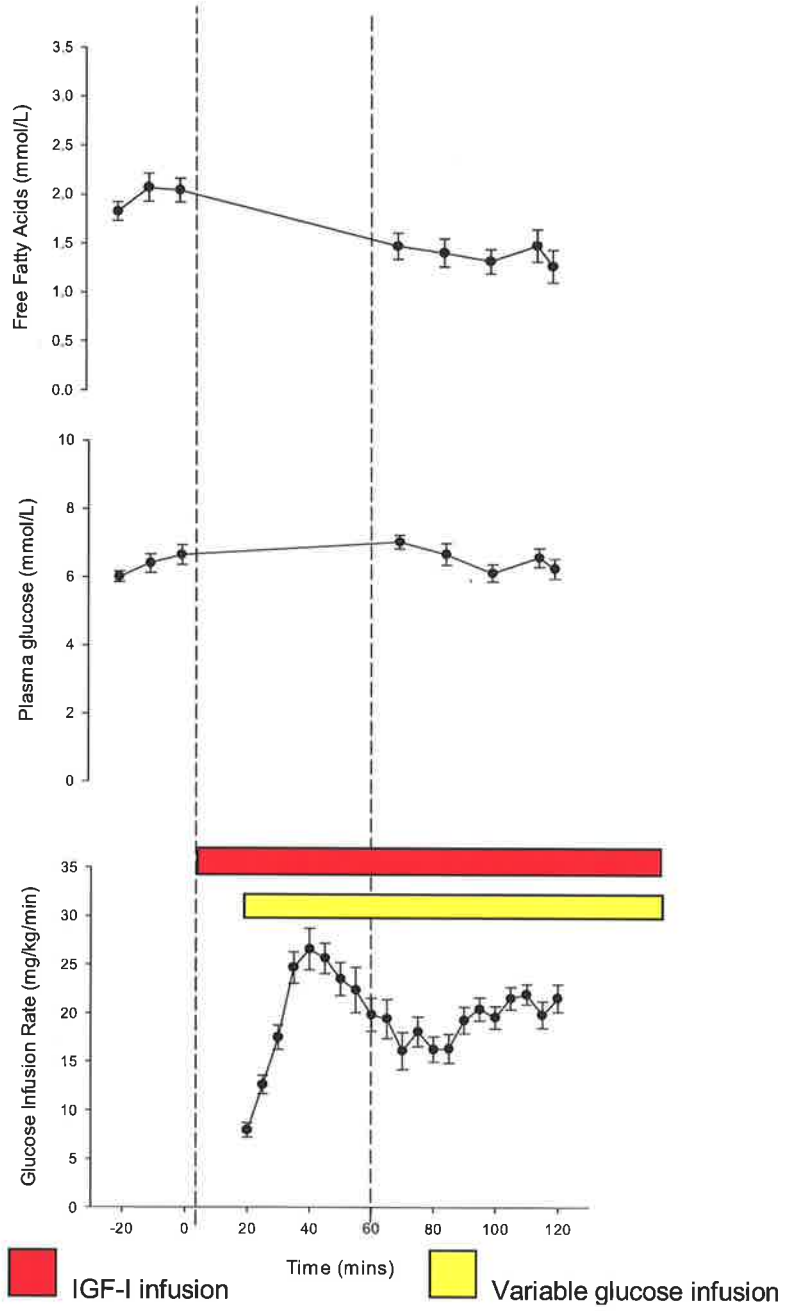

Figure 3.2: Plasma free fatty acids, plasma glucose and glucose infusion rate during the hyper-IGF-I-euglycaemic clamp

Animals are combined. All data is presented as mean ± SEM.

3.3.14 Post-mortem

Guinea pigs were sacrificed at 36 ± 2 days by an intraperitoneal overdose of sodium pentobarbitone (325 mg/ml, Lethabarb, Virbac, Australia), following an overnight fast. Blood samples were obtained by cardiac puncture immediately following death, into heparinised tubes, and plasma obtained after centrifugation at 4C and frozen at –20C until assay. All selected organs, individual muscles and adipose depots were dissected and weights recorded.

3.3.15 Statistics

The effect of birth weight class and sex and their interaction on parameters was determined by between factor analysis of variance with post-hoc analysis (SPSS Inc., Chicago). Associations between parameters were examined using Pearson's correlations analysis (SPSS Inc., Chicago). Unless otherwise stated, significance occurs at the level $p<0.05$.

3.4 Results

3.4.1 *Effect of birth weight class on circulating IGFs and IGFBPs*

Animals were classed into low (LBW) and high (HBW) birth weight categories according to the mean birth weight of the whole cohort (102.3g), and effects of birth weight class on the IGF axis determined (Table 3.1). Fasting plasma IGF-I prior to HIEC (IGF-I) tended to be lower in LBW animals (p=0.09) (Table 3.1, Figure 3.3). Fasting plasma IGF-I at post-mortem ($IGF\text{-}I_2$) was lower in LBW (-25%) (316 ± 38 $ng.ml^{-1}$), compared to HBW animals (425 ± 37 $ng.ml^{-1}$) (p=0.029) (Figure 3.3).

Secretion rates of IGF-I were lower (p=0.04) in LBW animals (-26%) compared to HBW, whereas IGF-I clearance rates did not differ between groups (Table 3.1).

Fasting plasma IGF-II prior to HIEC (p=0.006) was lower in LBW compared to HBW juveniles (-36%) (Table 3.1; Figure 3.3). Fasting plasma IGF-II was also lower at post-mortem in LBW animals (363 ± 51 $ng.ml^{-1}$) compared to HBW animals (523 ± 73 $ng.ml^{-1}$) (-31%) (p=0.049; Figure 3.3). Total plasma IGFs were lower in LBW compared to HBW animals (p=0.004) prior to HIEC (-32%) (Table 3.1).

Fasting plasma IGFBP capacity as indicated by interference in the IGF-I RIA, was reduced in LBW compared to HBW animals (-9%) (p=0.045; Table 3.1).

The ratio of IGF to IGFBP was calculated to determine the amount of circulating IGF compared to the amount of binding protein present in the blood. Plasma IGF-I:IGFBP was not different between LBW and HBW animals, however plasma IGF-II:IGFBP was lower in LBW compared to HBW animals in the fasting state (-29%) (p=0.004); and in the HIEC sample (-24%) (Table 3.1). Fasting plasma total IGF:IGFBP was also decreased in LBW compared to HBW animals (-25%) (p=0.006) (Table 3.1).

3.4.2 *Effect of birth weight class on circulating IGFs and metabolic sensitivity to IGF-I and IGF-I action on metabolism*

The effect of intravenous IGF-I infusion during the hyper-IGF-I-euglycaemic HIEC on circulating IGFs and IGFBPs is summarised in Table 3.1. The increase in plasma IGF-I from the fasted state to the plateau state (HIEC sample) (+8%) during the HIEC was higher in LBW compared to HBW animals (p=0.039). This was primarily due to the lower fasting plasma IGF-I in LBW animals, as the plateau plasma IGF-I concentrations did not differ with birth weight class (Table 3.1; Figure 3.4(b)).

IGF-I infusion reduced plasma IGF-II overall, but to a lesser extent in LBW (-47%) compared to HBW animals (-52%) (p=0.021) (Table 3.1; Figure 3.4(a)). Both fasting (p=0.006) and plateau (p=0.004) plasma IGF-II concentrations were lower in LBW compared to HBW animals (Table 3.1, Figure 3.4(a)).

IGF-I infusion increased plasma IGFBP similarly in LBW and HBW animals, and to similar plasma plateau values IGFBP levels during the HIEC (Table 3.1).

Plasma IGF-I:IGFBP did not differ between LBW and HBW animals during the HIEC (Table 3.1). Plateau plasma IGF-II:IGFBP ($p=0.006$) during the HIEC was lower in LBW compared to HBW animals (Table 3.1).

Metabolic sensitivity to IGF-I did not differ with birth weight class (Table 3.2). Similarly, IGF-I action on metabolism, as indicated by the disposition indices for glucose or for FFA did not differ with birth weight class (Table 3.2).

Table 3.1: The effect of birth weight class on the IGF axis in the juvenile guinea pig

	Low Birth Weight	*High Birth Weight*	*p-value*
Plasma			
Fasting IGF-I ($ng.ml^{-1}$)	323 ± 49	438 ± 47	0.09
HIEC IGF-I ($ng.ml^{-1}$)	1716 ± 54	1726 ± 47	ns
Δ IGF-I ($ng.ml^{-1}$)	1393 ± 25	1288 ± 49	0.039
Fasting IGF-II ($ng.ml^{-1}$)	421 ± 52	660 ± 45	0.006
HIEC IGF-II ($ng.ml^{-1}$)	224 ± 23	315 ± 16	0.004
Δ IGF-II ($ng.ml^{-1}$)	-197 ± 53	-346 ± 35	0.021
Total fasting IGF ($ng.ml^{-1}$)	743 ± 76	1099 ± 65	0.004
Total HIEC IGF ($ng.ml^{-1}$)	1939 ± 63	2041 ± 55	ns
Total Δ IGF ($ng.ml^{-1}$)	1196 ± 33	942 ± 44	0.001
IGF-I clearance ($*10^3$) ($\mu g.ml.ng^{-1}.kg^{-1}.min^{-1}$)	7.01 ± 0.2	6.99 ± 0.2	ns
IGF-I secretion ($\mu g.kg^{-1}.min^{-1}$)	2.24 ± 0.27	3.03 ± 0.31	0.04
Fasting IGFBP	753 ± 38	829 ± 22	0.045
HIEC IGFBP	1092 ± 110	1193 ± 55	ns
Δ IGFBP	-340 ± 79	-364 ± 51	ns
Fasting IGF-I:IGFBP	0.43 ± 0.05	0.52 ± 0.05	ns
HIEC IGF-I:IGFBP	1.62 ± 0.2	1.48 ± 0.08	ns
Fasting IGF-II:IGFBP	0.56 ± 0.06	0.79 ± 0.04	0.004
HIEC IGF-II:IGFBP	0.206 ± 0.01	0.27 ± 0.02	0.006
Total fasting IGF:IGFBP	0.97 ± 0.07	1.3 ± 0.07	0.006
Total HIEC IGF:IGFBP	1.83 ± 0.17	1.74 ± 0.09	ns

Data expressed as mean ± SEM.

Table 3.2: The effect of birth weight class on metabolic sensitivity to IGF-I in the juvenile guinea pig

	Low Birth Weight	*High Birth Weight*	*p-value*
IGF-I Sensitivity			
Plateau plasma IGF-I during HIEC ($ng.ml^{-1}$)	1716 ± 54	1726 ± 47	ns
Glucose			
Fasting plasma glucose (mmol/L)	6.11 ± 0.3	6.41 ± 0.3	ns
Unadjusted SSGIR ($mg.min^{-1}.kg^{-1}$)	19.3 ± 1.4	18.8 ± 1.2	ns
Adjusted SSGIR ($mg.ml.ng^{-1}.min^{-1}.kg^{-1}$) ($*10^2$)	2.56 ± 0.1	2.29 ± 0.2	ns
Free Fatty Acids			
Fasting plasma FFA (meq/L)	1.65 ± 0.2	2.05 ± 0.2	0.08
Δ FFA ($\Delta meq.ng^{-1}.ml^{-1}$)	0.61 ± 0.24	0.53 ± 0.07	ns
%Δ FFA ($\%\Delta meq.ng^{-1}.ml^{-1}$)	33.9 ± 11	27.2 ± 4	ns
Adjusted %Δ FFA ($\%\Delta meq.ng^{-2}$) ($*10^2$)	2.01 ± 0.67	1.62 ± 0.27	ns
Basal disposition indices			
Unadjusted glucose (IGF-I x $IGFS_{glu}$) ($mg.ng.min^{-1}.kg^{-1}.ml^{-1}$)	6423 ± 1534	8063 ± 923	ns
Adjusted glucose (IGF-I x $IGFS_{glu}$Adj) ($mg.ng^2.min^{-1}.kg^{-1}.ml^{-2}$)	1.93 ± 0.14	1.88 ± 0.11	ns
Unadjusted FFA (IGF-I x $IGFS_{FFA}$%) ($\%\Delta meq.ml^{-2}$)	9672 ± 2362	11117 ± 1658	ns
Adjusted FFA (IGF-I x $IGFS_{FFA}$%Adj) ($\%\Delta meq.ng^{-1}.ml^{-1}$)	5.7 ± 1.4	6.6 ± 1.0	ns

Data expressed as mean ± SEM. $IGFS_{glu}$ and $IGFS_{FFA}$% refer to IGF-I sensitivities unadjusted for plateau plasma IGF-I during HIEC.

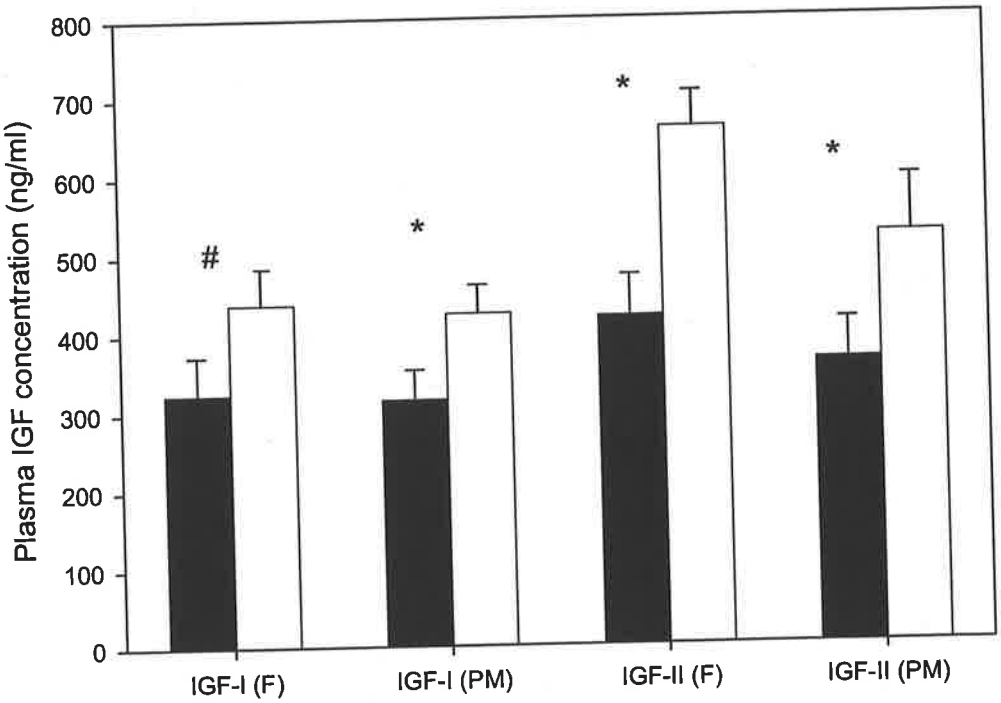

Figure 3.3: Effect of birth weight class on plasma IGF-I and –II concentrations in the juvenile guinea pig

Low birth weight is represented by black bars; high birth weight by white bars. * denotes significance at $p<0.05$; # $p<0.1$. PM refers to fasted plasma obtained at post-mortem; F refers to fasted plasma obtained prior to the HIEC.

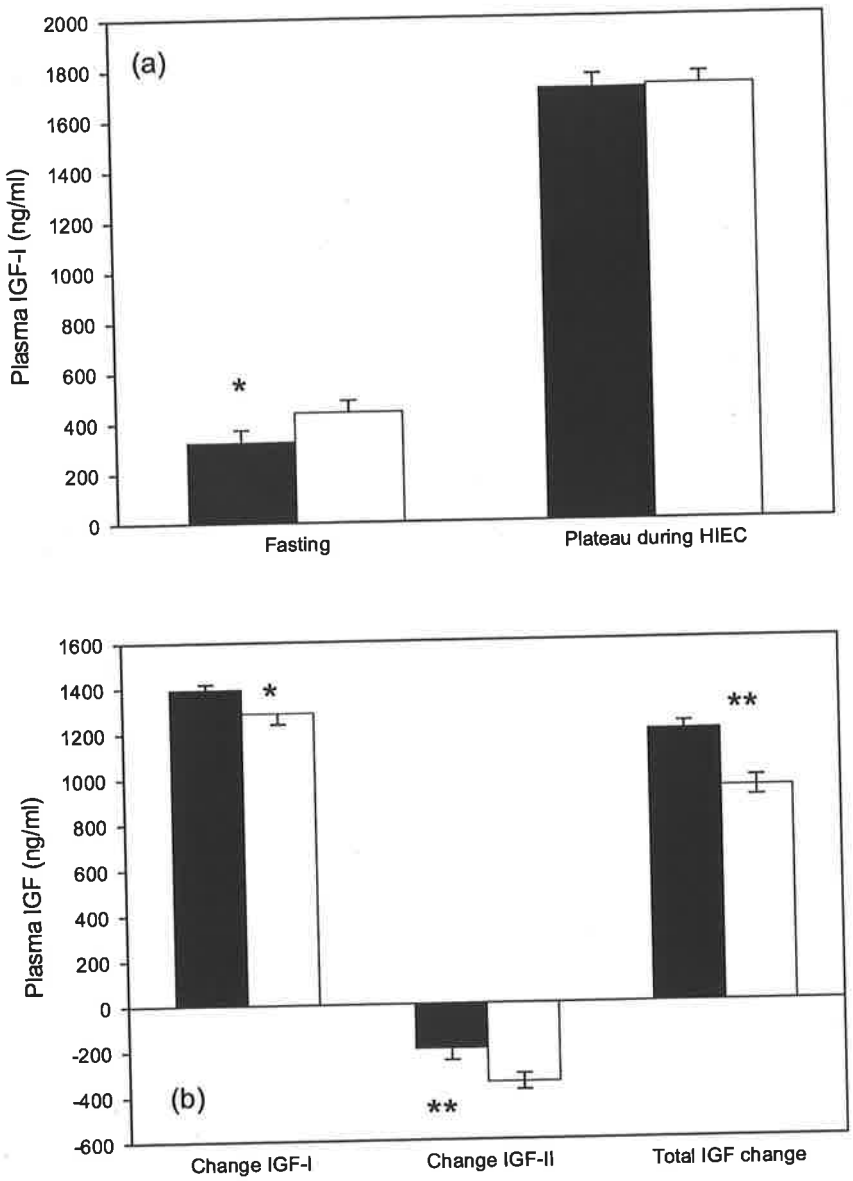

Figure 3.4: Effect of intravenous IGF-I infusion on plasma IGFs in the juvenile guinea pig

Low birth weight category is represented by black bars; high birth weight category by white bars. * denotes significance at $p<0.05$, ** at $p<0.001$. Figure 3.4(a): Fasting refers to the fasting plasma sample collected immediately before the HIEC; plateau is the average of two samples obtained during the last hour of the HIEC. Figure 3.4 (b): Change in IGF refers to the change in IGF from the fasted state before the HIEC to the plateau IGF concentration achieved during the last hour of the HIEC.

3.4.3 Size at birth, postnatal growth and circulating IGFs in the juvenile guinea pig

Weight (r=0.65), CRL (r=0.38), AC (r=0.39) and HL (r=0.53) at birth were positively correlated with plasma IGF-I (Figure 3.5). Birth AC correlated positively with IGF-I secretion (r=0.50) (Table 3.3).

Weight (r=0.52), AC (r=0.40), HL (r=0.46) and HW (r=0.61) at birth correlated positively with plasma IGF-II (Figure 3.5). Birth weight (r=0.64), HL (r=0.48) and HW (r=0.60) correlated positively with total plasma IGFs (Table 3.3). HL at birth correlated positively with plasma IGFBP (r=0.40) (Table 3.3).

Weight (r=0.58), CRL (r=0.43) and HL at birth (r=0.55) correlated positively with plasma IGF-I:IGFBP. In addition, weight (r=0.55), AC (r=0.51), HL (r=0.52), and HW (r=0.67) at birth were positively correlated with plasma IGF-II:IGFBP, and weight (r=0.62), AC (r=0.44), HL (r=0.49), and HW (r=0.66) at birth correlated positively with plasma total IGF:IGFBP (Table 3.3).

AGR_{weight} correlated positively with plasma IGF-I (r=0.62), IGF-I secretion (r=0.49), plasma IGF-II (r=0.42) and total plasma IGFs (r=0.42) (Table 3.4). In addition, AGR_{weight} correlated positively with plasma IGF-I:IGFBP (r=0.47; Table 3.4). AGR_{HL} (r=0.50) and AGR_{HW} (r=0.61) both correlated positively with plasma IGFBP (Table 3.4).

FGR_{weight} correlated negatively with plasma total IGF:IGFBP ratio (r=-0.42; Table 3.4). FGR_{CRL} correlated negatively with many parameters of circulating

IGFs, including plasma IGF-I (r=-0.48), IGF-II (r=-0.59), total IGFs (r=-0.54), IGF-II:IGFBP (r=-0.56) and total IGF:IGFBP (r=-0.46). FGR_{HW} positively correlated with plasma IGFBP (r=0.55) (Table 3.4).

3.4.4 *Size at birth, postnatal growth and IGF-I sensitivity and action in the juvenile guinea pig*

As birth weight increased, IGF-I sensitivity of glucose metabolism (as determined by glucose infusion rate adjusted for IGF-I concentration over the last hour of the clamp; adjusted SSGIR) decreased, such that animals with lowest birth weights had the highest IGF-I sensitivity of glucose metabolism (r=-0.51; Figure 3.6). This negative correlation with IGF-I sensitivity of glucose metabolism was apparent with other birth parameters, including CRL (r=-0.53), AC (r=-0.57) and HW (r=-0.57) (Table 3.3, Figure 3.6). Weight (r=-0.49), CRL (r=-0.51) and AC (r=-0.55) at birth were also negatively correlated with unadjusted SSGIR (Table 3.3). Size at birth in terms of weight (r=-0.49), CRL (r=-0.51) and AC (r=-0.55) correlated negatively with the adjusted disposition index for glucose (Table 3.3).

IGF-I sensitivity of glucose metabolism (adjusted) correlated negatively with AGR_{weight} (r=-0.91), but positively with FGR_{CRL} (r=0.60) (Table 3.5). The adjusted disposition index for glucose was negatively correlated with AGR_{weight} (r=-0.77), but positively correlated with FGR_{CRL} (r=0.56) and FGR_{AC} (r=0.59) (Table 3.5). The adjusted disposition indice for FFA was positively correlated with AGR_{weight} (r=0.52), but the remaining disposition indices of glucose and FFA were not associated with any measures of size at birth or postnatal growth (Table 3.5).

Table 3.3: IGF axis and size at birth in the juvenile guinea pig

	Weight	*CRL*	*AC*	*Head Length*	*Head Width*
IGF axis parameter	***r***	***r***	***r***	***r***	***r***
Post-mortem plasma					
IGF-I ($ng.ml^{-1}$)	0.65**	0.38*	0.39*	0.53*	0.35#
IGFBP	0.24	0.06	0.34#	0.40*	0.07
IGF-I:IGFBP	0.58*	0.43*	0.26	0.55*	0.22
IGF-II ($ng.ml^{-1}$)	0.52*	0.08	0.40*	0.46*	0.61*
IGF-II:IGFBP	0.55*	0.09	0.51*	0.52*	0.67**
Total IGF ($ng.ml^{-1}$)	0.64**	0.12	0.28	0.48*	0.60**
Total IGF:IGFBP	0.62**	0.10	0.44*	0.49*	0.66**
IGF-I abundance					
IGF-I clearance ($\mu g.ml.ng^{-1}.kg^{-1}.min^{-1}$)	-0.06	-0.06	-0.04	-0.03	-0.27
IGF-I secretion ($\mu g.kg^{-1}.min^{-1}$)	0.34	0.10	0.50*	0.30	0.31
IGF-I sensitivity					
Glucose					
SSGIR ($mg.min^{-1}.kg^{-1}$)	-0.49*	-0.51*	-0.55*	-0.45#	-0.39#
Adjusted SSGIR ($mg.ml.ng^{-1}.min^{-1}.kg^{-1}$)	-0.51*	-0.53*	-0.57*	-0.46*	-0.53*
Free Fatty Acids					
%Δ FFA (%Δ$meq.ng^{-1}.ml^{-1}$)	0.06	0.39#	-0.16	0.21	-0.17
Adjusted %Δ FFA (%Δ$meq.ng^{-2}$)	0.04	0.37#	-0.16	0.19	-0.19
Basal disposition Index					
Glucose (IGF-I x $IGFS_{glu}$)	-0.05	-0.25	0.04	-0.09	0.09
Glucose (IGF-IxIGFS_{glu}Adj)	-0.49*	-0.51*	-0.55*	-0.45#	-0.39#
FFA (IGF-I x $IGFS_{FFA}$%)	0.04	0.37#	-0.16	0.19	-0.19
FFA (IGF-I x $IGFS_{FFA}$%Adj)	0.32	0.44#	0.25	0.45#	0.01

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. CRL refers to crown-rump length; AC to abdominal circumference. Units for basal disposition indices are as follows: Glucose (IGF-I x $IGFS_{glu}$) ($mg.ng.min^{-1}.kg^{-1}.ml^{-1}$); Glucose (IGF-IxI$GFS_{glu}$Adj) ($mg.ng^{2}.min^{-1}.kg^{-1}.ml^{-2}$); FFA (IGF-I x $IGFS_{FFA}$%) (%Δ$meq.ml^{-2}$); FFA (IGF-I x $IGFS_{FFA}$%Adj) (%Δ$meq.ng^{-1}.ml^{-1}$).

Table 3.4: IGF axis and postnatal growth in the juvenile guinea pig

	Absolute Growth Rate					Fractional Growth Rate				
	Weight	***CRL***	***AC***	***HL***	***HW***	***Weight***	***CRL***	***AC***	***HL***	***HW***
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Post-mortem plasma										
Plasma IGF-I	0.62*	-0.11	0.10	-0.06	-0.18	-0.19	-0.48*	-0.15	-0.22	-0.24
Plasma IGFBP	-0.16	-0.14	-0.07	0.50*	0.61*	-0.12	-0.31	-0.18	0.33	0.55*
Plasma IGF-I:IGFBP	0.47*	-0.11	-0.05	-0.01	-0.20	-0.21	-0.43#	-0.21	-0.16	-0.24
Plasma IGF-II	0.42*	-0.34	0.11	-0.19	-0.07	-0.18	-0.59*	-0.03	-0.33	-0.15
Plasma IGF-II:IGFBP	0.33	-0.32	0.09	-0.24	-0.12	-0.28	-0.56*	-0.10	-0.38#	-0.21
Total plasma IGF	0.42*	-0.31	0.30	-0.06	-0.04	-0.32#	-0.54*	0.16	-0.22	-0.13
Total IGF:IGFBP	0.25	-0.27	0.20	-0.14	-0.10	-0.42*	-0.46*	0.01	-0.29	-0.20
IGF-I abundance										
IGF-I clearance ($\mu g.ml.ng^{-1}.kg^{-1}.min^{-1}$)	-0.04	0.41	-0.44#	0.30	-0.02	-0.15	0.36	-0.46#	0.32	-0.07
IGF-I secretion ($\mu g.kg^{-1}.min^{-1}$)	0.49*	0.05	0.12	-0.08	0.01	0.07	-0.14	-0.19	-0.16	-0.10

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. CRL refers to crown-rump length; AC to abdominal circumference; HL to head length and HW to head width.

Table 3.5: IGF-I sensitivity and postnatal growth in the juvenile guinea pig

	Absolute Growth Rate					Fractional Growth Rate				
	Weight	***CRL***	***AC***	***HL***	***HW***	***Weight***	***CRL***	***AC***	***HL***	***HW***
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
IGF-I sensitivity										
Glucose										
SSGIR (mg/min/kg)	-0.77**	0.34	0.23	0.13	0.16	-0.04	0.56*	0.59*	0.27	0.24
Adjusted SSGIR ($mg.ml.ng^{-1}.min^{-1}.kg^{-1}$)	-0.81**	0.49#	-0.07	0.21	0.16	-0.08	0.70*	0.42	0.41	0.28
Free Fatty Acids										
%Δ FFA (%Δ$meq.ng^{-1}.ml^{-1}$)	0.14	-0.33	-0.23	-0.08	-0.47#	-0.02	-0.31	-0.16	-0.14	-0.43#
Adjusted %Δ FFA (%Δ$meq.ng^{-2}.ml$)	0.12	-0.27	-0.25	-0.05	-0.46#	-0.02	-0.26	-0.17	-0.11	-0.42
Disposition Index										
Glucose (IGF-I x $IGFS_{glu}$)	-0.09	0.21	0.38	-0.02	0.16	0.02	0.23	0.39	0.02	0.11
Glucose (IGF-IxIGFS_{glu}Adj)	-0.77**	0.34	0.23	0.13	0.16	-0.04	0.56*	0.59*	0.27	0.24
FFA (IGF-I x $IGFS_{FFA}$%)	0.12	-0.27	-0.25	-0.05	-0.46#	-0.02	-0.26	-0.17	-0.11	-0.42
FFA (IGF-I x $IGFS_{FFA}$%Adj)	0.52*	-0.16	-0.24	-0.09	-0.42	0.02	-0.31	-0.42	-0.20	-0.46#

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. CRL refers to crown-rump length; AC to abdominal circumference; HL to head length and HW to head width. Units for basal disposition indices are as follows: Glucose (IGF-I x $IGFS_{glu}$) ($mg.ng.min^{-1}.kg^{-1}.ml^{-1}$); Glucose (IGF-IxIGF$S_{glu}$Adj) ($mg.ng^{2}.min^{-1}.kg^{-1}.ml^{-2}$); FFA (IGF-I x $IGFS_{FFA}$%) (%Δ$meq.ml^{-2}$); FFA (IGF-I x $IGFS_{FFA}$%Adj) (%Δ$meq.ng^{-1}.ml^{-1}$).

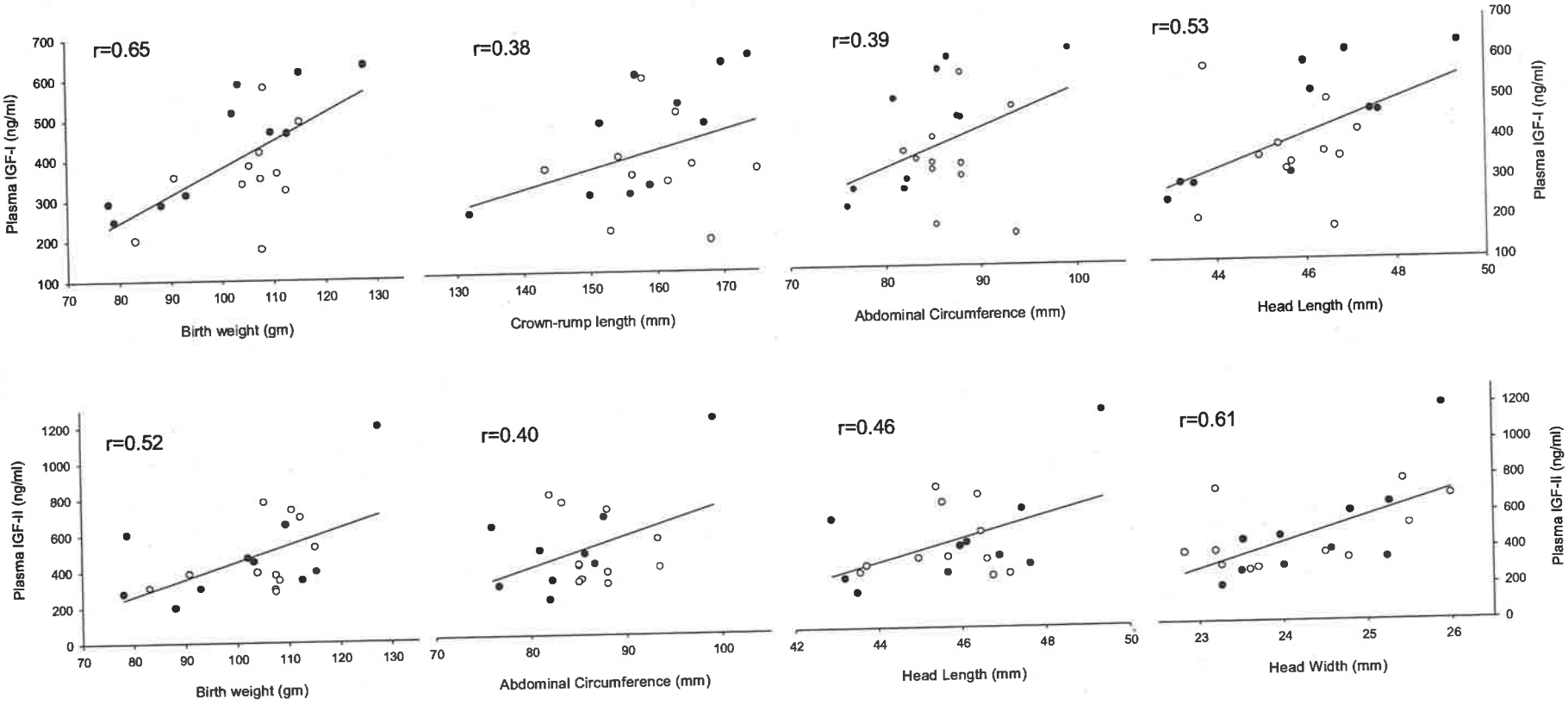

Figure 3.5: Plasma IGF-I and IGF-II concentrations and size at birth in the juvenile guinea pig

Males are represented by closed, and females by open circles. All correlations are significant at $p<0.05$.

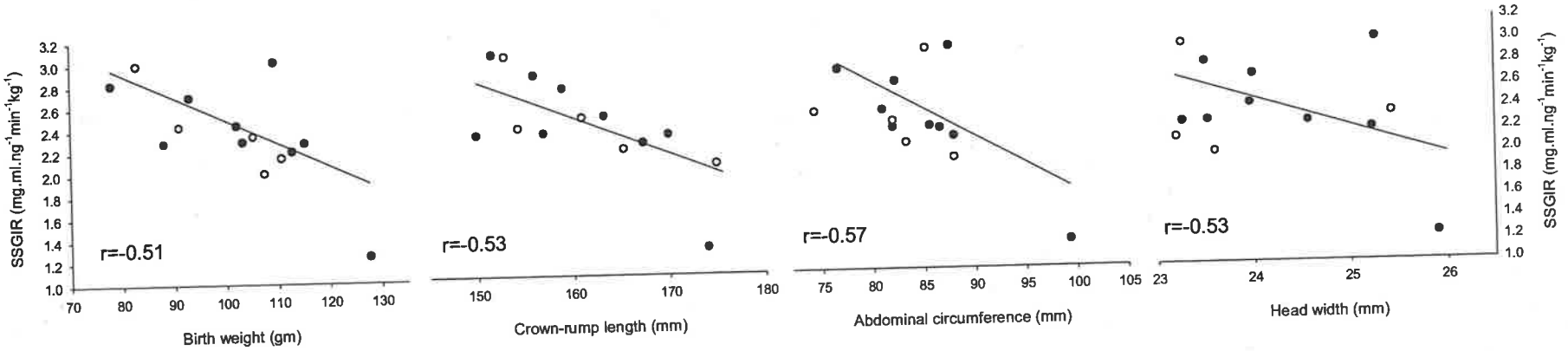

Figure 3.6: IGF-I sensitivity of glucose metabolism and size at birth in the juvenile guinea pig

Males are represented by closed, and females by open circles. IGF-I sensitivity of glucose metabolism is represented by corrected SSGIR ($*10^2$). All correlations are significant at $p<0.05$.

3.5 Discussion

This is the first study to our knowledge to examine the IGF-IGFBP system in the juvenile guinea pig, and its relationship to size at birth and subsequent postnatal growth. Importantly, this study has revealed how the IGF axis responds following times of prenatal restraint, as indicated by small size at birth, following spontaneous fetal growth restriction which shares many characteristics with human IUGR also resulting from a less favourable intra-uterine environment.

In the juvenile guinea pig, endogenous production of IGF-I and –II was decreased in animals small at birth, as indicated by lower circulating levels of both IGF-I and IGF-II under fasted conditions, and a reduced rate of IGF-I secretion. The apparent bioavailability of these growth factors appears to be decreased in LBW animals, as indicated by less IGF relative to IGFBPs.

LBW guinea pigs were more sensitive to the actions of IGF-I in terms of glucose metabolism. Not only were animals of the lowest birth weights more IGF sensitive in glucose metabolism, but also those that were smaller in length, abdominal circumference and head measures at birth. LBW animals had normal plasma glucose levels, with a tendency to have lower plasma FFA compared to HBW animals.

Increased fractional growth rates for length and abdominal circumference were also positively associated with increased IGF sensitivity of glucose metabolism, such that the animals displaying catch-up growth were more sensitive to IGF.

These results indicate that an adverse intrauterine environment and reduced substrate supply alters the IGF axis postnatally, and that catch-up growth in offspring that are small at birth is at least could be driven, at least in part, by an increased sensitivity to the actions of IGF. Whether decreased hormone abundance is driving the increase in sensitivity, or vice versa, remains to be established.

In the current study LBW guinea pigs have lower plasma IGF concentrations at 36 days of age, but plasma IGF is not related to postnatal catch-up growth. Similarly, in the human IUGR infant, plasma IGF-I is lower in IUGR infants compared to controls at 5 days of age postnatally (Thieriot-Prevost et al., 1988), and plasma IGF-I levels are reported as being reduced, or at best, normal, in the first few months of life and up to 1 year of age in the presence of catch-up growth in the human IUGR infant (Bennett et al., 1983; Giudice et al., 1995; Leger et al., 1996; Ogilvy-Stuart et al., 1998; Ozkan et al., 1999). However, serum IGF-II levels have been reported as being higher in IUGR infants undergoing catch-up growth, compared to those infants not catching up at 3 months of age (Garcia et al., 1996). In the current study, although plasma IGF-I levels were positively correlated with absolute growth rates, lower plasma IGF-I and –II concentrations were seen in the animals with increased fractional growth rates of length. Therefore, those animals growing faster in absolute terms have increased IGFs, but catch-up growth is not dependent on increased IGF concentrations. Although the low birth weight guinea pigs in this study have increased fractional growth rates of weight, they have not caught up to their normal birth weight counterparts at the time of IGF measurements, and are

more than likely in the midst of their catch-up growth phase (see Chapter 2). Therefore it appears that, in this cohort of animals, circulating IGF concentrations do not play a role in driving postnatal catch-up growth. It seems that the smaller guinea pigs have similar IGF axes profiles to human IUGR infants, as opposed to children who have completed their catch-up growth phase.

The IGFs are most often found complexed with IGF binding proteins (IGFBPs) in the circulation (Le Roith et al., 2001; Sara & Carlsson-Skwirut, 1986; Tanaka, 1996). Plasma IGFBP-3 levels are lower in SGA neonates at 5 days of age (Cance-Rouzaud et al., 1998), but higher in short IUGR children (age ~ 8 years) compared to short normal birth weight children (Cutfield et al., 2002). In the present study, apparent plasma IGFBP concentrations were decreased in low birth weight animals, however we cannot make the distinction between different forms of IGFBPs. Therefore, one IGFBP form may be increased, and another decreased, and the overall concentration would not alter significantly. However, we did observe a decrease in the ratio of total IGF to IGFBP, which suggests that although IGFBP concentrations are decreased in LBW animals, there is still more circulating binding protein in relation to IGFs. Recent studies have also suggested a role for the IGFBPs in growth, as transgenic mice over-expressing hIGFBP-5 have reduced birth weights, and they remain growth retarded. These mice show assymetrical growth retardation, with increased weights of brain and liver relative to body weight (Salih et al., 2004). In addition, in the current study plasma IGFBP levels did not correlate with fractional growth rates of weight or length. Similarly, plasma IGFBP-1 and IGFBP-3 levels are not different between

human IUGR neonates who undergo catch-up growth to 6-9 months of age, and those who don't (Ozkan et al., 1999). These results suggest that spontaneous fetal growth restriction in the guinea pig alters the expression of IGFBPs postnatally, but the level of IGFBP does not play a significant role in catch-up growth. Further study to elucidate which IGFBP forms are expressed differently is required.

The hyper-IGF-I-euglycemic clamp is a tool that is useful in determining an organisms' sensitivity of glucose metabolism to IGF-I, through measurement of the rate of glucose infusion needed to maintain blood glucose levels in response to elevation of circulating IGF-I by infusion of recombinant IGF-I. This is the first study to our knowledge to report IGF-I sensitivity of glucose metabolism in the juvenile guinea pig and the effect of size at birth, and provides us with valuable information on the activity of the IGF axis in this species. Juvenile guinea pigs with the lowest birth weights had increased sensitivity to IGF-I in terms of glucose metabolism, as determined by an increased glucose infusion rate over the last hour of the clamp. In addition, not only were the smallest animals in terms of weight more sensitive to the anabolic or metabolic actions of IGF-I, but also in those small in terms of length, abdominal circumference, and head measures. In the current study, we could not measure the exact sites of increased sensitivity of glucose metabolism to IGF-I. However, a previous study has shown that an infusion of 0.57nmol/kg/min (in the current study IGF-I was infused at a rate comparable to ~ 1.56nmol/kg/min) to rats results in a glucose metabolic rate (as measured by 2-deoxy-D-glucose uptake) at comparable levels in quadriceps, soleus and

diaphragm muscles (Moxley et al., 1990). However, the effect of IGF-I infusion at this dose was much lower in the liver (Moxley et al., 1990). This suggests that in the current study, the increased sensitivity to IGF-I may be in the skeletal muscle, however this is purely speculation at this point.

In the current study, infusion of IGF-I to weanling guinea pigs resulted in no change to plasma free fatty acid concentrations. Similarly, infusion of IGF-I to healthy human volunteers does not change plasma free fatty acid concentration (Russell-Jones et al., 1995), and plasma FFA levels remain unchanged when IGF-I is infused into the awake fasted rat (Jacob et al., 1989). In addition, no association between size at birth, catch-up growth and free fatty acid utilisation were seen. Interestingly, plasma IGF-II was suppressed in LBW animals following IGF-I infusion, suggesting a negative feedback mechanism when plasma IGF-I concentrations dramatically increase. IGF-I delivered to pigs at 4μg/h from day 3 to day 10 of postnatal age does not alter concentrations of IGF-II (Schoknecht et al., 1997), however this dose is much lower than the dose delivered in the current study. As IGF-I and IGF-II bind and act through the same receptor (Zapf et al., 1999), an increase in IGF-I will reduced binding of IGF-II to its receptor, and a negative feedback mechanism may exist when IGF-II levels increase in circulation.

Although no relationship was seen between FGR of weight and sensitivity of glucose metabolism to IGF-I, those animals with an increased FGR for length had increased IGF-I sensitivity to glucose metabolism measures, suggesting a possible role for catch-up growth in skeletal length. The IGFs stimulate bone

elongation, increasing chondrocyte proliferation and hypertrophy in vitro (Wang et al., 1999), and IGF-I stimulates proliferation of chicken embryo sternal chondrocytes and increases matrix production, as well as inducing a strong transient increase in levels of collagen production (Bohme et al., 1992). Therefore the IGF axis may play a greater role in skeletal (therefore bone growth) than in stimulating body weight gain in the juvenile guinea pig undergoing catch-up growth.

It may be possible that the increase in sensitivity to the actions of IGF-I seen in the LBW animals is due to a change in IGF receptor number or affinity in the individual tissues that are most IGF-I sensitive to glucose metabolism. Changes in erythrocyte IGF-I receptor affinity and number have been described in IUGR children aged 4-16 years. Receptor affinity is significantly reduced, whereas receptor number is increased, and in those children with these abnormalities, plasma IGF-I levels are significantly lower than in control children, and IUGR children that do not have a changed IGF-I receptor profile (Ducos et al., 2001). Further studies should therefore focus on the expression of IGF receptors in tissues that are most responsive to IGF-I and –II.

In conclusion, being small at birth leads to reduced circulating concentrations of IGFs and IGFBPs, in conjunction with increased sensitivity of glucose metabolism to the actions of IGF-I. We suggest that in utero programming of the IGF axis occurs in spontaneous fetal growth restriction of the guinea pig, and that this has profound effects on postnatal growth and its control by the IGF axis. Whether this programming of the IGF axis has long-term effects on an

animal's health and longevity are yet to be determined. Low serum IGF-I levels have been associated with the development of cardiovascular disease (Janssen 1998), whereas higher IGF-concentrations correlate with higher systolic blood pressures (Fall et al., 1995; Kajantie et al., 2003), increased fibrinogen levels, and insulin resistance (Kajantie et al., 2003). Therefore if the altered circulating IGF concentrations seen in the current study following IUGR persist into adult life, they may contribute to onset of adult disease.

Chapter 4

Circulating IGFs and sensitivity to IGF-I, and body composition in the juvenile guinea pig

4.1 Introduction

The insulin-like growth factors (IGFs) act on a range of tissues and organs in the body to induce proliferation and/or differentiation. As discussed in Chapter 3, small size at birth and its associated catch-up growth in infants and children are characterised by marked but somewhat variable alterations in the somatotropic and IGF axis. Previous studies have investigated the relationship between circulating IGFs and muscularity or adiposity in childhood and adult life. However, the link between intrauterine growth restriction, postnatal catch-up growth and body composition during catch-up growth has not been studied extensively, particularly in relation to what role the IGFs play in this series of events. In addition, very little is known about the relationship between IGF sensitivity and an organisms' body composition. Obesity appears to be a long-term consequence of fetal growth restriction (Rogers & Group., 2003), however the mechanisms involved need to be elucidated further. Therefore this study was undertaken to determine the effect of fetal growth restriction and catch-up growth on body composition during the latter, and what role the IGFs may play in determining both growth and body composition following fetal growth restriction in the juvenile guinea pig.

IUGR in human populations leads to a relative reduction in skeletal muscle mass, at birth (Yajnik et al., 2002) and in childhood and adolescence (Hediger et al., 1998; Singhal et al., 2003). In addition, early postnatal catch-up growth may independently predict risk of adiposity, with catch-up growth up to 2 years

of age predicting an increased body fat mass in children (Levy-Marchal et al., 2004; Ong et al., 2000).

The postnatal role of IGF-I has been more extensively characterised than that of IGF-II. However, both IGFs are the only known growth factors to stimulate both proliferation and differentiation in muscle cells. Addition of IGF-I to myotube cultures increases protein synthesis rates, and decreases protein degradation (Oksbjerg et al., 2004). Transgenic mice expressing a dominant negative IGF-I receptor specifically in skeletal muscle show growth retardation soon after birth, both in weight and length (Fernandez et al., 2002). In addition, the IGFBPs may affect the role that the IGFs play in muscle growth, as IGFBPs are produced by muscle cell cultures and may act to either inhibit or potentiate the effects of the IGFs (Oksbjerg et al., 2004).

Receptors for IGFs have been characterised in porcine adipose tissue, adipocytes and preadipocytes, as well as insulin/IGF-I hybrid receptors being present in 3T3-L1 preadipocytes (Louveau & Gondret, 2004). Growing adipocytes are thought to secrete growth factors that induce proliferation of preadipocytes, including the IGFs (Zizola et al., 2002). IGF-I increases preadipocyte replication and differentiation in stromal-vascular (S-V) cells (Marques et al., 2000), and promotes differentiation of ovine preadipocytes (Soret et al., 1999). The increase in IGF-I transcripts in primary cultures of preadipocytes treated with exogenous IGF-I suggests a positive feedback mechanism for IGF-I in differentiation (Boney et al., 1994). IGFBP-I may also regulate adipogenesis, with an increasing amount of IGFBP-I being associated

with a decrease in proliferation of porcine adipose tissue stem cells (Hausman et al., 2001).

Although various studies have investigated the long-term effects in terms of body composition following IUGR, very few researchers have looked at the factors linking IUGR to obesity later in life. Perturbations in the IGF system appears to be a potential candidate in providing the link between IUGR, catch-up growth and adverse adult outcomes, due to its defined role in growth of multiple tissue types. Therefore we investigated the relationship between plasma IGF concentrations, metabolic sensitivity to IGF-I and IGFBP abundance with vital organ size, muscularity and adiposity at 36 days of age in the juvenile guinea pig. We have reported that LBW animals have increased fractional growth rates, indicating the presence of a catch-up growth mechanism (Chapter 2), and that these animals have decreased plasma IGFs, yet increased sensitivity to IGF-I of glucose but not FFA (Chapter 3).

4.2 General Aim

To investigate the relationships between body composition and the IGF axis in the in the juvenile guinea pig.

Specific Aim 1:

To investigate the relationship between size and body composition including vital organ size, muscularity and adiposity, and the IGF axis in the juvenile guinea pig.

4.3 Materials and methods

4.3.1 Mating and housing of guinea pigs

Guinea pigs (IMVS tri coloured, Gilles Plains Resource Centre, SA) were caged individually in plastic tubs with wire lids, and maintained in a room with a 12:12h light:dark cycle at 25°C, with *ad libitum* access to guinea pig/rabbit ration (Ridley Agriproducts, Australia). Males used for mating were fed lucerne in addition to the guinea pig chow. All guinea pigs also had *ad libitum* access to tap water, containing 400mg/L vitamin C. Females and males were mated as described in Chapter 2.3.2. Upon birth, guinea pigs were classed as being low or high birth weight according to the mean birth weight of the cohort (102.3g).

4.3.2 Size at birth and postnatal growth measurements

A description of the total number of animals used in each experiment can be found in Appendix A, and total numbers of litters used is summarised in Appendix C. Guinea pigs were weighed at birth, and size in terms of weight, crown-rump length (CRL), abdominal circumference (AC), head width (HW), and head length (HL) measured at birth and subsequently at 5-day intervals (see Table 2.1). All pups were weaned at 25 days of age onto normal guinea pig chow/rabbit ration and fed *ad libitum* with access to water containing 400mg/L vitamin C. Absolute growth rates were recorded for each growth measurement for each guinea pig (AGR) by calculating the slope of the regression line of postnatal age and size, and then expressed in terms of

individual birth size to give a measure of catch up growth (fractional growth rate: FGR).

4.3.3 Insertion of vascular catheters and maintenance

At 28 ± 1 days of age, catheters were inserted into the external jugular vein and the right common carotid artery as described in Chapter 3.3.3. Catheterised pups were weighed daily. Catheters were flushed daily with 500µl heparinised saline (50µU/ml), and sealed with a clean plug.

4.3.4 Hyper-IGF-I-euglycaemic clamp and IGF-I sensitivity of glucose and FFA metabolism

At 32 ± 1 days of age, IGF-I sensitivity of glucose metabolism was measured by HIEC as described in Chapter 3.3.4. Blood glucose in the arterial blood samples taken during the hyper-IGF-I-euglycemic clamp was measured using a glucometer (Hemocue AB, Sweden). Plasma glucose concentrations were measured prior to and during the final 60 mins of the HIEC (t=-20, -10, 0, 70, 85, 100, 115, 120 minutes), using the COBAS MIRA automated sample system, as described in Chapter 3.3.10. Plasma free fatty acid (FFA) concentrations were measured prior to and during the final 60 mins of the HIEC (t=-20, -10, 0, 70, 85, 100, 115, 120 minutes). These measurements were performed using the COBAS MIRA, as described in Chapter 3.3.11.

4.3.5 Measurement of plasma IGF-I, IGF-II and IGFBP concentrations

Plasma IGF-I, -II and IGFBPs were separated as described in Chapter 3.3.5. Plasma IGF-I, IGF-II and IGFBP concentrations were measured by specific RIAs, as described in Chapters 3.3.6, 3.3.7 and 3.3.8.

4.3.6 Post mortem

Post-mortems were performed on 21 juvenile guinea pigs at 36 ± 2 days of age as described in Chapter 2.3.4.

4.3.7 Statistics

Associations between parameters were examined using Pearson's correlations analysis (SPSS Inc., Chicago). Unless otherwise stated, significance occurs at the level $p<0.05$.

4.4 Results

4.4.1 *Circulating IGFs, sensitivity to IGF-I and organ size in the juvenile guinea pig*

Juvenile body weight and length at 36 days of age were positively correlated with fasting plasma concentrations of IGF-I (weight r=0.73; length r=0.54), IGF-II (weight r=0.41; length r=0.50) and total IGFs (weight r=0.63; length r=0.62). Juvenile weight:length correlated positively with plasma IGF-I (r=0.58) and plasma total IGFs (r=0.47) (Table 4.1). Juvenile body weight (r=-0.60) and length (r=-0.64) correlated negatively with IGF-I sensitivity of glucose metabolism ($SSGIR_{adj}$), and juvenile body weight also correlated negatively with the adjusted glucose disposition index (DI) (r=-0.52; Table 4.1).

Current FGR_{CRL} correlated negatively with plasma IGF-II (r=0.53), and current FGR_{weight} correlated negatively with IGF-I sensitivity of glucose metabolism (Table 4.1).

Brain (r=0.44), kidney (r=0.49; Figure 4.1(a)) and heart (r=0.60) weights were positively correlated with plasma IGF-I. Kidney weight correlated positively with (r=0.38), and GIT weight (r=-0.71), and the relative weights of the adrenals (r=-0.37), brain (r=-0.45) and GIT (r=-0.74) correlated negatively with plasma IGF-II (Table 4.2). Kidney weight (r=0.50; Figure 4.1(b)) and heart weight (r=0.42), correlated positively with, and relative weights of the brain (r=-0.49) and GIT (r=-0.61) correlated negatively with plasma total IGFs (Table 4.2). GIT weight

correlated negatively with IGF-I sensitivity of glucose metabolism (r=-0.82). Brain weight correlated negatively with IGF-I sensitivity of FFA metabolism (adjusted %Δ FFA) (r=-0.52), and the adjusted FFA DI (r=-0.57; Table 4.2). Thyroid absolute (r=0.61) and relative (r=0.72) weight correlated positively with IGF-I sensitivity of FFA metabolism, and with FFA DI (r=0.63, r=0.74 respectively) (Table 4.2).

Table 4.1: Current size, current postnatal growth rates and plasma IGFs and metabolic sensitivity to IGF-I in the juvenile guinea pig

	Plasma IGF-I	***Plasma IGF-II***	***Plasma total IGFs***	***$SSGIR_{adj}$***	***Adjusted glucose DI***	***Adjusted %Δ FFA***	***Adjusted FFA DI***
Current size/growth	***r***	***r***	***r***	***r***			
Weight (g)	0.73**	0.41*	0.63**	-0.60*	-0.52*	-0.06	0.09
CRL (mm)	0.54*	0.50*	0.62*	-0.64*	-0.43#	0.13	0.36
Wt:CRL ($g.mm^{-1}$)	0.58*	0.34#	0.47*	-0.25	0.48#	-0.49#	-0.09
BMI ($g.mm^{-3}$)	0.27	0.04	0.13	0.14	0.42	-0.42	-0.18
FGR of weight	-0.06	0.04	-0.12	-0.48*	0.08	0.24	0.43#
FGR of CRL	-0.42#	-0.53*	-0.46#	0.60#	0.58#	-0.40	-0.34

** Denotes significance at $p<0.001$; *$p<0.05$; # $p<0.1$. DI is disposition index, %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; $SSGIR_{adj}$ is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I.

Table 4.2: Organ size, plasma IGFs and metabolic sensitivity to IGF-I in the juvenile guinea pig

	Plasma IGF-I	*Plasma IGF-II*	*Total plasma IGFs*	*$SSGIR_{adj}$*	*Adjusted glucose DI*	*Adjusted %Δ FFA*	*Adjusted FFA DI*
Organ	*r*	*r*	*r*	*r*			
Adrenal (g)	0.28	-0.19	-0.02	0.27	0.23	0.09	0.09
(%BW)	-0.07	-0.37*	-0.31#	0.46#	0.39#	0.14	0.15
Kidney (g)	0.49*	0.38*	0.50*	-0.40#	-0.35	-0.05	-0.06
(%BW)	-0.05	0.08	0.04	-0.05	-0.04	-0.03	-0.03
Pancreas (g)	0.28	-0.11	0.04	0.19	0.20	0.13	0.10
(%BW)	-0.03	-0.26	-0.21	0.34	0.32	0.14	0.12
GIT (g)	0.12	-0.71*	-0.43	-0.82*	-0.41	-0.21	-0.26
(%BW)	-0.18	-0.74*	-0.61*	-0.41	-0.11	-0.26	-0.29
Brain (g)	0.44*	-0.10	0.11	0.11	0.10	-0.52*	-0.57*
(%BW)	-0.33#	-0.45*	-0.49*	0.32	0.45#	-0.43#	-0.45#
Liver (g)	0.23	0.06	0.15	-0.27	-0.14	0.08	0.04
(%BW)	-0.28	-0.19	-0.26	0.06	0.09	0.08	0.06
Lungs (g)	0.28	-0.16	-0.01	-0.40	-0.41#	0.09	0.06
(%BW)	-0.12	-0.33#	-0.30#	-0.08	-0.14	0.18	0.17
Heart (g)	0.60*	0.20	0.42*	-0.44#	-0.28	0.37	0.29
(%BW)	0.31#	0.04	0.16	-0.27	-0.13	0.42#	0.34
Thyroid (g)	-0.04	0.29	0.21	0.06	-0.24	0.61*	0.63*
(%BW)	-0.35#	-0.14	-0.26	0.27	-0.07	0.72*	0.74*

** Denotes significance at $p<0.001$; *$p<0.05$; # $p<0.1$. EDL is extensor digitorum longus; %BW is weight expressed as a percentage of body weight; DI is disposition index, %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; $SSGIR_{adj}$ is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I.

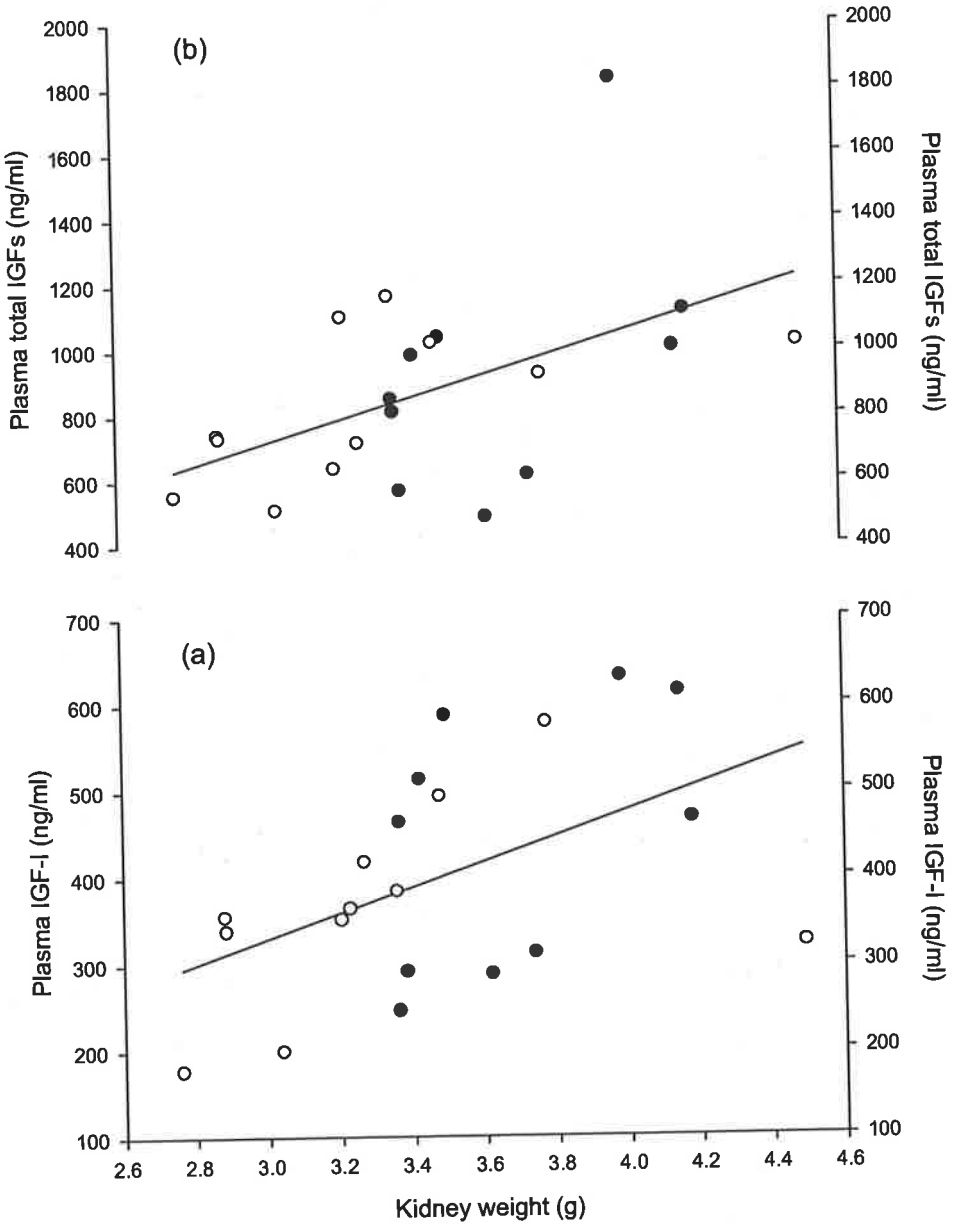

Figure 4.1: Kidney weight and plasma IGF concentrations in the juvenile guinea pig

Males are represented by closed, and females by open circles. Kidney weight was positively correlated with (a) plasma IGF-I ($r=0.49$) and (b) plasma total IGFs ($r=0.50$) ($p<0.05$ for each).

4.4.2 Organ size and circulating IGFBP and IGF:IGFBP ratios in the juvenile guinea pig

Juvenile body weight tended to correlate positively with plasma IGFBP concentrations ($p<0.1$). Absolute or relative weights of organs did not correlate with plasma IGFBP (Table 4.3).

Plasma IGF-I, IGF-II and total IGF concentrations were expressed as a ratio of their corresponding IGFBP concentration in the fasted state. This provides us with an index of circulating IGF bioavailability. Juvenile body weight ($r=0.58$) and length ($r=0.60$) correlated positively with plasma IGF-I:IGFBP. Kidney ($r=0.46$), brain ($r=0.48$) and heart weight ($r=0.73$), as well as with the relative weight of heart ($r=0.54$) correlated positively with plasma IGF-I:IGFBP. Juvenile body length ($r=0.46$) and kidney weight ($r=0.38$) correlated positively with plasma IGF-II:IGFBP (Table 4.3).

Juvenile body weight tended to correlate positively with plasma total IGF:IGFBP ($p<0.1$), but absolute or relative organ weights were not correlated with plasma total IGF:IGFBP (Table 4.3).

Current FGR_{CRL} was negatively correlated with IGF-II:IGFBP (Table 4.3).

Table 4.3: Organ size and circulating IGFBP concentrations, and IGF:IGFBP ratios in the juvenile guinea pig

	PLASMA			
	IGFBP	*IGF-I:IGFBP*	*IGF-II:IGFBP*	*Total IGF:IGFBP*
	r	*r*	*r*	*r*
Current size				
Weight (g)	0.30#	0.58*	0.26	0.30#
CRL (mm)	0.07	0.60*	0.46*	0.07
Wt:CRL ($g.mm^{-1}$)	0.37#	0.33	0.24	0.26
BMI ($g.mm^{-3}$)	0.32	-0.04	-0.04	-0.03
Current growth				
Current FGR_{weight}	0.25	-0.06	-0.03	-0.19
Current FGR_{CRL}	-0.09	-0.42	-0.56*	-0.46#
Organ				
Adrenal (g)	-0.16	0.26	-0.12	-0.16
(%BW)	-0.28	-0.05	-0.25	-0.28
Kidney (g)	-0.01	0.46*	0.38*	-0.01
(%BW)	-0.21	-0.01	0.19	-0.21
Pancreas (g)	0.04	0.31	-0.05	0.04
(%BW)	-0.05	-0.03	-0.17	-0.05
GIT (g)	-0.07	0.05	-0.47	-0.07
(%BW)	-0.19	-0.20	-0.47	-0.19
Brain (g)	-0.07	0.48*	-0.13	-0.07
(%BW)	-0.32#	-0.15	-0.35#	-0.32#
Liver (g)	-0.06	0.41*	0.07	-0.06
(%BW)	-0.26	-0.01	-0.10	-0.26
Lungs (g)	0.26	0.29	-0.29	-0.26
(%BW)	0.11	-0.04	-0.39#	0.11
Heart (g)	0.14	0.73**	0.15	0.14
(%BW)	0.12	0.54*	0.05	0.02
Thyroid (g)	0.11	-0.03	0.27	0.11
(%BW)	0.01	-0.35#	0.15	0.01

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. %BW refers to weight expressed as a percentage of total body weight.

4.4.3 Skeletal muscle mass, circulating IGFs and metabolic sensitivity to IGF-I in the juvenile guinea pig

Summed absolute muscle mass was positively correlated with plasma IGF-I (r=0.68; Figure 4.2(a)). Weights of all individual skeletal muscles, excluding plantaris were positively correlated with plasma IGF-I ($p<0.05$; see Table 4.4). In addition, the relative weight of EDL muscle was positively correlated with plasma IGF-I (r=0.38; Table 4.4).

Summed muscle weight tended to be positively associated with plasma IGF-II ($p<0.1$). Biceps femoris muscle absolute (r=0.53) and relative weight (r=0.38) were both positively correlated with plasma IGF-II. However, plantaris muscle absolute (r=-0.52), and relative weight (r=-0.65) was negatively correlated to plasma IGF-II (Table 4.4).

Summed muscle weight (r=0.53; Figure 4.2(b)), and gastrocnemious (r=0.38), biceps femoris (r=0.72), EDL (r=0.39) and diaphragm muscle (r=0.45) weights, and the relative weight of biceps femoris muscle (r=0.43) correlated positively with total plasma IGF (Table 4.4).

Summed muscle mass (r=-0.61; Figure 4.2(c)), and summed relative muscle mass (r=-0.41) were negatively correlated to metabolic sensitivity to IGF-I. Biceps femoris (r=-0.71) and vastus lateralis (r=-0.53) muscle weights, and relative weight of the biceps femoris muscle (r=-0.62) were negatively correlated with IGF-I sensitivity to glucose metabolism (Table 4.4). Summed muscle mass

(r=-0.51), absolute and relative weights of biceps femoris muscle (r=-0.70; r=-0.67 respectively), and weight of vastus lateralis muscle (r=-0.48) also correlated negatively with the adjusted glucose disposition index (DI) (Table 4.4).

Diaphragm muscle absolute and relative weight (r=-0.58; r=-0.62 respectively), and tibialis relative weight (r=-0.50) were negatively correlated, and biceps femoris relative weight positively correlated (r=0.52) with IGF-I sensitivity of FFA metabolism (Table 4.4). Measures of lean body mass did not correlate with the FFA disposition index.

Table 4.4: Skeletal muscle weights, metabolic sensitivity to IGFs and plasma IGFs in the juvenile guinea pig

	Plasma IGF-I	*Plasma IGF-II*	*Plasma total IGFs*	*$SSGIR_{adj}$*	*Adjusted glucose DI*	*Adjusted %Δ FFA*	*Adjusted FFA DI*
Muscle	*r*	*r*	*r*	*r*			
Plantaris (g)	0.03	-0.52*	-0.38*	0.12	0.16	-0.30	0.01
(%BW)	-0.18	-0.65*	-0.56*	0.20	0.21	-0.27	-0.03
Gastrocnemious (g)	0.53*	0.20	0.38*	-0.44#	-0.36	0.02	-0.19
(%BW)	0.04	-0.12	-0.07	-0.14	-0.11	0.05	-0.41#
Tibialis (g)	0.49*	-0.10	0.25	-0.28	-0.11	-0.47#	0.39
(%BW)	0.36#	-0.19	0.02	-0.14	0.02	-0.50*	0.44#
Semitendinosus (g)	0.45*	-0.10	0.19	-0.22	-0.11	-0.07	0.29
(%BW)	0.11	-0.27	-0.16	-0.02	0.09	-0.06	0.32
Biceps Femoris (g)	0.72*	0.53*	0.72**	-0.71*	-0.70*	0.31	0.21
(%BW)	0.33#	0.38*	0.43*	-0.62*	-0.67*	0.52*	0.30
Vastus Lateralis (g)	0.43*	0.07	0.24	-0.53*	-0.48*	-0.09	0.16
(%BW)	0.01	-0.21	-0.16	-0.30	-0.29	-0.06	0.19
EDL (g)	0.51*	0.22	0.39*	-0.32	-0.07	-0.41#	-0.06
(%BW)	0.38*	0.13	0.26	-0.21	0.05	-0.45#	-0.06
Biceps Brachii (g)	0.50*	0.07	0.27	-0.44#	-0.36	0.04	0.13
(%BW)	0.09	-0.22	-0.13	-0.25	-0.19	0.09	0.15
Diaphragm (g)	0.42*	0.35#	0.45*	-0.09	0.03	-0.58*	-0.34
(%BW)	-0.01	0.16	0.12	0.24	0.34	-0.62*	-0.47#
Summed Muscle (g)	0.68**	0.31#	0.53*	-0.61*	-0.51*	-0.02	0.13
(%BW)	0.28	0.04	0.15	-0.41*	-0.34	0.04	0.17

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. EDL refers to extensor digitorum longus; %BW refers to weight expressed as a percentage of total body weight. DI is disposition index, %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; $SSGIR_{adj}$ is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I.

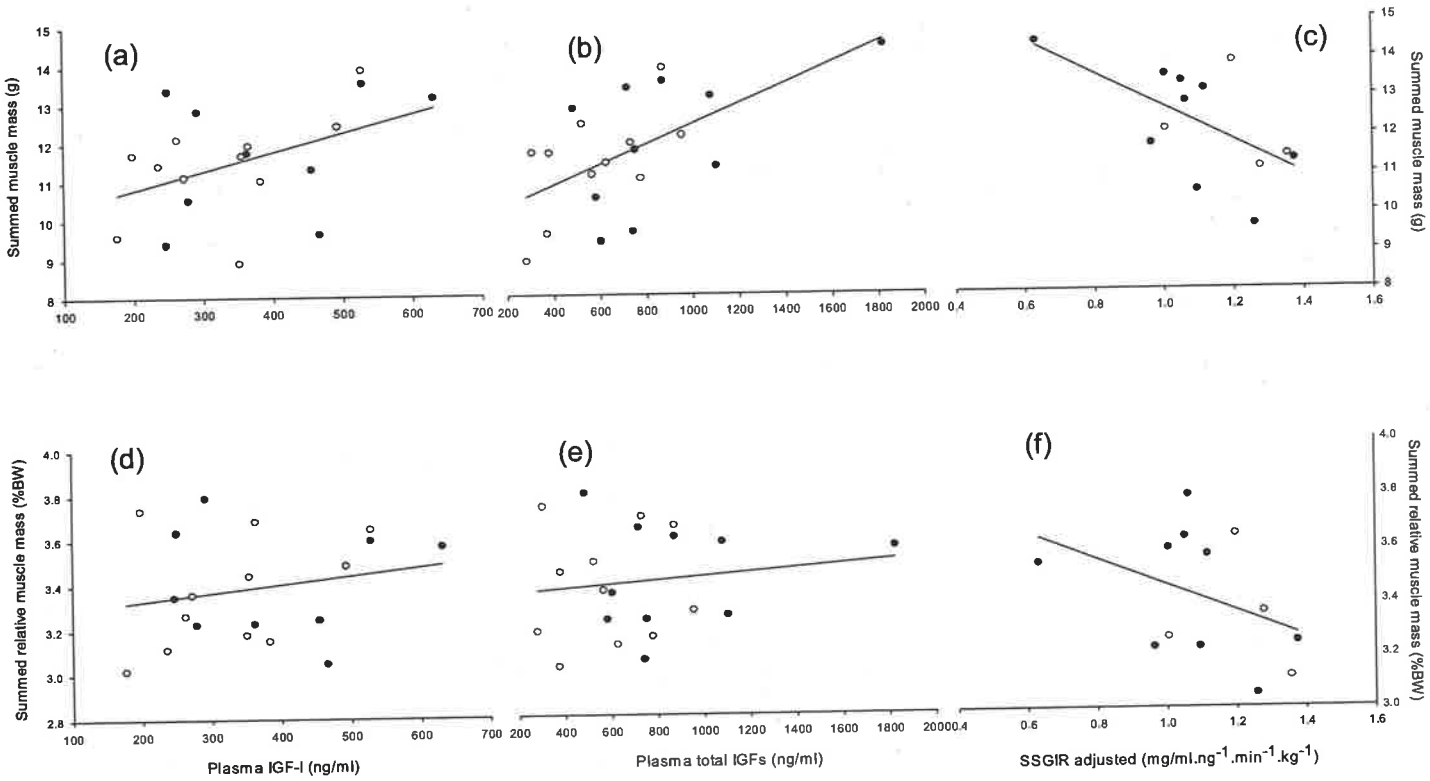

Figure 4.2: The IGF axis and skeletal muscle mass in the juvenile guinea pig

Males are represented by closed, and females by open circles. Summed muscle mass was positively correlated with (a) plasma IGF-I ($r=0.68$; $p<0.001$), (b) total plasma IGFs ($r=0.53$; $p<0.05$), and (c) SSGIR ($r=-0.61$; $p<0.05$). Summed relative muscle mass was not correlated with (d) plasma IGF-I ($r=0.28$), (e) plasma total IGFs ($r=0.15$), but was negatively correlated with SSGIR ($r=-0.41$; $p<0.05$).

4.4.4 Skeletal muscle mass, IGFBP concentrations and IGF:IGFBP ratios in the juvenile guinea pig

Summed muscle mass tended to correlate positively with plasma IGFBP ($p<0.1$). Gastrocnemious ($r=0.40$), biceps femoris ($r=0.50$) and biceps brachii ($r=0.38$) muscle weights, as well as with the biceps femoris muscle relative weight ($r=0.44$) were positively correlated with plasma IGFBP (Table 4.5).

Summed muscle mass ($r=0.45$) was positively correlated with plasma IGF-I:IGFBP. Tibialis ($r=0.64$) and biceps femoris ($r=0.50$) muscle weights, as well as tibialis relative weight ($r=0.47$) were positively correlated with plasma IGF-I:IGFBP (Table 4.5).

In contrast, plantaris muscle absolute ($r=-0.63$) and relative ($r=-0.73$) weight, as well as biceps brachii muscle relative weight ($r=-0.41$) were negatively correlated with plasma IGF-II:IGFBP (Table 4.5).

Plantaris muscle weight ($r=-0.51$) and relative weight ($r=-0.66$) were negatively correlated with plasma total IGF:IGFBP. Biceps femoris ($r=0.46$) and diaphragm ($r=0.38$) muscle weights were also positively correlated with plasma total IGF:IGFBP (Table 4.5).

Table 4.5: Skeletal muscle mass, plasma IGFBP and IGF:IGFBP ratios in the juvenile guinea pig

	PLASMA			
	IGFBP	***IGF-I:IGFBP***	***IGF-II:IGFBP***	***Total IGF:IGFBP***
Muscle	***r***	***r***	***r***	***r***
Plantaris (g)	0.09	-0.16	-0.63**	-0.51*
(%BW)	0.03	-0.32#	-0.73**	-0.66**
Gastrocnemious (g)	0.40*	0.28	-0.01	0.11
(%BW)	0.29	-0.19	-0.28	-0.29
Tibialis (g)	-0.04	0.64*	-0.03	0.26
(%BW)	-0.18	0.47*	-0.16	0.09
Semitendinosus (g)	0.33#	0.27	-0.12	0.04
(%BW)	0.26	0.02	-0.34#	-0.25
Biceps Femoris (g)	0.50*	0.50*	0.31#	0.46*
(%BW)	0.44*	0.16	0.19	0.20
Vastus Lateralis (g)	0.37#	0.30	-0.15	-0.04
(%BW)	0.25	0.05	-0.38#	-0.37#
EDL (g)	0.22	0.36#	0.08	0.24
(%BW)	0.15	0.25	0.02	0.15
Biceps Brachii (g)	0.38*	0.40#	-0.15	0.01
(%BW)	0.29	-0.01	-0.41*	-0.37#
Diaphragm (g)	0.14	0.14	0.30	0.38*
(%BW)	0.06	-0.26	0.20	0.16
Summed Muscle (g)	-0.38#	0.45*	0.10	0.27
(%BW)	-0.24	0.04	-0.16	-0.11

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. EDL refers to extensor digitorum longus; %BW refers to weight expressed as a percentage of total body weight.

4.4.5 Adiposity and the IGF axis in the juvenile guinea pig

Summed absolute and relative body fat correlated positively with plasma IGF-I (r=0.55, Figure 4.3(a) and r=0.40 respectively) and plasma total IGFs (r=0.55, Figure 4.3(c) and r=0.44 respectively). Summed fat mass was positively correlated with plasma IGF-II (r=0.41, Figure 4.3(b)) (Table 4.6). Summed muscle:fat correlated negatively with plasma IGF-I (r=-0.46), plasma IGF-II (r=-0.41) and plasma total IGFs (r=-0.56) (Table 4.6).

Shoulder fat (r=0.59), retroperitoneal fat (r=0.57), perirenal fat (r=0.44), epididymal fat (r=0.62) and groin fat (r=0.38) weights correlated positively with plasma IGF-I (Table 4.5). Epididymal fat (r=0.66) and groin fat (r=0.65) weights correlated positively with plasma IGF-II. Relative weights of shoulder fat (r=0.44) and retroperitoneal fat (r=0.48) correlated positively with plasma IGF-I, and relative weights of epididymal fat (r=0.57) and groin fat (r=0.56) correlated positively with plasma IGF-II (Table 4.6).

Shoulder fat (r=0.48), parametrial fat (r=0.54), retroperitoneal fat (r=0.44), epididymal fat (r=0.74) and groin fat (r=0.65) weights were positively correlated with total plasma IGF concentration (Table 4.5). Relative groin fat weight was positively correlated with plasma total IGF (r=0.53) (Table 4.6).

Summed fat mass tended to correlate negatively with IGF-I sensitivity of glucose metabolism ($p<0.1$). Groin fat weight (r=-0.59) and relative weight (r=-0.50) were negatively correlated with IGF-I sensitivity of glucose metabolism (Table 4.6). Dorsal fat weight (r=-0.50), and absolute and relative weights of

groin fat (r=-0.61, r=-0.54 respectively) were negatively correlated with the adjusted glucose DI.

Dorsal fat absolute (r=0.70) and relative weight (r=0.74) correlated positively with IGF-I sensitivity of FFA metabolism. Neck fat absolute and relative weights tended correlate positively with IGF-I sensitivity of FFA metabolism ($p<0.1$; Table 4.6).

Summed absolute and relative adipose tissue weights (r=0.57 and r-0.54 respectively) were positively correlated with plasma IGFBP. Dorsal fat (r=0.56), shoulder fat (r=0.42), retroperitoneal fat (r=0.50), perirenal fat (r=0.42) and groin fat (r=0.38) weights, as well with relative weights of dorsal fat (r=0.52), shoulder fat (r=0.40), retroperitoneal fat (r=0.50) and perirenal fat (r=0.41) were positively correlated with plasma IGFBP (Table 4.7).

Measures of adiposity were not related to plasma IGF-I:IGFBP (Table 4.6). Groin fat weight and relative weight (r=0.49; r=0.44 respectively) and epididymal fat weight and relative weight (r=0.76; r=0.84 respectively) correlated positively with plasma IGF-II:IGFBP (Table 4.7).

Epididymal fat weight (r=0.82) and parametrial fat weight (r=0.65), and their relative weights (r=0.60; r=0.81) respectively were positively correlated with plasma total IGF:IGFBP. Groin fat weight was positively correlated with plasma total IGF:IGFBP (r=0.46) (Table 4.7).

Table 4.6: Adiposity, metabolic sensitivity to IGF-I and plasma IGFs in the juvenile guinea pig

	Plasma IGF-I	***Plasma IGF-II***	***Total plasma IGFs***	***$SSGIR_{adj}$***	***Adjusted glucose DI***	***Adjusted %Δ FFA***	***Adjusted FFA DI***
Adipose depot	***r***	***r***	***r***	***r***			
Dorsal Fat (g)	0.40*	0.26	0.37#	-0.26	-0.50*	0.70*	-0.05
(%BW)	0.20	0.14	0.19	-0.11	-0.37	0.74*	-0.10
Shoulder Fat (g)	0.59*	0.30#	0.48*	-0.24	-0.30	0.01	-0.07
(%BW)	0.44*	0.20	0.35#	-0.11	-0.20	0.06	-0.08
Neck Fat (g)	0.15	0.16	0.19	-0.12	-0.34	0.48#	-0.14
(%BW)	-0.02	0.10	0.07	-0.01	-0.25	0.51#	-0.11
Parametrial Fat (g)	0.33	0.46#	0.54*	-0.88#	-0.56	-0.03	0.23
(%BW)	0.16	0.40	0.41	-0.83#	-0.59	0.17	0.36
Retroperitoneal Fat (g)	0.57*	0.26	0.44*	-0.13	-0.16	0.13	-0.03
(%BW)	0.48*	0.20	0.36#	-0.02	-0.09	0.19	-0.02
Perirenal Fat (g)	0.44*	0.15	0.31#	-0.16	-0.14	-0.02	-0.01
(%BW)	0.31#	0.07	0.19	-0.05	-0.04	0.02	-0.03
Epididymal Fat (g)	0.62*	0.66*	0.74*	-0.24	-0.19	-0.04	0.10
(%BW)	0.26	0.57*	0.53#	0.08	0.09	-0.12	0.02
Groin Fat (g)	0.38*	0.65**	0.66*	-0.59*	-0.61*	0.22	0.09
(%BW)	0.24	0.56*	0.53*	-0.50*	-0.54*	0.28	0.12
Summed Fat (g)	0.55*	0.41*	0.55*	-0.38#	-0.47#	0.30	0.03
(%BW)	0.40*	0.34#	0.44*	-0.24	-0.37	0.38	0.02
Summed muscle:fat (g)	-0.46*	-0.41*	-0.56*	0.24	-0.03	-0.42#	-0.31

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. %BW refers to weight expressed as a percentage of total body weight. DI is disposition index, %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; $SSGIR_{adj}$ is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I.

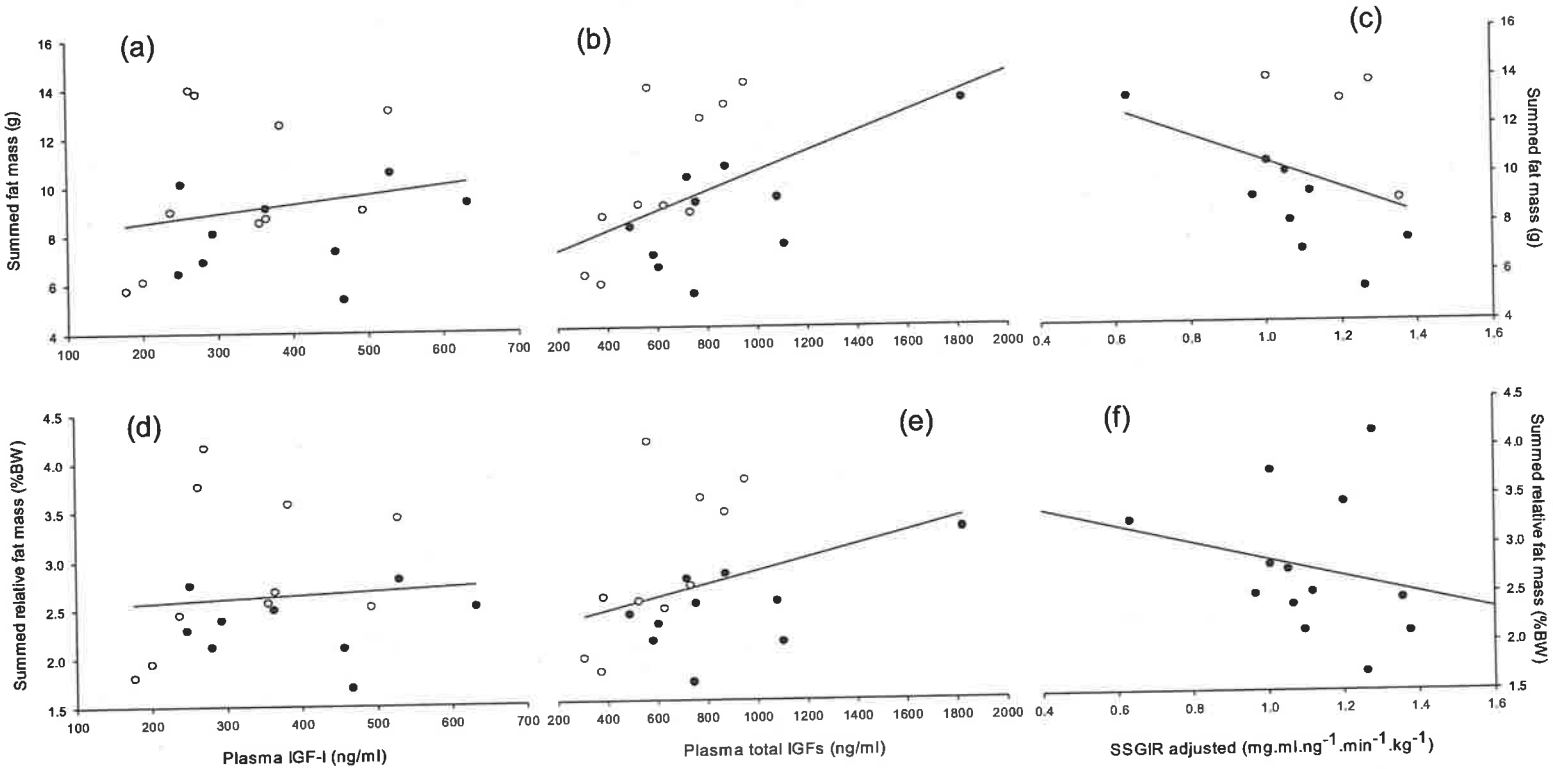

Figure 4.3: Adiposity and the IGF axis in the juvenile guinea pig

Males are represented by closed, and females by open circles. Summed fat mass was positively correlated to (a) plasma IGF-I ($r=0.55$; $p<0.05$), (b) total plasma IGFs ($r=0.55$; $p<0.05$), and tended to correlate negatively with (c) SSGIR ($r=-0.38$; $p<0.1$). Summed relative fat mass was not correlated with (d) plasma IGF-I or (f) SSGIR, but was positively correlated with (e) plasma total IGFs ($r=0.44$; $p<0.05$).

Table 4.7: Adiposity, plasma IGFBP and plasma IGF:IGFBP ratios in the juvenile guinea pig

	PLASMA			
	IGFBP	***IGF-I:IGFBP***	***IGF-II:IGFBP***	***Total IGF:IGFBP***
Adipose depot	***r***	***r***	***r***	***r***
Dorsal Fat (g)	0.56*	0.23	0.03	0.07
(%BW)	0.52*	0.06	-0.07	-0.08
Shoulder Fat (g)	0.42*	0.35#	0.11	0.24
(%BW)	0.40*	0.17	0.02	0.11
Neck Fat (g)	0.26	0.06	0.06	0.05
(%BW)	0.20	-0.07	0.03	-0.03
Parametrial Fat (g)	-0.06	0.35	0.48#	0.65*
(%BW)	-0.16	0.34	0.49#	0.60*
Retroperitoneal Fat (g)	0.50*	0.38#	0.07	0.22
(%BW)	0.50*	0.30	0.02	0.14
Perirenal Fat (g)	0.42*	0.33#	-0.02	0.10
(%BW)	0.41*	0.22	-0.10	-0.01
Epididymal Fat (g)	0.01	0.46	0.76*	0.82*
(%BW)	-0.27	0.12	0.84**	0.81*
Groin Fat (g)	0.38*	0.27	0.49*	0.46*
(%BW)	0.34#	0.17	0.44*	0.37#
Summed Fat (g)	0.57*	0.34	0.17	0.26
(%BW)	0.54*	0.21	0.13	0.16
Sum muscle:fat (g)	-0.46*	-0.32	-0.24	-0.25

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. %BW refers to weight expressed as a percentage of total body weight.

4.5 Discussion

We have shown that juvenile guinea pigs of larger size at 36 days of age have increased circulating concentrations of IGF-I and –II. Increased current fractional growth rate (at 36 days of age) of weight is associated with decreased IGF-I sensitivity of glucose metabolism, and an increased current FGR of crown-rump length is associated with decreased plasma IGF-II. Increased plasma IGF levels are associated with an increase in summed skeletal muscle mass, as are increased plasma ratios of IGF-I:IGFBP. An increased IGF-I sensitivity of glucose metabolism is associated with increased muscle mass relative to juvenile body weight, whereas an increased IGF-I sensitivity of FFA metabolism appears to be associated with decreased muscle growth.

Increased plasma IGF levels are also associated with increased summed fat mass, and in particular with an increased relative summed fat mass. Increased adiposity (absolute and relative) of selected adipose depots is associated with a decreased IGF-I sensitivity of glucose metabolism, whereas increased adiposity is associated with increased IGF-I sensitivity of FFA metabolism. This is the first study to investigate the relationship of size and body composition to metabolic sensitivity of IGF-I, as well as circulating IGFs, in the juvenile guinea pig.

The increased plasma IGF concentrations seen in the largest animals at 36 days of age agrees with previous studies in which plasma IGF-I concentrations are highest in heavy and tall children at 4 and 7 years of age (Fall et al., 1995), and IGF-I correlates positively with height in infants (Juul et al., 1995). The most

likely source of these circulating IGFs are the liver and adipose tissue, with expression being increased in these tissues, compared to others, in young adult female guinea pigs (Sohlstrom et al., 1998). In addition, the large size in the juvenile guinea pig was characterised by increased plasma IGF-I:IGFBP, which is probably a reflection of increased plasma IGF-I levels as those of plasma IGFBP did not differ with size in the juvenile guinea pig. Plasma IGFBP-3 is higher in short pre-pubertal children with IUGR (Cutfield et al., 2002), and the IGF-I:IGFBP-3 ratio is inversely related to adult ponderal index, a relationship which strengthens with age (Ben-Shlomo et al., 2003). We have not assayed for specific binding proteins, and the IGFBP concentration we measure reflects interference in the RIA by all binding proteins, therefore we cannot comment on the effects of size on individual binding protein abundance and their ratios to IGFs.

Current fractional growth rate of length was negatively correlated with plasma IGF-II. It was previously reported in the current study that an increased fractional growth rate calculated in terms of birth weight was also associated with decreased plasma IGF-II (see Chapter 3). It is interesting that an increased current fractional growth rate of weight is associated with a decreased IGF-I sensitivity of glucose metabolism, as in Chapter 3 we reported that increased birth fractional growth rates were associated with increased sensitivities of glucose metabolism to IGF-I. It has recently been shown in the neonatal lamb, following placental restriction, that current fractional growth rates are associated with increased IGF-I sensitivity of glucose and FFA metabolism (De Blasio, 2004). The mechanism behind this discrepancy is currently unclear, but it is

possible that size at 36 days of age in the juvenile guinea pig is not an indicator of metabolic sensitivity to IGF-I, as birth weight is.

A large kidney absolute and relative weight was associated with increased plasma IGFs. This is of particular interest because the kidney is a known target of IGF-I and its actions (Monzavi & Cohen, 2002) and although the liver is the major site of IGF synthesis, the kidney is likely to produce small amounts (O'Dell & Day, 1998). Experiments in animals have shown that administration of IGF-I promotes cellular hypertrophy and hyperplasia (Rabkin & Schaefer, 2004), and increases renal plasma flow, glomerular filtration rate (GFR) and proximal and distal tubular reabsorption of fluid and sodium (Monzavi & Cohen, 2002; Rabkin & Schaefer, 2004; Zapf et al., 1999). In the early stages of diabetes in the human, kidney hypertrophy is observed, and renal blood flow and GFR increase, suggesting a role for IGF-I in the development of diabetic glomerular disease (Rabkin & Schaefer, 2004). Therefore the increased plasma IGF concentrations observed in juvenile guinea pigs of increased birth weight (see Chapter 3) may be a precursor to the development of adult diabetes. On the other hand, the observed association between circulating IGFs and kidney size may be due to the effect of decreased plasma IGF concentrations inhibiting kidney growth. Although we do not know the expression of IGFs in fetal life in these guinea pigs, the kidney does continue growth after birth, and therefore the decreased plasma IGFs seen in postnatal life may still effect kidney growth to an extent. IGFBPs also have profound effects on the kidney, delivering IGF-I to its target cells, and exerting IGF-independent effects (Rabkin & Schaefer,

2004), however in the present study we did not observe any significant association between IGFBPs and kidney size.

In the current study, increased plasma IGFs predicted increased adipose tissue mass, in relative to body weight terms. We have already observed that the animals with the highest birth weights have increased plasma IGF concentrations (Chapter 3), and high birth weight also predicts an increase in adipose tissue mass (Chapter 2). Therefore the significant correlation between plasma IGFs and body composition was expected in light of other findings in this cohort. IGF-I does influence adipocyte proliferation, increasing preadipocyte replication and differentiation in S-V adipocyte cells, and promoting differentiation of ovine preadipocytes (Soret et al., 1999). In 7- and 8-year old children, IGF-I levels are correlated with increased percentage of body fat and fat-free soft tissue (Garnett et al., 2004). Therefore it appears that in pre-pubertal juvenile guinea pigs, IGFs play a significant role in increasing adipose tissue mass, as is observed in pre-pubertal human subjects. Negative associations between plasma IGF-I and BMI, WHR and abdominal fat content have been reported in adults (Juul, 2003), and therefore these associations between the IGF axis and adiposity may only be transient, and change following puberty.

Increased plasma IGFs also predicted increased skeletal muscle mass, in relative terms, although the increase in adipose tissue appeared to be greater, due to the association between decreased summed muscle:fat ratio and plasma IGFs. The IGFs stimulate proliferation and differentiation in muscle cells

(Oksbjerg et al., 2004), and IGF-I stimulates glucose and amino-acid uptake by muscle, and also increases protein synthesis rate in humans (Turkalj et al., 1992) and pigs (Davis et al., 2002). LBW guinea pigs have reduced muscle mass (Chapter 2) and reduced plasma IGFs (chapter 3). The association between IUGR, low plasma IGFs and reduced skeletal muscle mass suggests that the IUGR associated with a relative paucity of muscle mass in postnatal life in several populations (Li et al., 2003; Yajnik, 2001) may be partly due to a reduction in circulating IGFs postnatally, inhibiting muscle growth.

In this study, there was also a tendency for an increase in IGFBP to be associated with increasing muscle weight. The IGFBPs have been suggested to have a role in muscle growth, as IGFBPs are produced by muscle cell cultures (Oksbjerg et al., 2004). Depending on the muscle cell type, different IGFBPs are produced. For example, human muscle cells produce IGFBP-2, -3 and –4 (Crown et al., 2000), whereas porcine myoblast cells produce IGFBP-3 and –5 (Johnson et al., 2003). However it is not known whether the IGFBPs act to inhibit or potentiate the effects of the IGFs in muscle cells (Oksbjerg et al., 2004). In this study, it appears that IGFBPs may play a role in muscle growth, which may support the notion that these binding proteins have individual roles in endocrine functions, in addition to their actions on IGFs.

IGFBP-I may regulate adipogenesis, with an increasing amount of IGFBP-I being associated with a decrease in proliferation of porcine adipose tissue stem cells (Hausman et al., 2001). IGFBP-2 mRNA is decreased in differentiating preadipocyte primary cultures, whereas IGFBP-3 and –4 mRNA do not change,

suggesting that the modulation of IGF-I by these binding proteins does not relate specifically to adipogenesis (Boney et al., 1994). In contrast, in the present study we observed increased plasma IGFBP and summed absolute and relative adipose tissue mass in the juvenile guinea pig. However, the IGFBP is measured as interference in the IGF-I radioimmunoassay and does not differentiate between different binding protein types. Therefore, in the juvenile guinea pig, increasing adiposity is associated with an increase in plasma IGFBP concentrations, but further analysis needs to be carried out to determine which binding proteins are upregulated.

In contrast to the effects of plasma IGFs on body composition, IGF-I sensitivity of glucose metabolism was associated with reduced muscle mass, as was IGF-I sensitivity of FFA metabolism. Low birth weight in these guinea pigs leads to increased IGF-I sensitivity of glucose metabolism (Chapter 3), and these animals have increased fractional growth rates, however at the age of post-mortem (36 days), these animals have not completed their catch-up growth period, and are still smaller than their high birth weight counter parts (Chapter 2). Therefore the increased metabolic sensitivity to IGF-I that is observed may have long-term effects on body composition that are not apparent at this early age.

IGF-I sensitivity of glucose metabolism was also associated with reduced adipose tissue mass. Although IGF-I sensitivity of FFA metabolism was not associated with absolute or relative weights of most adipose organs, a positive relationship with weight of dorsal fat and neck fat was apparent. Therefore,

increased IGF-I sensitivity of FFA metabolism may predict increased adiposity in the juvenile guinea pig. The lack of significance seen with other fat mass depots may be due to the low number of animals studied (n=14), reducing statistical power.

In conclusion, increased absolute and relative skeletal muscle and adipose tissue mass is associated with increased plasma IGF concentrations in the juvenile guinea pig, however IGF-I sensitivity of glucose metabolism predicts reduced lean and adipose tissue mass, indicative of effects of spontaneous fetal growth restriction on body composition at this early age. Although IGF-I has been implicated in muscle and adipose tissue growth, these effects observed probably reflect a positive relationship between current size and plasma IGFs, as opposed to a specific effect on muscularity and adiposity. The long-term effects of the increased metabolic sensitivity to IGF-I and decreased plasma IGFs seen in low birth weight animals on body composition remain to be elucidated.

Chapter 5

Spontaneous fetal growth restriction, catch-up growth and the thyroid hormone and HPA axes

5.1 Introduction

Intrauterine growth restriction results in altered postnatal growth during infancy (Karlberg et al., 1997), however the physiological basis for this catch-up growth is unknown. The major endocrine regulators of early postnatal infant growth include the TH and HPAA axes (Fowden, 1995), which also interact in postnatal catch-up growth. The association of IUGR with catch-up growth suggests that an altered intrauterine environment is the main cause of altered postnatal development. There is evidence in humans and other species for alterations in both these axes, at least in children and certainly adults following IUGR.

Thyroid hormone (TH) exists in two main forms, triiodothyronine (T_3) and thyroxine (T_4), both being α-amino acid derivatives of tyrosine (Lazar, 1993). The effect of IUGR on postnatal thyroid hormone status is largely unknown. However, postnatally, total and free plasma T_3 and T_4 are reduced in very low birth weight babies (Kilby et al., 1998; Martin et al., 2001). In SGA children (mean age 6.7 years) who have shown full catch-up growth, plasma TSH is significantly higher compared to AGA children. Plasma free T_3 is also higher in these children, however plasma rT_3, free T_4, and free T_3:rT_3 ratio are similar between groups (Radetti et al., 2004).

The placentally restricted neonatal lamb, which is hypothyroid in utero, has elevated plasma T_4 levels in the first 24h following birth (Mellor & Pearson, 1977), but it is not known if these persist. In the guinea pig, IUGR caused by

uterine artery ligation at 28-30 days gestation does not result in any change to plasma total T_3 or T_4 in offspring at 8 weeks of age (Briscoe et al., 2004).

The HPA axis may be programmed through the prenatal environment, leading to altered HPAA function postnatally (Challis et al., 2001; Matthews, 2002). Urinary cortisol/creatinine ratios are also higher in IUGR infants than controls when measured from 5-20 weeks postnatally (Jackson et al., 2004). Plasma cortisol levels are inversely associated with birth weight in children who were born IUGR, and currently aged ~ 9 years. Cortisol is lowest in those children exhibiting catch-up growth (corrected height > 0 z-score), compared to those children without catch-up growth (corrected height < 0 z-score) (Cianfarani et al., 2002). In addition, a recent study has shown children with higher plasma cortisol/cortisone ratios have slower growth rates between 0 and 12 years of age, compared to those children that have lower ratios (Tenhola et al., 2005), suggesting an inhibitory role for cortisol in catch-up growth.

Under-nutrition for 10 days in late gestation in the sheep reduces baseline plasma cortisol concentrations in the offspring at 30 months of age compared to ad libitum fed controls (Bloomfield et al., 2003). Maternal protein deprivation in rats does not have any long-term effects on plasma cortisol levels in adult male offspring, with no differences in basal plasma corticosterone secretory patterns between IUGR and control groups (Nolan et al., 2001). Bilateral uterine artery ligation in the guinea pig at 28-30 days gestation causes a 100% increase in plasma cortisol in 8-week old offspring (Briscoe et al., 2004).

Therefore, the actions of cortisol in pre- and post-natal life has been well characterised in humans and other species. In general, IUGR is associated with increased cortisol levels postnatally, however in the presence of catch-up growth, cortisol levels are reduced. The effect of IUGR on postnatal HPAA function has been less extensively studied, and in particular circulating cortisol during postnatal catch-up growth in the IUGR guinea pig has not been defined. In addition, few studies have focused on the role of the thyroid hormone axis during postnatal catch-up growth following IUGR. The HPA axis and thyroid hormone axis are related, as cortisol is the major hormone that stimulates the deiodination of T_4 to T_3, leading to a prepartum rise in T_3 (Forhead et al., 2000). Therefore it is of great interest as to what role each of these axes plays in postnatal catch-up growth, and whether they are closely correlated with each other during this period. In this study we have measured free and total thyroid hormones in fasted plasma samples in the juvenile guinea pig. In addition, we have measured salivary cortisol levels from birth to post-mortem at day 36. Various studies have reported the significant correlation between plasma and salivary cortisol in humans (Aardal & Holm, 1995), and guinea pigs (Fenske 1997). Salivary cortisol is lower than plasma levels because 11-hydroxysteroid is present in salivary glands, which metabolises cortisol to cortisone (Aardal & Holm, 1995). Measuring salivary cortisol has experimental advantages over that of plasma cortisol because of the lack of corticosteroid-binding globulin in saliva, therefore cortisol measured in saliva reflects the biologically active free fraction (Fenske 1997). This study is unique as salivary cortisol has never been measured during the catch-up growth period in the juvenile guinea pig, nor has the plasma concentrations of thyroid hormone been reported during this period

following IUGR in this species. In addition, we have characterised the relationships of salivary cortisol and thyroid hormones to size, body composition and catch-up growth in the juvenile guinea pig.

5.2 General Aim

To investigate the role that cortisol and thyroid hormones play in postnatal catch-up growth following IUGR in the juvenile guinea pig.

Specific Aim 1:

To characterise circulating plasma thyroid hormones and their associations with size at birth, current postnatal growth rates and body composition in the juvenile guinea pig.

Specific Aim 2:

To characterise salivary cortisol concentrations and their associations with size at birth, current postnatal growth rates and body composition in the juvenile guinea pig.

Specific Aim 3:

To characterise the associations between salivary cortisol and plasma thyroid hormone concentrations in the juvenile guinea pig.

5.3 Materials and methods

5.3.1 Mating and housing of animals

Guinea pigs (IMVS tri coloured, Gilles Plains Resource Centre, SA) were caged individually in plastic tubs with wire lids, and maintained in a room with a 12:12h light:dark cycle at 25°C, with *ad libitum* access to guinea pig/rabbit ration (Ridley Agriproducts, Australia). Males used for mating were fed lucerne in addition to the guinea pig chow. All guinea pigs also had *ad libitum* access to tap water, containing 400mg/L vitamin C. Females and males were mated as described in Chapter 2.3.2. Upon birth, guinea pigs were classed as being low or high birth weight according to the mean birth weight of the cohort (102.3g).

5.3.2 Size at birth and postnatal growth measurements

A description of the total number of animals used in each experiment can be found in Appendix A, and total numbers of litters used is summarised in Appendix C. Guinea pigs were weighed at birth, and size in terms of weight, crown-rump length (CRL), abdominal circumference (AC), head width (HW), and head length (HL) measured at birth and subsequently at 5-day intervals (see Table 2.1). All pups were weaned at 25 days of age onto normal guinea pig chow/rabbit ration and fed *ad libitum* with access to water containing 400mg/L vitamin C. Absolute growth rates were recorded for each growth measurement for each guinea pig (AGR) by calculating the slope of the regression line of postnatal age and size, and then expressed in terms of individual birth size to give a measure of catch up growth (fractional growth rate:

FGR). Current fractional growth rates (CFGR) were calculated by expressing the AGR in terms of individual current size at 36 days of age.

5.3.3 Saliva collection

At 5, 10, 15, 20 and 30 days of age, saliva was sampled from juvenile guinea pigs. Chewing and therefore saliva secretion was stimulated by placing a cotton bud in the guinea pig's mouth for approximately 2 minutes. The cotton buds were placed into an eppendorf tube and centrifuged for 2 minutes at 12,400 rpm (Eppendorf centrifuge 5415D). The buds were discarded, and the saliva centrifuged for a further 0.5 minutes to remove any solid particles. The supernatant was transferred to a pre-weighed eppendorf tube, weighed to give mass/volume, and frozen at -20^0C.

5.3.4 Measurement of salivary cortisol

Cortisol was measured in guinea pig saliva by specific RIA. The anti cortisol used was Immunogen cortisol-3-O-carboxymethyloxime:BSA (ICN diagnostics) raised in rabbit, and the secondary antibody was GAR (goat anti-rabbit IgG) (Silenus). The radioactive label was cortisol-3-(O-carboxymethyl) oximono-(2-[I^{125}] iodohistamine (Amersham Pharmacia Biotech UK), and the standards made from hydrocortisone (Sigma).

Guinea pig saliva was diluted 1:10 in Tris-HCl buffer (pH 7.4). The label was diluted to 10,000-counts/100 µl in Tris-HCl buffer, and the antisera 1:100 in buffer. To make the standards, 100 µl of 1000 nmol/L stock was added to 900 µl

of Tris-HCl buffer to make the top standard 100 nmol/L, then serial dilutions were performed to achieve the following concentrations: 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 nmol/L. One hundred µl of each standard or sample was added into pre-labelled tubes, along with 100 µl of buffer and 100 µl of antisera. Tubes were vortexed, and then 100 µl of label was added, and following another vortex the tubes were incubated at 37°C for 1 hour. One hundred µl of GAR, diluted in Tris-HCl buffer, was then added to all tubes except totals, and 1ml of 20% PEG (polyethylene glycol) added to all tubes, vortexed briefly, and then spun for 30 minutes at 4,000 rpm at 6°C. The supernatant was aspirated from all tubes, and then I^{125} radioactivity measured using a gamma counter. The concentrations calculated by the counter were multiplied by the dilution factor (1:10) to give a final concentration. The inter- and intra-assay co-efficients of variation for the salivary cortisol assay were 6.6% and 5.6% respectively.

5.3.5 *Post mortem*

Post-mortems were performed on 22 juvenile guinea pigs at 36 ± 2 days of age as described in Chapter 2.3.4.

5.3.6 *Measurement of free and total thyroid hormones*

Free thyroid hormone concentrations were measured in plasma, collected at post-mortem by heart puncture at 36 ± 2 days of age. All thyroid hormones were measured using specific RIAs (Cis-Bio International, France).

5.3.6.1 *Plasma free T_3*

Free T_3 is measured to determine bioavailable T_3. The RIA-gnost FT_3 kit determines the concentration of free T_3 by incubation of the serum sample with polyclonal solid-phase antibody-coated tubes (rabbit anti-T3). Fifty µl of standard (supplied) or plasma sample was pipetted on to the base of the coated tubes in duplicate. This was followed by the addition of 1000 µl of iodine^{125}-FT_3 tracer, which acts to occupy the free binding sites of the antibody. The tubes were shaken on a horizontal shaker (250-300 rpm) for 120 min at room temperature. The solution was removed by decanting, and the tubes placed upside down on absorbent paper towel for ~ 3 mins. The radioactivity of the tubes was measured for 1 minute in the ^{125}I channel of a gamma scintillation counter (1261 Multigamma, LKB Pharmacia and Wallace Oy). The intra-assay co-efficient of variation was 5.8%.

5.3.6.2 *Plasma free T_4*

The RIA-gnost free T_4 kit measures free serum thyroxine (T_4) by incubation of serum with a polyclonal solid-phase antibody (sheep anti-T_4). One hundred µl of standard (supplied) or plasma sample was pipetted onto the bottom of the tubes in duplicate. One thousand µl of iodine^{125}-FT_4 tracer was added to each tube, which acts to occupy the free binding sites of the antibody. The tubes were shaken on a horizontal shaker (250-300 rpm) for 120 min at room temperature. The solution was removed by decanting, and the tubes placed upside down on absorbent paper towel for ~ 3 mins. The radioactivity of the tubes was measured for 1 minute in the ^{125}I channel of a gamma scintillation counter (1261

Multigamma, LKB Pharmacia and Wallace Oy). The intra-assay co-efficient of variation was 1.6%.

5.3.6.3 *Plasma total T_3*

The RIA-gnost T_3 kit measures total triiodothyronine (T_3) in serum by competitive protein binding analysis. The T_3 to be measured is displaced from the binding proteins using the displacement reagent 8-anilion-1-sulphonic acid (ANSA) and competes with ^{125}I-T_3 for binding sites of a specific T_3 antibody (rabbit anti-T_3) that are available in limited numbers. The quantity of bound T_3 tracer is consequently inversely proportional to the T_3 concentration in the sample or standard. Standards and unknown plasma samples (50 µl) were pipetted into the bottom of test tubes coated with anti-T_3 antibodies. One ml of ^{125}I-T_3 tracer was added to each tube and the tubes were incubated on a horizontal shaker (250-300 rpm) for 2 hours at room temperature. The tubes were then decanted and left on absorbent paper for 3 minutes. The radioactivity in each tube was measured for 1 minute in the ^{125}I channel of a gamma scintillation counter (1261 Multigamma, LKB Pharmacia and Wallace Oy). The intra-assay co-efficient of variation was 6.5%.

5.3.6.4 *Plasma total T_4*

The RIA-gnost T_4 kit measures total thyroxine (T_4) in serum, giving a measurement of total bound and unbound hormone in the circulation. Using the displacement reagent 8-anilino-1-sulfonic acid (ANSA), T_4 is displaced from its binding proteins and competes with ^{125}I-T_4 for binding sites of a specific antibody (sheep anti-T_4). Twenty µl of standard (supplied) or plasma sample

was pipetted onto the bottom of the tubes in duplicate. 1000µl of tracer (^{125}I-T_4) was then added, and the tubes shaken on a horizontal shaker (250-300 rpm) for ~ 2 hours at room temperature. The solution was removed by decanting and the tubes placed upside down on absorbent paper towel for ~ 3 mins. The radioactivity in each tube was measured for 1 minute in the ^{125}I channel of a gamma scintillation counter (1261 Multigamma, LKB Pharmacia and Wallace Oy). The intra-assay co-efficient of variation was 9.2%.

5.3.7 *Statistics*

The effect of birth weight class and sex and their interaction on parameters was determined by between factor analysis of variance with post-hoc analysis (SPSS Inc., Chicago). Associations between parameters were examined using Pearson's correlations analysis (SPSS Inc., Chicago). Unless otherwise stated, significance occurs at the level $p<0.05$.

5.4 Results

5.4.1 The effect of birth weight class on plasma thyroid hormones in the juvenile guinea pig

Birth weight class in the juvenile guinea pig did not affect plasma total T_4 levels. LBW class tended to reduce plasma free T_4 levels (p=0.059) (Table 5.1). LBW class also tended to increase plasma total T_3 (p=0.077), but there was no significant effect of birth weight class on plasma free T_3 levels (Table 5.1). Birth weight class did not affect total T_3:T_4, but did tend to increase plasma free T_3:T_4 (p=0.058) (Table 5.1).

5.4.2 Thyroid hormones, size at birth and postnatal growth in the juvenile guinea pig

Size at birth did not correlate with plasma total T_4 in the juvenile guinea pig. However, weight (r=0.40), AC (r=0.58), HW (r=0.47) (Figure 5.1) and weight:length (r=0.44) at birth correlated positively with plasma free T_4. Size at birth did not correlate with plasma total and free T_3 levels, or with total T_3:T_4 (Table 5.2). Weight (r=-0.58), AC (r=-0.71), HL (r=-0.46), HW (=-0.55), BMI (r=-0.55), weight:length (r=-0.64) (Figure 5.2), and ponderal index (r=-0.39) at birth correlated negatively with free T_3:T_4 (Table 5.2).

Postnatal growth did not correlate with plasma total T_4 levels in the juvenile guinea pig. FGR_{weight} tended to correlate negatively with, and AGR_{CRL} and

AGR_{AC} positively correlate, with plasma free T_4 ($p<0.1$; Table 5.2). Postnatal growth did not correlate with plasma total T_3 and plasma free T_3 in the juvenile guinea pig, or with plasma total T_3:T_4 (Table 5.2). However, FGR_{weight} ($r=0.50$) and FGR_{CRL} ($r=0.52$) correlated positively with, (Figure 5.3), and AGR_{CRL} ($r=-0.52$) and AGR_{AC} ($r=-0.60$) correlated negatively, with plasma free T_3:T_4 (Table 5.2).

Table 5.1: Effect of birth weight class on plasma thyroid hormones in the juvenile guinea pig

	Low Birth Weight	*High Birth Weight*	*p-value*
Plasma thyroid hormones			
Total T_3 (nmol/L)	0.923 ± 0.090	0.774 ± 0.026	0.077
Free T_4 (pmol/L)	26.7 ± 4.0	35.6 ± 3.4	0.059
Total T_4 (nmol/L)	13.1 ± 2.6	16.3 ± 2.0	ns
Free T_3 (pmol/L)	2.75 ± 0.29	2.96 ± 0.18	ns
Free T_3:T_4 (pmol/L)	0.126 ± 0.019	0.089 ± 0.006	0.058
Total T_3:T_4 (nmol/L)	0.101 ± 0.024	0.072 ± 0.012	ns

Data expressed as mean ± SEM.

Table 5.2: Size at birth and plasma thyroid hormones in the juvenile guinea pig

	PLASMA					
	Free T_3	*Free T_4*	*Total T_3*	*Total T_4*	*Free T_3:T_4*	*Total T_3:T_4*
	r	*r*	*r*	*r*	*r*	*r*
Size at birth						
Weight (g)	0.15	0.40*	-0.29	0.04	-0.58*	-0.13
CRL (mm)	0.15	0.07	-0.27	0.11	-0.12	-0.17
AC (mm)	0.18	0.58*	-0.35	0.08	-0.71**	-0.06
Head length (mm)	0.07	0.20	-0.18	-0.02	-0.46*	0.01
Head width (mm)	0.01	0.47*	-0.08	0.15	-0.55*	-0.32
Weight:length (g.cm)	0.11	0.44*	-0.23	-0.01	-0.64**	-0.08
Ponderal Index ($g.cm^{-3}$)	-0.12	0.24	0.15	-0.09	-0.39*	0.14
BMI ($g.cm^{-2}$)	-0.02	0.37#	-0.03	-0.07	-0.55*	0.05
Absolute Growth Rate						
Weight (g/day)	-0.05	0.07	-0.08	-0.25	-0.14	-0.16
CRL (cm/day)	0.17	0.38#	-0.35	0.14	-0.52*	-0.27
AC (mm/day)	0.14	0.40#	-0.07	0.01	-0.60*	0.35
Head length (mm/day)	0.03	-0.22	-0.22	-0.17	0.27	0.31
Head Width (mm/day)	-0.17	-0.04	-0.32	-0.15	-0.17	0.33
Fractional Growth Rate						
Weight (%/day)	-0.22	-0.36#	0.29	-0.26	0.50*	0.01
CRL (%/day)	0.13	-0.20	-0.15	0.07	0.52*	-0.15
AC (%/day)	0.07	0.11	0.11	0.05	-0.23	0.39#
Head length (%/day)	0.01	-0.26	-0.18	-0.14	0.35	0.33
Head width (%/day)	-0.14	-0.08	-0.27	-0.16	-0.07	0.39#

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. CRL refers to crown-rump length; AC to abdominal circumference; BMI to body mass index.

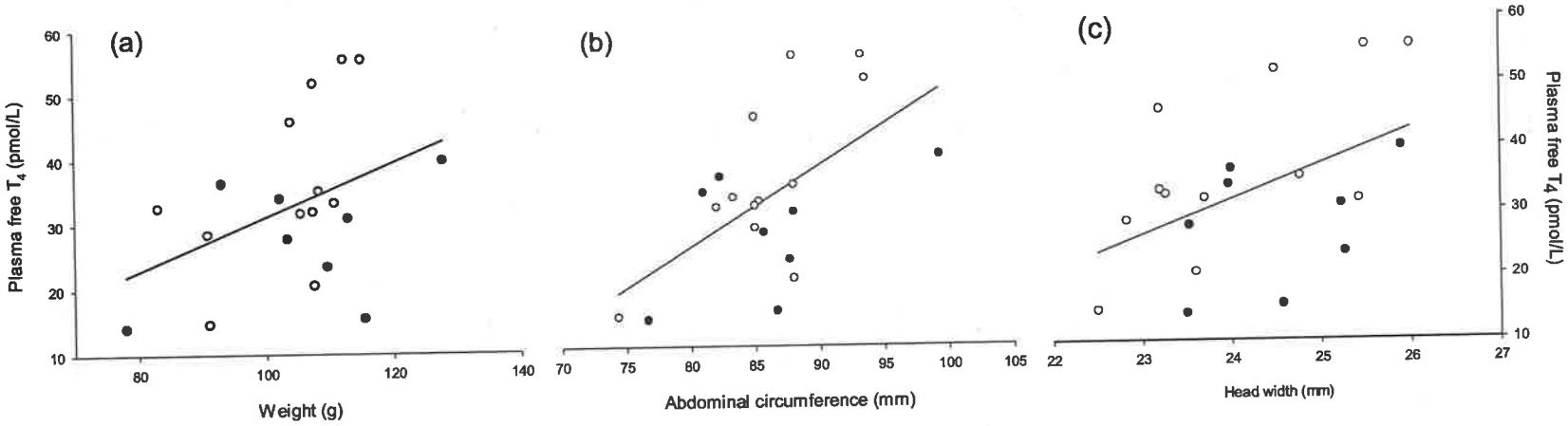

Figure 5.1: Plasma free T_4 and size at birth in the juvenile guinea pig

Males are represented by closed, and females by open circles. Plasma free T_4 was positively correlated with (a) birth weight (r=0.40), (b) AC (r=0.58) and (c) HW (r=0.47) (all $p<0.05$).

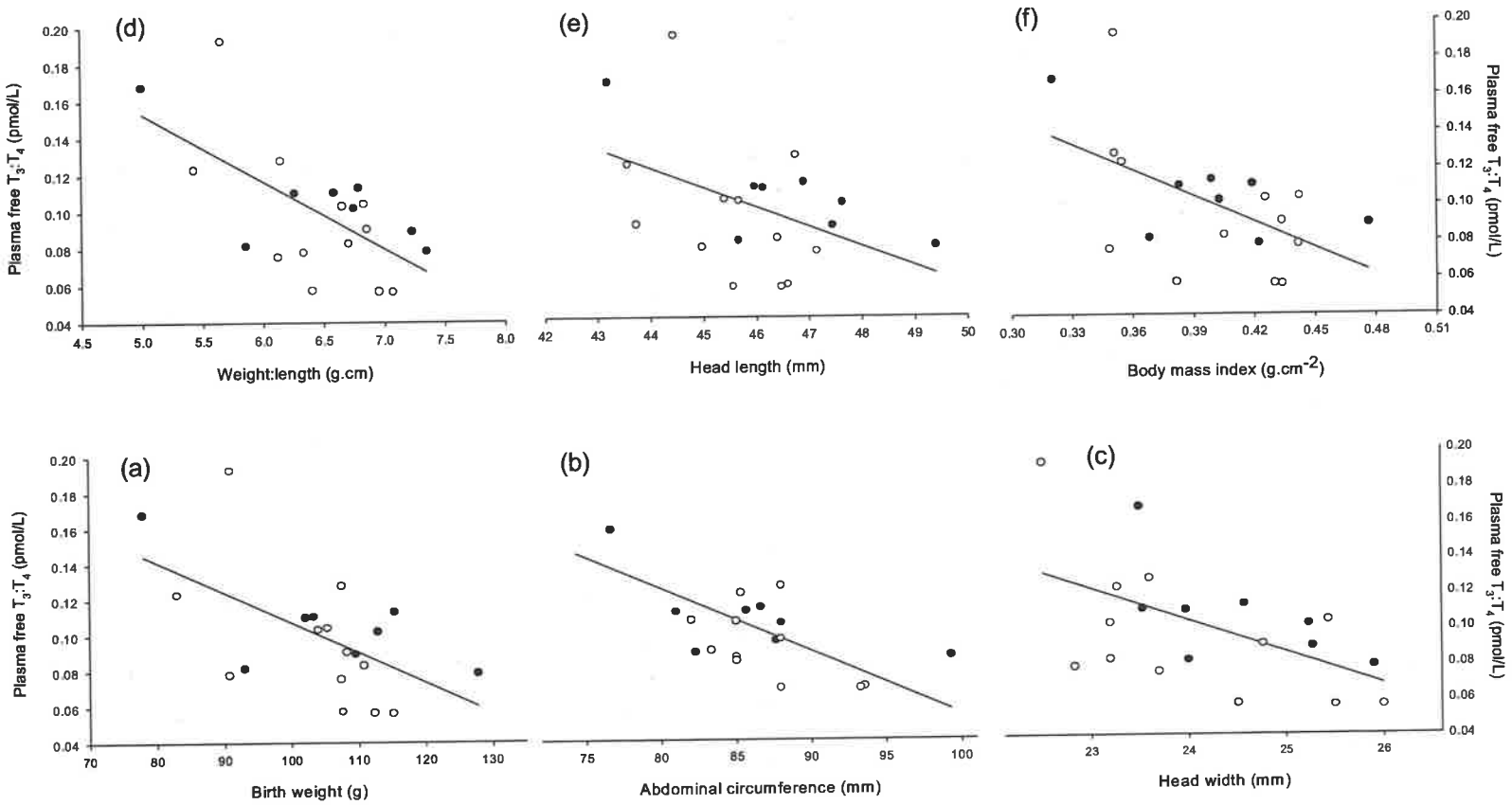

Figure 5.2: Plasma free T_3:T_4 and size at birth in the juvenile guinea pig

Males are represented by closed, and females by open circles. Plasma free T_3:T_4 was negatively correlated with (a) birth weight (r=-0.58), (b) AC (r=-0.71), (c) HW (r=-0.55), (d) HL (r=-0.46), (e) weight:length (r=-0.64), and (f) BMI (r=-0.55) (all $p<0.05$).

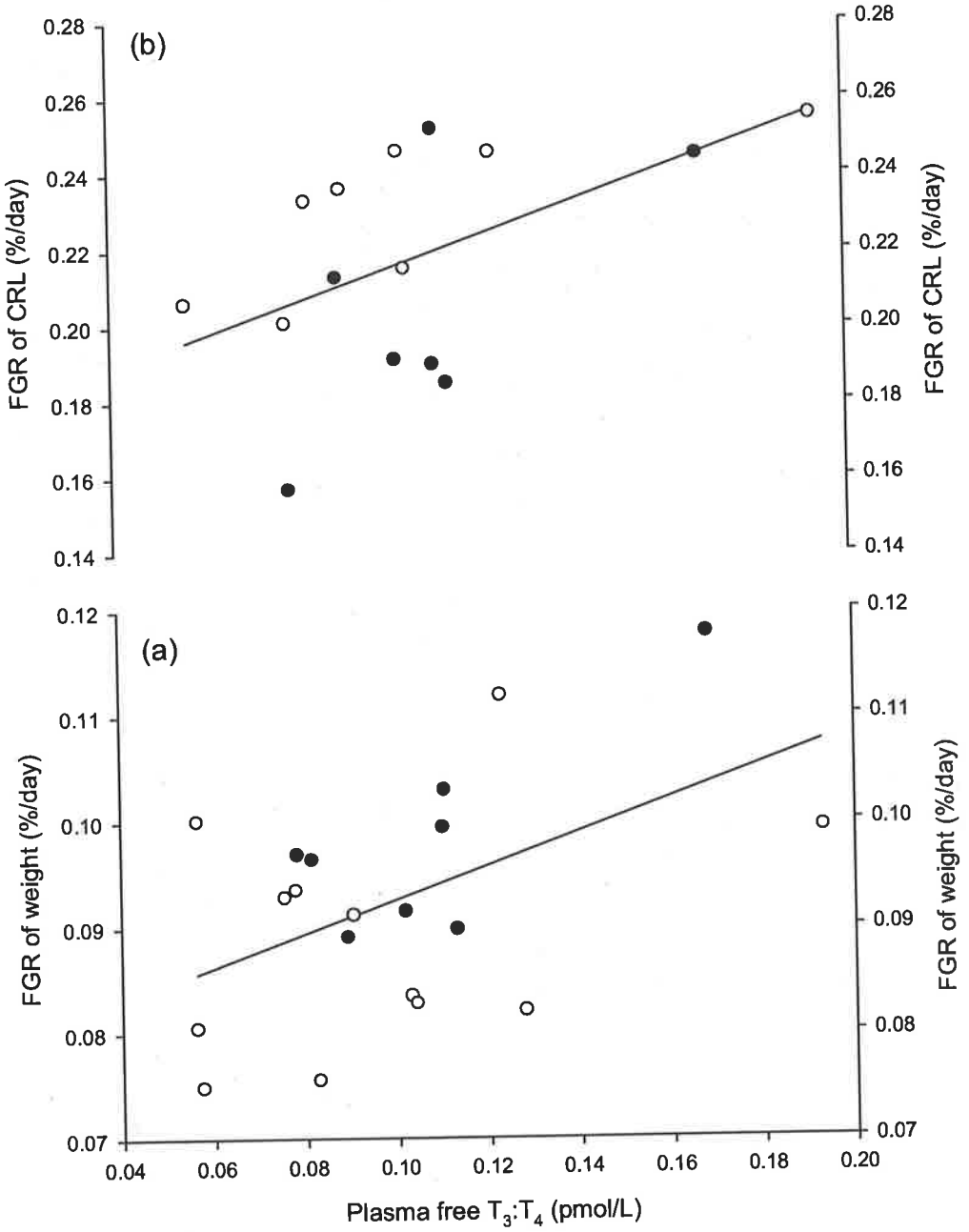

Figure 5.3: Plasma free T_3:T_4 and postnatal growth in the juvenile guinea pig

Males are represented by black, and females by open circles. FGR of CRL is represented as %/day x 100. Plasma free T_3:T_4 was positively correlated with (a) FGR of weight ($r=0.50$) and (b) FGR of CRL ($r=0.52$), $p<0.05$ for both.

5.4.3 Organ size and plasma thyroid hormones in the juvenile guinea pig

Body weight or length in the juvenile guinea pig were not associated with plasma thyroid hormone concentrations, with the exception of plasma free T_3, which tended to correlate positively with body weight ($p<0.1$) (Table 5.3). Juvenile BMI was positively correlated with plasma free T_3 ($r=0.40$).

Current FGR_{weight} was positively correlated with plasma free T_3 ($r=-0.40$). Current FGR_{CRL} was positively correlated with plasma total T_4 ($r=0.53$) negatively correlated with plasma total T_3:T_4 ($r=-0.59$) (Table 5.3).

GIT relative weight ($r=-0.65$) and brain weight ($r=-0.61$; Table 5.3) correlated negatively with plasma total T_4. Brain absolute ($r=-0.42$), and relative ($r=-0.63$) weight correlated negatively with plasma free T_4. Brain weight was also negatively correlated with plasma total T_3 ($r=-0.56$; Table 5.3). Kidney relative weight was negatively correlated with ($r=-0.56$), and thyroid relative weight positively correlated ($r=0.40$) with plasma free T_3. Interestingly, plasma free T_3 was the only hormone associated with the thyroid gland weight. Brain absolute ($r=0.54$) and relative ($r=0.73$) weight correlated positively with plasma free T_3:T_4 (Table 5.3).

Table 5.3: Organ size and plasma thyroid hormones in the juvenile guinea pig

	PLASMA					
	Free T_3	*Free T_4*	*Total T_3*	*Total T_4*	*Free T_3:T_4*	*Total T_3:T_4*
	r	*r*	*r*	*r*	*r*	*r*
Current size						
Weight (g)	0.31#	0.28	-0.29	-0.22	-0.24	-0.09
CRL (mm)	-0.15	0.01	0.10	-0.19	-0.11	-0.09
Wt:CRL ($g.mm^{-1}$)	0.32#	0.27	-0.33	-0.20	-0.13	-0.29
BMI ($g.mm^{-3}$)	0.40*	0.26	-0.32	-0.09	-0.07	-0.17
FGR_{weight}	-0.40*	-0.23	0.27	-0.04	0.11	-0.14
FGR_{CRL}	0.38#	-0.03	0.01	0.53*	0.43#	-0.59*
Organ						
Adrenal (g)	0.28	0.22	-0.13	0.22	-0.07	-0.26
(%BW)	0.13	0.07	-0.01	0.33#	0.05	-0.25
Kidney (g)	-0.30#	-0.10	-0.32	-0.31#	0.01	0.04
(%BW)	-0.56*	-0.33#	-0.05	-0.15	0.20	0.06
Pancreas (g)	0.11	-0.23	0.20	-0.31#	0.13	-0.05
(%BW)	-0.03	-0.33#	0.33	-0.23	0.22	-0.02
GIT (g)	-0.06	-0.26	0.38	-0.50	0.39	0.39
(%BW)	-0.13	-0.42	0.31	-0.65*	0.61#	0.61#
Brain (g)	-0.01	-0.42*	-0.56*	-0.61*	0.54*	-0.07
(%BW)	-0.29#	-0.63**	-0.03	-0.34#	0.73**	0.02
Liver (g)	-0.08	-0.12	-0.15	-0.05	0.18	0.02
(%BW)	-0.29#	-0.30	0.08	0.09	0.35#	0.05
Lungs (g)	-0.25	-0.27	-0.40#	-0.25	0.16	0.06
(%BW)	-0.34#	-0.37#	0.03	-0.09	0.26	0.11
Heart (g)	0.11	-0.20	0.24	-0.14	0.22	-0.19
(%BW)	-0.04	-0.35#	0.40#	-0.04	0.36#	-0.20
Thyroid (g)	0.36#	-0.20	-0.06	0.05	-0.23	0.31
(%BW)	0.40*	-0.01	0.09	0.06	0.13	-0.15

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. GIT refers to gastrointestinal tract. %BW refers to organ weight expressed as a percentage of total body weight.

5.4.4 Plasma thyroid hormones and body composition in the juvenile guinea pig

EDL absolute (r=-0.54) and relative (r=-0.56) weights were negatively correlated with plasma total T_4, and summed absolute and relative muscle weights also tended to correlate negatively with total T_4 ($p<0.1$; Table 5.4). Plantaris weight (r=0.38) was positively correlated with plasma free T_4. Tibialis weight was negatively correlated with plasma total T_3 (r=-0.52), whereas gastrocnemius (r=0.37) and biceps brachii (r=0.42) weights were positively correlated with plasma free T_3. EDL absolute (r=0.43) and relative (r=0.52) weights, as well as tibialis relative weight (r=0.40) were positively correlated with plasma free T_3:T_4 (Table 5.4).

Plasma total T_3 and total T_4 were not associated with any measures of adiposity. Dorsal, shoulder and neck fat weights tended to correlate positively with plasma free T_4 ($p<0.1$; Table 5.5). Parametrial fat relative weight correlated negatively with plasma free T_3 (r=-0.55). Neck fat weight was negatively correlated with plasma free T_3:T_4 (r=-0.41), and perirenal fat relative weight was negatively correlated with plasma total T_3:T_4 (r=-0.51) (Table 5.5).

Table 5.4: Skeletal muscle mass and plasma thyroid hormones in the juvenile guinea pig

	PLASMA					
	Free T_3	*Free T_4*	*Total T_3*	*Total T_4*	*Free T_3:T_4*	*Total T_3:T_4*
Muscle	*r*	*r*	*r*	*r*	*r*	*r*
Plantaris (g)	-0.24	0.38*	-0.14	0.03	-0.18	-0.12
(%BW)	0.17	0.31#	-0.11	0.07	-0.11	-0.07
Gastrocnemious (g)	0.37*	0.22	0.05	-0.23	-0.06	-0.21
(%BW)	0.24	0.03	0.30	-0.16	0.17	-0.19
Tibialis (g)	0.01	-0.21	-0.52*	-0.34#	0.26	-0.20
(%BW)	-0.11	-0.33#	-0.46#	-0.31#	0.40*	-0.20
Semitendinosus (g)	0.34#	0.11	0.15	-0.17	-0.03	-0.17
(%BW)	0.27	-0.02	0.36	-0.12	0.11	-0.13
Biceps Femoris (g)	0.11	0.11	0.09	-0.33#	-0.15	0.07
(%BW)	-0.13	-0.07	0.44#	-0.27	-0.02	0.17
Vastus Lateralis (g)	0.15	0.25	-0.17	-0.26	-0.22	0.04
(%BW)	-0.03	0.10	-0.05	-0.20	-0.09	0.14
EDL (g)	0.21	-0.26	-0.15	-0.54*	0.43*	-0.25
(%BW)	0.17	-0.33#	-0.08	-0.56*	0.52*	-0.25
Biceps Brachii (g)	0.42*	0.28	0.08	-0.13	-0.11	-0.23
(%BW)	0.35#	0.18	0.31	-0.02	0.04	-0.23
Diaphragm (g)	0.12	0.13	-0.22	-0.22	-0.06	-0.41#
(%BW)	-0.09	-0.03	-0.03	-0.14	0.09	-0.39#
Summed muscle (g)	0.24	0.15	-0.02	-0.33#	-0.09	-0.12
(%BW)	0.05	-0.07	0.36	-0.34#	0.13	-0.09
Sum muscle:fat (g)	-0.09	-0.20	0.11	-0.12	0.27	0.11

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. EDL refers to extensor digitorum longus.

%BW refers to organ weight expressed as a percentage of total body weight.

Table 5.5: Adiposity and plasma thyroid hormones in the juvenile guinea pig

	PLASMA					
	Free T_3	*Free T_4*	*Total T_3*	*Total T_4*	*Free T_3:T_4*	*Total T_3:T_4*
Adipose depot	*r*	*r*	*r*	*r*	*r*	*r*
Dorsal Fat (g)	0.21	0.33#	0.15	0.14	-0.37#	-0.16
(%BW)	0.11	0.26	0.35	0.24	-0.32#	-0.20
Shoulder Fat (g)	0.16	0.32#	-0.17	-0.04	-0.25	-0.28
(%BW)	0.06	0.27	-0.08	0.02	-0.21	-0.31
Neck Fat (g)	0.15	0.35#	0.05	0.28	-0.47*	-0.07
(%BW)	0.08	0.30	0.14	0.32	-0.42#	-0.04
Parametrial Fat (g)	-0.36	0.25	-0.53	0.08	-0.40	0.28
(%BW)	-0.55*	0.13	-0.35	0.11	-0.38	0.46
Retroperit'eal Fat (g)	0.26	0.08	-0.09	-0.10	-0.12	-0.33
(%BW)	0.21	0.03	-0.02	0.02	-0.09	-0.36
Perirenal Fat (g)	0.29	0.21	-0.15	0.13	-0.20	-0.47#
(%BW)	0.23	0.15	-0.11	0.17	-0.15	-0.51*
Epididymal Fat (g)	0.22	0.39	-0.14	-0.27	-0.69	0.06
(%BW)	-0.02	0.23	-0.03	-0.10	-0.61	0.02
Groin Fat (g)	-0.04	0.11	-0.20	-0.08	-0.23	0.20
(%BW)	-0.12	0.04	-0.13	-0.02	-0.18	0.27
Summed Fat (g)	0.15	0.16	-0.02	0.07	-0.21	-0.29
(%BW)	0.07	0.14	0.09	0.12	-0.20	-0.35

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. %BW refers to organ weight expressed as a percentage of total body weight.

5.4.5 Plasma thyroid hormones and the IGF axis in the juvenile guinea pig

Fasting plasma IGF-I correlated negatively with plasma total T_4 (r=-0.45; p=0.02) (Figure 5.4), while plasma IGF-I and IGF-II were not correlated with any other plasma thyroid hormone measures (Table 5.6). Plasma IGF-I:IGFBP correlated negatively with plasma total T_4 (r=-0.48), and plasma IGF-II:IGFBP correlated negatively with plasma free T_3:T_4 (r=-0.40) (Table 5.6).

IGF-I sensitivity of glucose metabolism was not associated with plasma thyroid hormone concentrations, but IGF-I sensitivity of FFA metabolism correlated positively with plasma total T_3 (Table 5.6).

Table 5.6: Plasma thyroid hormones and the IGF axis in the juvenile guinea pig

	PLASMA					
	Free T_3	***Free T_4***	***Total T_3***	***Total T_4***	***Free T_3:T_4***	***Total T_3:T_4***
Adipose depot	*r*	*r*	*r*	*r*	*r*	*r*
Post-mortem plasma						
IGF-I ($ng.ml^{-1}$)	-0.03	-0.20	-0.19	-0.45*	-0.03	-0.04
IGFBP	0.33#	0.11	0.40#	-0.03	-0.01	-0.19
IGF-I:IGFBP	-0.22	-0.42#	-0.32	-0.48*	0.12	0.19
IGF-II ($ng.ml^{-1}$)	0.10	0.27	0.02	-0.08	-0.35#	-0.08
IGF-II:IGFBP	0.01	0.27	-0.13	-0.12	-0.40*	-0.01
Total IGF ($ng.ml^{-1}$)	0.04	0.09	0.04	-0.23	-0.29	-0.07
Total IGF:IGFBP	-0.13	0.11	-0.24	-0.27	-0.35#	0.06
IGF-I sensitivity						
Glucose						
Adjusted SSGIR ($mg.ml.ng^{-1}.min^{-1}.kg^{-1}$)	0.06	-0.19	-0.05	0.22	0.25	-0.18
Free Fatty Acids						
Adjusted %Δ FFA ($\%\Delta meq.ng^{-2}.ml$)	0.19	0.31	0.77**	0.43#	-0.36	0.07
Basal disposition Index						
Glucose (IGF-IxIGFS$_{glu}$Adj)	-0.07	-0.33	-0.24	-0.13	0.30	-0.30
FFA (IGF-I x IGFS$_{FFA}$%Adj)	0.10	0.11	0.40	0.04	-0.26	0.05

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. %BW refers to weight expressed as a percentage of total body weight. %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; $SSGIR_{adj}$ is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I. Units for basal disposition indices are as follows: Glucose (IGF-IxIGFS$_{glu}$Adj) ($mg.ng^{2}.min^{-1}.kg^{-1}.ml^{-2}$); FFA (IGF-I x IGFS$_{FFA}$%Adj) ($\%\Delta meq.ng^{-1}.ml^{-1}$).

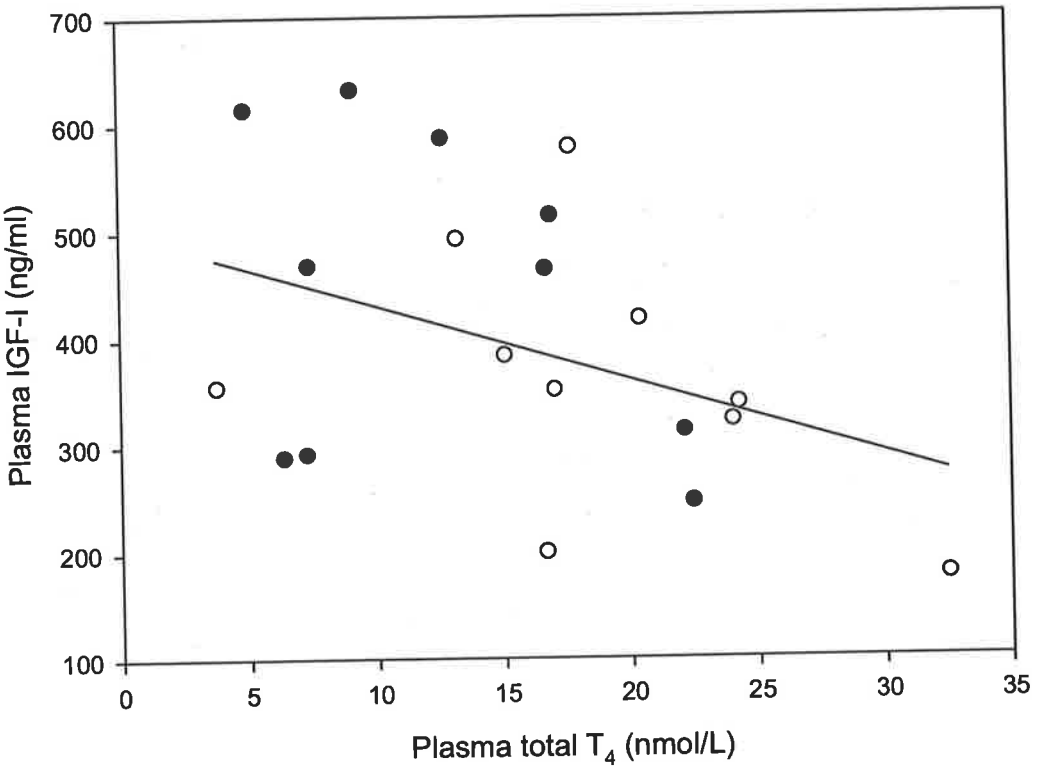

Figure 5.4: Plasma total T_4 and plasma IGF-I in the juvenile guinea pig

Males are represented by closed, and females by open circles. Plasma total T_4 was positively correlated with plasma IGF-I ($r=-0.45$; $p=0.02$).

5.4.6 The effect of birth weight class on salivary cortisol in the juvenile guinea pig

Birth weight tended to alter salivary cortisol ($p=0.07$; results not shown), however, were no significant effects of sex ($p=0.37$) or the interaction of birth weight and sex ($p=0.13$) on salivary cortisol. However, age significantly affected salivary cortisol ($p=0.01$; results not shown). Using birth weight, crown-rump length or weight:length at birth as covariates did not change these results (results not shown). Salivary cortisol at day 5 tended to be higher in the LBW animals than in HBW animals ($p<0.1$). Day 10 salivary cortisol concentrations were higher in the LBW group (8.5 ± 1.4 nmol/L) compared to the HBW group (4.1 ± 0.5 nmol/L) ($p=0.008$; Table 5.7; Figure 5.5B). Females (4.4 ± 0.4 nmol/L) had higher salivary cortisol at day 20 than males (2.9 ± 0.8 nmol/L; $p=0.045$; Figure 5.5A). No sex differences were seen in salivary cortisol at any other ages (Figure 5.5A). However, when males and females were analysed separately, salivary cortisol was increased at all ages between 5 and 25 days in LBW compared to HBW males (Figure 5.6A). Female LBW animals had significantly higher salivary cortisol at day 10 compared to HBW females, but had lower salivary cortisol at day 20 (Figure 5.6B).

Table 5.7: Effect of birth weight class on salivary cortisol concentrations in the juvenile guinea pig

	SALIVARY CORTISOL (nmol/L)		
Age (days)	***Low Birth Weight***	***High Birth Weight***	***p-value***
5	8.2 ± 1.6	5.6 ± 0.6	0.09
10	8.5 ± 1.4	4.1 ± 0.5	0.008
15	5.1 ± 1.0	5.3 ± 1.1	ns
20	4.0 ± 0.9	3.6 ± 0.5	ns
30	8.5 ± 2.2	11.0 ± 1.9	ns

Data expressed as mean ± SEM; statistics are 1-tailed independent t-tests, 2 sample unequal variance.

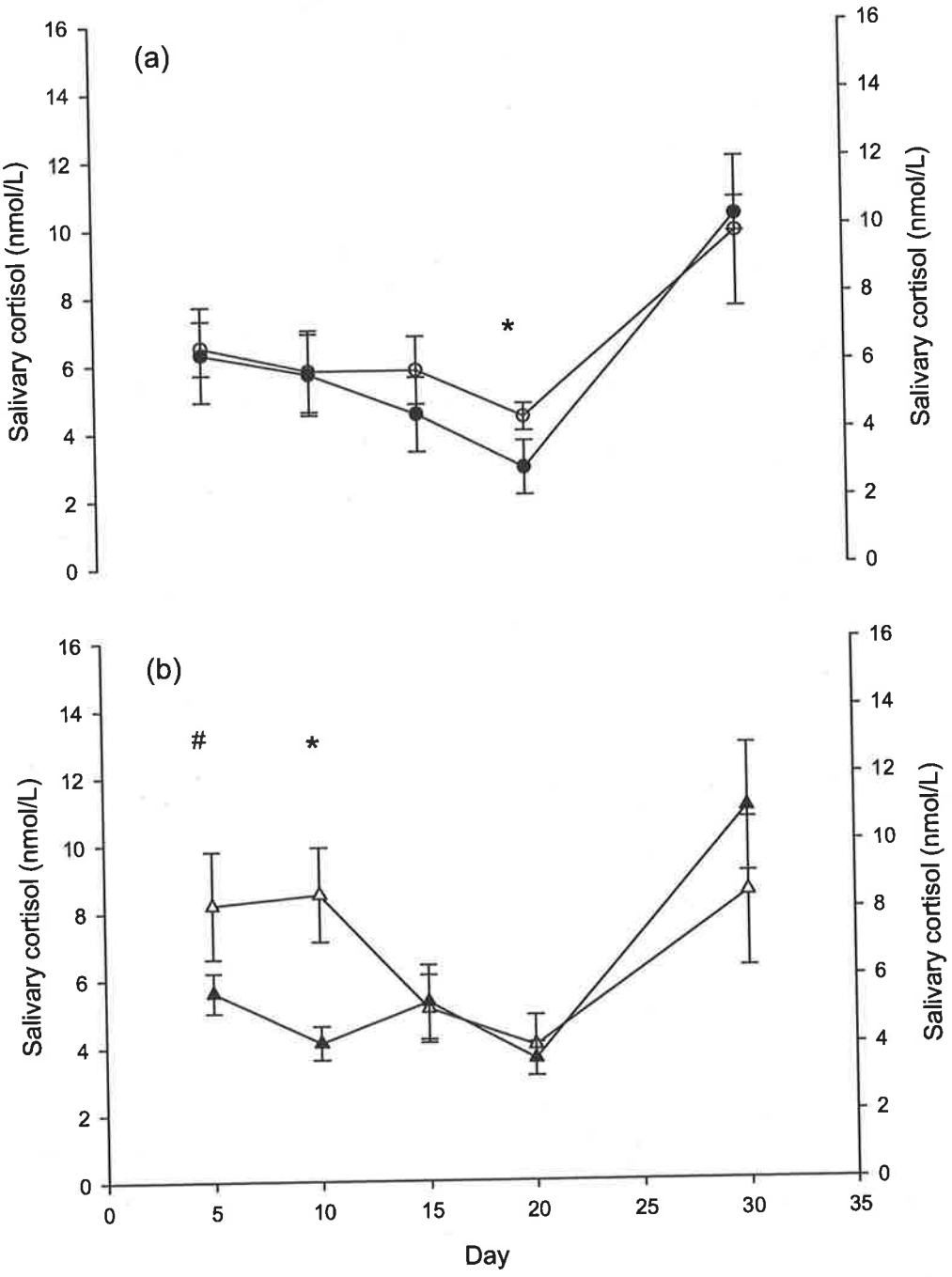

Figure 5.5: Effect of birth weight class and sex on salivary cortisol concentrations in the juvenile guinea pig

(a): Sex and salivary cortisol. Males are represented by closed, and females by open circles. (b): Birth weight class and salivary cortisol. Low birth weight is represented by open, and high birth weight by closed triangles. Significance denoted by * $p<0.05$.

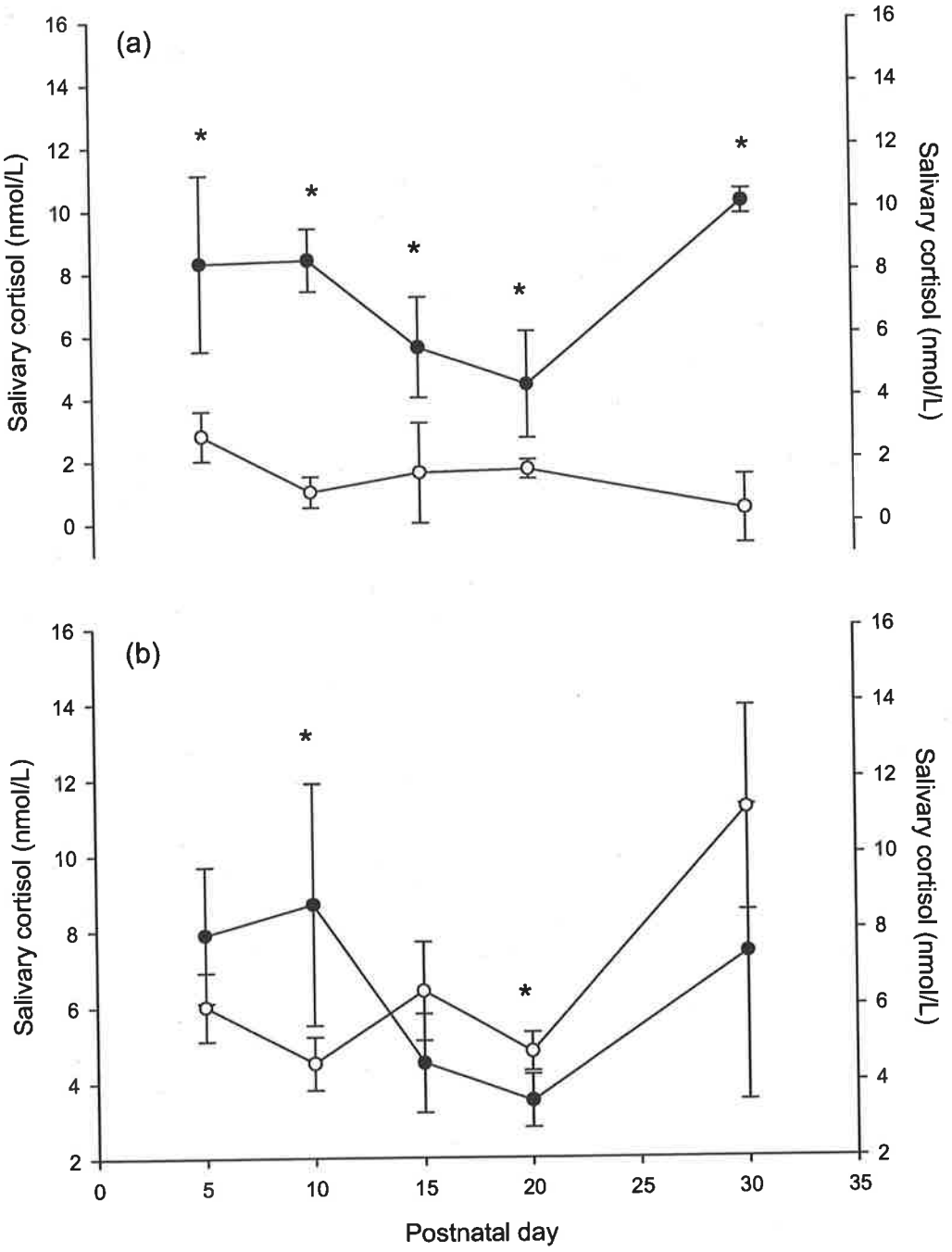

Figure 5.6: Effect of birth weight class on salivary cortisol in male and female juvenile guinea pigs in males

(a): Salivary cortisol concentrations in males. Low birth weight is represented by closed, and high birth weight by open circles.

(b): Salivary cortisol concentrations in females. Low birth weight is represented by closed, and high birth weight by open circles. Significance denoted by * $p<0.05$

5.4.7 Size at birth, postnatal growth and salivary cortisol in the juvenile guinea pig

Weight (r=-0.50; r=-0.53) (Figure 5.6), HL (r=-0.53; r=-0.46) and weight:length (r=-0.56; r=-0.52 respectively) at birth were negatively correlated with salivary cortisol in all animals combined at measured at 5 and 10 days of age. BMI at birth correlated negatively with cortisol at 5 days of age (r=-0.51), and AC (r=-0.42) and HW (r=-0.52) at birth correlated negatively with that at 10 days of age. In addition, birth CRL correlated negatively with salivary cortisol at 20 days of age (r=-0.40) (Table 5.8).

AGR_{weight} correlated negatively with salivary cortisol at 20 days of age (r=-0.65). AGR_{HW} correlated negatively with salivary cortisol at 30 days of age (r=-0.59), as did AGR_{HL} (r=-0.60).

FGR_{CRL} correlated positively with salivary cortisol at 5 days of age (r=0.43) (Figure 5.7). FGR_{AC} was positively correlated with cortisol at day 10 (r=0.45) (Figure 5.7), and also tended to at 15 and 20 days of age. FGR_{HW} (r=-0.62) and FGR_{HL} (r=-0.64) correlated negatively with salivary cortisol at 30 days of age (Table 5.8).

Birth weight correlated negatively with salivary cortisol at 5 days of age (r=-0.71), 10 days (r=-0.68) and 20 days (r=-0.70) in male guinea pigs, but correlations between birth weight and salivary cortisol at these ages in female guinea pigs did not reach statistical significance (results not shown). In addition, FGR_{weight} correlated negatively with salivary cortisol at 20 days of age in female

guinea pigs (r=-0.82), but not in males. There were no sex differences for any other variables measured (results not shown).

Table 5.8: Size at birth, postnatal growth and salivary cortisol in the juvenile guinea pig

	Age (days)				
	5	10	15	20	30
	r	r	r	r	r
Size at birth					
Weight (g)	-0.50*	-0.53*	0.01	-0.26	0.21
CRL (mm)	-0.13	-0.33#	0.08	-0.40*	0.30
AC (mm)	-0.30	-0.42*	-0.24	-0.31	0.49#
Head length (mm)	-0.53*	-0.46*	-0.04	-0.28	0.25
Head width (mm)	-0.32	-0.52*	-0.08	0.01	0.44#
Weight:length (g:cm)	-0.56*	-0.52*	-0.03	-0.13	0.11
Ponderal Index ($g.cm^{-3}$)	-0.38#	-0.08	-0.09	0.32#	-0.15
BMI ($g.cm^{-2}$)	-0.51*	-0.33#	-0.08	0.13	-0.03
Absolute Growth Rate					
Weight (g/day)	-0.27	-0.36#	-0.38#	-0.65**	0.11
CRL (cm/day)	-0.15	-0.03	-0.04	-0.13	0.42#
AC (mm/day)	-0.17	0.24	0.23	0.23	-0.11
Head length (mm/day)	0.14	0.16	0.23	0.22	-0.60*
Head Width (mm/day)	0.20	0.20	0.25	0.35#	-0.59*
Fractional Growth Rate					
Weight (%/day)	0.38#	0.32#	-0.28	-0.12	-0.09
CRL (%/day)	0.43*	0.06	-0.20	0.01	-0.17
AC (%/day)	-0.03	0.45*	0.37#	0.39#	-0.33
Head length (%/day)	0.23	0.26	0.22	0.27	-0.64*
Head width (%/day)	0.24	0.32#	0.25	0.32#	-0.62*

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. CRL refers to crown-rump length; AC to abdominal circumference; BMI to body mass index.

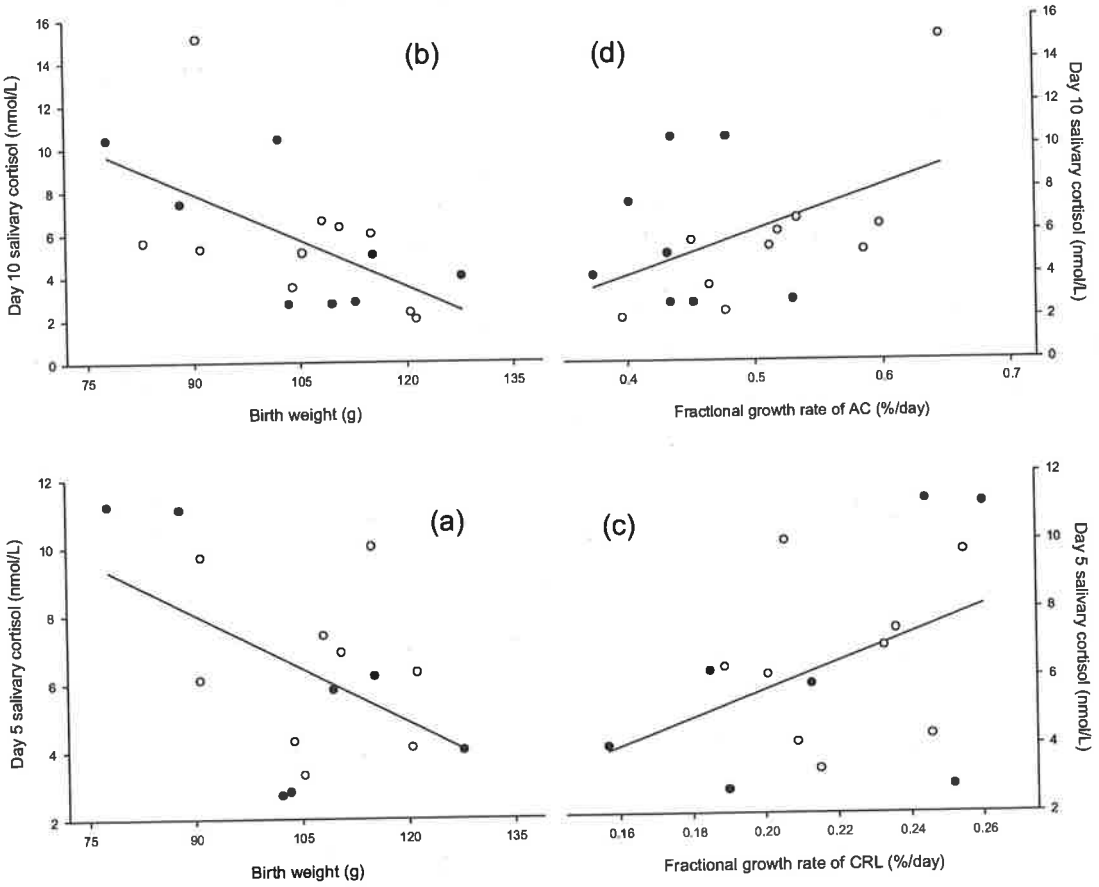

Figure 5.7: Size at birth, postnatal growth and salivary cortisol at 5 and 10 days of age in the juvenile guinea pig

Males are represented by closed, and females by open circles. Correlations are significant at $p<0.05$. Fractional growth rates of CRL and AC are represented as %/day * 100. (a) birth weight ($r=-0.50$) correlated negatively with salivary cortisol at 5 days of age, and (b) correlated negatively ($r=-0.53$) with salivary cortisol at 10 days of age; (c) FGR of CRL ($r=0.43$) correlated positively with salivary cortisol at 5 days; (d) FGR of AC ($r=0.45$) correlated positively with salivary cortisol at 10 days.

5.4.8 Organ size and salivary cortisol in the juvenile guinea pig

Body weight at 36 days of age correlated negatively with cortisol at 5 (r=-0.56) and 20 (r=-0.63) days of age. Body length was also negatively correlated with cortisol at 20 days of age (r=-0.60) (Table 5.9).

The association of salivary cortisol with organ size in the juvenile guinea pig varied with age, with more negative correlations apparent at later ages (Table 5.9). Adrenal weights were not associated with salivary cortisol at any age. Lung relative weight correlated positively with salivary cortisol at 5 days of age (r=0.49) and lung absolute weight correlated negatively with salivary cortisol at 20 days (r=-0.61). Brain weight correlated negatively with salivary cortisol at 15 (r=-0.44) and 20 (r=-0.64) days of age, and brain relative weight correlated positively with salivary cortisol at 5 days of age (r=0.69). Thyroid absolute (r=-0.55) and relative (r=-0.49) weight correlated negatively with salivary cortisol at 5 days of age, but thyroid absolute weight correlated positively with cortisol at 15 days of age (r=0.58) (Table 5.9). GIT absolute (r=-0.70) and relative (r=-0.63) weight were negatively correlated with salivary cortisol at 15 days, but were negatively correlated with that at 20 days of age (r=-0.74, r=-0.64 respectively). Liver absolute (r=0.49) and relative (r=0.48) weight were positively correlated with salivary cortisol at 15 days of age, but liver weight correlated negatively with cortisol at 30 days (r=-0.57). Heart weight correlated positively with salivary cortisol at 15 days of age (r=0.44). Kidney weight correlated negatively with salivary cortisol at 20 days of age (r=-0.46), as did lung weight (r=-0.61) (Table 5.9). Summed absolute visceral organ weight was positively correlated with salivary cortisol at 5 (r=0.46) and 10 (r=0.43) days of

age. Summed relative visceral organ weight correlated negatively with salivary cortisol at 15 (r=-0.58) and 20 (r=-0.59) days of age (Table 5.9). No major sex differences were observed between organ size and salivary cortisol (results not shown). Therefore, reduced organ size in the juvenile guinea pig was associated with increased salivary cortisol after 10 days of postnatal life.

Table 5.9: Organ size and salivary cortisol in the juvenile guinea pig

	Age (days)				
	5	10	15	20	30
	r	r	r	r	r
Current size					
Weight (g)	-0.56*	-0.38#	-0.09	-0.63*	-0.11
Length (cm)	-0.29	-0.33	0.09	-0.60*	0.16
Organ					
Adrenal (g)	0.05	-0.18	0.24	-0.19	0.02
(%BW)	0.22	-0.07	0.28	0.06	0.09
Kidney (g)	0.11	-0.40#	-0.08	-0.46*	-0.19
(%BW)	0.45#	-0.18	-0.02	0.07	-0.12
Pancreas (g)	-0.23	-0.35#	-0.09	-0.38#	0.28
(%BW)	-0.02	-0.19	-0.09	-0.17	0.28
GIT (g)	0.12	-0.13	-0.70*	-0.74*	0.17
(%BW)	0.33	0.09	-0.63*	-0.64*	0.35
Brain (g)	0.31	-0.27	-0.44*	-0.64*	-0.03
(%BW)	0.69*	0.09	-0.26	0.09	0.06
Liver (g)	0.14	0.01	0.49*	-0.15	-0.57*
(%BW)	0.39	0.20	0.48*	0.31	-0.48#
Lungs (g)	0.26	-0.22	-0.19	-0.51*	-0.23
(%BW)	0.49*	0.03	-0.11	-0.10	-0.19
Heart (g)	-0.38#	-0.30	0.36#	-0.35#	0.06
(%BW)	-0.23	-0.21	0.44*	-0.10	0.11
Thyroid (g)	-0.55*	0.12	0.58*	0.10	0.06
(%BW)	-0.49*	-0.01	-0.13	-0.17	0.33
Sum visceral (g)	0.12	-0.23	-0.58*	-0.59*	0.11
(%BW)	0.46*	0.43*	-0.13	-0.16	-0.18

** Denotes significance at p<0.001; * p<0.05; # p<0.1. GIT refers to gastro-intestinal tract; %BW refers to weight expressed as a percentage of body weight.

5.4.9 Body composition and salivary cortisol in the juvenile guinea pig

Summed absolute muscle mass correlated negatively with salivary cortisol at 20 days (r=-0.68), whereas summed relative muscle mass correlated negatively with cortisol at 15 days of age (r=-0.45). Gastrocnemious weight correlated negatively with salivary cortisol at 20 days of age (r=-0.57). Tibialis absolute (r=-0.57) and relative (r=-0.43) weight correlated negatively with salivary cortisol at 10 (r=-0.59; r=-0.53) and 20 (r=-0.57; r=-0.43 respectively) days of age. Semitendinosus absolute weight correlated negatively with salivary cortisol at 5 (r=-0.46) and 20 days of age (r=-0.68) and its' relative weight correlated negatively with cortisol at 20 days (r=-0.51). Biceps femoris weight correlated negatively with salivary cortisol at 20 days of age (r=-0.45), as did vastus lateralis (r=-0.52) and biceps brachii (r=-0.54) weight, and relative EDL weight (r=-0.50) (Table 5.9). Day 15 cortisol was negatively correlated with, diaphragm muscle absolute weight (r=-0.59) correlated negatively with salivary cortisol at 10 (r=-0.46) and 15 (r=-0.59) days of age, and relative weight (r=-0.55) correlated negatively with salivary cortisol at 15 days of age. Plantaris relative weight correlated positively with cortisol at 5 days of age (r=0.47) (Table 5.10).

Absolute and relative weights of individual adipose tissue organs did not correlate with salivary cortisol at 10, 20 or 30 days of age. Retroperitoneal fat in absolute (r=-0.58) and relative (r=-0.56) terms was negatively correlated with salivary cortisol at 5 days of age. Parametrial fat absolute (r=0.66) and relative (r=0.72) weight correlated positively with salivary cortisol at 15 days of age (Table 5.11).

Summed absolute fat mass was positively correlated with salivary cortisol at 15 days of age in female guinea pigs (r=-0.61), but not in males (r=-0.20).

Table 5.10: Skeletal muscle mass and salivary cortisol in the juvenile guinea pig

	Age (days)				
	5	***10***	***15***	***20***	***30***
Muscle	***r***	***r***	***r***	***r***	***r***
Plantaris (g)	0.37#	0.09	-0.35#	-0.35#	0.12
(%BW)	0.47*	0.16	-0.36#	-0.28	0.17
Gastrocnemious (g)	-0.15	-0.11	-0.17	-0.57*	0.01
(%BW)	0.21	0.15	-0.15	-0.27	0.10
Tibialis (g)	0.04	-0.59*	-0.29	-0.57*	-0.09
(%BW)	0.19	-0.53*	-0.31	-0.43*	-0.06
Semitendinosus (g)	-0.46*	-0.22	-0.32	-0.68**	-0.07
(%BW)	-0.21	-0.04	-0.35#	-0.51*	-0.01
Biceps Femoris (g)	-0.27	-0.11	-0.15	-0.45*	-0.31
(%BW)	-0.04	0.08	-0.13	-0.07	-0.27
Vastus Lateralis (g)	0.05	-0.02	-0.29	-0.52*	-0.15
(%BW)	0.31	0.18	-0.28	-0.27	-0.10
EDL (g)	-0.01	-0.16	-0.38#	-0.60*	-0.18
(%BW)	0.20	-0.04	-0.40#	-0.50*	-0.13
Biceps Brachii (g)	-0.18	-0.07	-0.07	-0.54*	0.06
(%BW)	0.10	0.14	-0.04	-0.25	0.14
Diaphragm (g)	-0.01	-0.46*	-0.59*	-0.30	-0.05
(%BW)	0.28	-0.27	-0.55*	0.14	0.03
Summed muscle (g)	-0.25	-0.25	-0.34#	-0.68**	-0.18
(%BW)	0.15	0.09	-0.45*	-0.37#	-0.13

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. EDL refers to extensor digitorum longus muscle; %BW refers to weight expressed as a percentage of body weight.

Table 5.11: Adiposity and salivary cortisol in the juvenile guinea pig

	Age (days)				
	5	10	15	20	30
Adipose depot	r	r	r	r	r
Dorsal Fat (g)	-0.45#	0.05	0.26	-0.26	-0.34
(%BW)	-0.31	0.17	0.34#	-0.04	-0.31
Shoulder Fat (g)	-0.07	-0.28	-0.09	-0.24	-0.06
(%BW)	0.06	-0.23	-0.07	-0.05	-0.04
Neck Fat (g)	-0.27	-0.02	0.33#	-0.08	-0.19
(%BW)	-0.18	0.03	0.34#	0.05	-0.15
Parametrial Fat (g)	-0.16	0.28	0.66*	-0.01	-0.46
(%BW)	-0.17	0.37	0.72*	0.04	-0.46
Retroperit'eal Fat (g)	-0.58*	-0.26	0.20	-0.31	-0.35
(%BW)	-0.56*	-0.22	0.28	-0.21	-0.37
Perirenal Fat (g)	-0.39#	-0.27	0.21	-0.27	-0.13
(%BW)	-0.31	-0.21	0.28	-0.15	-0.11
Epididymal Fat (g)	-0.52	-0.57#	-0.24	-0.50#	-0.31
(%BW)	-0.32	-0.48	-0.20	-0.15	-0.48
Groin Fat (g)	-0.39#	-0.15	0.20	-0.15	-0.28
(%BW)	-0.35	-0.09	0.27	-0.02	-0.29
Summed Fat (g)	-0.47#	-0.24	0.20	-0.29	-0.38
(%BW)	-0.39#	-0.18	0.31	-0.10	-0.39

** Denotes significance at p<0.001; * p<0.05; # p<0.1. %BW refers to weight expressed as a percentage of body weight.

5.4.10 Salivary cortisol and plasma thyroid hormones in the juvenile guinea pig

Plasma total T_3 and T_4 tended to decrease with increasing salivary cortisol at 5 days of age ($p<0.1$), and plasma total T_4 tended to decrease with increasing salivary cortisol 10 days of age ($p<0.1$). Plasma total T_3 correlated positively with salivary cortisol at 30 days of age ($r=0.67$). Plasma total T_3:T_4 was positively correlated with salivary cortisol at 10 days ($r=0.66$), and tended to correlate negatively with salivary cortisol at 30 days of age ($p<0.1$) (Table 5.12). Plasma free T_3 was negatively correlated with salivary cortisol at 5 days of age ($r=-0.49$), but was not associated with salivary cortisol at any other age examined (Table 5.12). Plasma free T_4 correlated positively with salivary cortisol at 30 days of age ($r=0.67$). Plasma free T_3:T_4 did not correlate with salivary cortisol at any age examined (Table 5.12).

5.4.11 Salivary cortisol and the IGF axis in the juvenile guinea pig

Plasma IGF-I correlated negatively with salivary cortisol at 5 days ($r=-0.45$) and 20 days ($r=-0.53$) of age (Table 5.13). Plasma IGF-II correlated negatively with salivary cortisol at 5 days of age ($r=-0.45$), as did plasma total IGFs ($r=-0.62$). Plasma IGFBP correlated negatively with salivary cortisol at 5 days of age ($r=-0.48$), and plasma IGF-I:IGFBP correlated negatively with salivary cortisol at 20 days ($r=-0.53$). IGF-I sensitivity of FFA metabolism correlated negatively with salivary cortisol at 5 days of age ($r=-0.67$), as did basal disposition index of FFA ($r=-0.73$) (Table 5.13).

Table 5.12: Salivary cortisol and plasma thyroid hormones in the juvenile guinea pig

	Age (days)				
	5	10	15	20	30
Plasma TH	r	r	r	r	r
Total T_3	-0.52#	0.20	0.06	0.18	0.67*
Total T_4	-0.40#	-0.35#	-0.02	0.39#	0.36
Total T_3:T_4	0.25	0.66*	0.25	-0.15	-0.53#
Free T_3	-0.49*	-0.24	-0.02	-0.01	0.40
Free T_4	-0.21	-0.13	-0.25	0.19	0.67*
Free T_3:T_4	0.35	0.04	0.08	0.01	-0.30

** Denotes significance at p<0.001; * p<0.05; # p<0.1.

Table 5.13: Salivary cortisol and the IGF axis in the juvenile guinea pig

	Age (days)				
	5	10	15	20	30
	r	r	r	r	r
Post-mortem plasma					
IGF-I ($ng.ml^{-1}$)	-0.45*	-0.32	0.02	-0.53*	-0.07
IGFBP	-0.48*	0.14	-0.14	-0.01	-0.01
IGF-I:IGFBP	-0.34	-0.31	0.25	-0.53*	-0.23
IGF-II ($ng.ml^{-1}$)	-0.47*	-0.29	0.10	0.16	0.24
IGF-II:IGFBP	-0.31	-0.36#	0.10	0.10	0.32
Total IGF ($ng.ml^{-1}$)	-0.62*	-0.37#	0.27	0.02	0.14
Total IGF:IGFBP	-0.21	-0.42#	0.10	-0.04	0.28
IGF-I sensitivity					
Glucose					
Adjusted SSGIR ($mg.ml.ng^{-1}.min^{-1}.kg^{-1}$)	0.22	0.09	0.08	0.46#	-0.15
Free Fatty Acids					
Adjusted %Δ FFA (%Δ$meq.ng^{-2}.ml$)	-0.67*	0.28	0.32	-0.33	0.56
Basal disposition Index					
Glucose (IGF-IxIGFS$_{glu}$Adj)	0.09	-0.22	-0.33	0.09	-0.73#
FFA (IGF-I x IGFS$_{FFA}$%Adj)	-0.73*	-0.15	-0.12	-0.49#	0.41

** Denotes significance at $p<0.001$; *$p<0.05$; # $p<0.1$. %Δ FFA is the percentage change in FFA from basal to plateau values in the last hour of the clamp; Adjusted SSGIR is the glucose infusion rate over the last hour of the clamp, adjusted for plateau plasma IGF-I. Units for basal disposition indices are as follows: Glucose (IGF-IxIGFS$_{glu}$Adj) ($mg.ng^{2}.min^{-1}.kg^{-1}.ml^{-2}$); FFA (IGF-I x IGFS$_{FFA}$%Adj) (%Δ$meq.ng^{-1}.ml^{-1}$).

5.5 Discussion

This is the first study to characterise plasma thyroid hormones and salivary cortisol during the catch-up growth period following fetal growth restriction in the guinea pig. In particular, this study has shown that low birth weight animals, and those animals with increased postnatal fractional growth rates of weight and length, have decreased plasma free T_4 levels, but increased ratios of plasma free T_3:T_4, at 36 days of age, possibly reflecting increased conversion of T_4 to the metabolically active form T_3.

Salivary cortisol concentrations are increased in LBW males compared to HBW males at all ages examined, however in LBW females, salivary cortisol is increased at 10 days of age, but decreased at 20 days of age, compared to HBW females. Salivary cortisol is also highest in the first 10 days of postnatal life in low birth weight animals (combined) undergoing catch-up growth in length and abdominal circumference, however, cortisol is lower at 30 days of life in those animals with increased fractional growth rates of head size. In female animals only, increased fractional growth rates of weight are associated with increased salivary cortisol at 20 days of age. Therefore it appears that fetal growth restriction in the guinea pig, followed by postnatal catch-up growth, is characterised by an increase in salivary cortisol concentrations in the period immediately following birth, however after ~ 10 days of life there is a sex difference, with salivary cortisol increased in LBW males but not in LBW females compared to their HBW counterparts. Sex differences in programming

of the HPA axis have been previously reported, In the rat, following maternal prenatal stress, female offspring have increased ACTH and corticosterone concentrations (Davies & Norman, 2002; McCormick et al., 1995; Weinstock et al., 1998), but this does not occur in males (McCormick et al., 1995), indicating that the female axis may be more susceptible to programming following maternal stress. However, there is much variation in reported outcomes in male animals following prenatal stress (Matthews, 2002).

Plasma free thyroid hormone concentrations, and an increased plasma free T_3:T_4 ratio also predict an increased muscle mass in the juvenile guinea pig, whereas increased cortisol concentrations after day 10 of postnatal life predict reduced weights of individual muscles in absolute and relative terms.

Plasma free T_3 and T_4 are reduced in blood from very low birth weight babies (Kilby et al., 1998; Martin et al., 2001), and free T_4 increases with increasing birth weight in pre-term infants in the first 5 weeks of life (Ng et al., 2001). We have also now shown that plasma free T_4 is reduced postnatally in the juvenile guinea pig following spontaneous fetal growth restriction. Similarly, plasma total T_4 and T_3 were reduced in the first few hours of life, and at 11 days of age following spontaneous fetal growth restriction in the lamb, and TH correlated positively with birth weight at these ages (Cabello & Levieux, 1981).

The increased plasma free T_3:T_4 ratio in LBW animals compared to HBW animals suggests that deiodinase activity may be increased in low birth weight animals postnatally, and this persists into the catch-up growth period, at least until 36 days of age. Maternal nutrient deprivation at day 50 of gestation in the

guinea pig also increases D2 deiodinase mRNA expression in the fetal brain (Chan et al., 2005). It has been suggested that an increase in deiodinase expression may act to protect the fetus by maintaining circulating T_3 levels, in response to the hypothyroxinemia observed in IUGR fetuses (Berthon et al., 1993; Mellor & Pearson, 1977). Whether this effect exists postnatally in the guinea pig is unknown.

In the guinea pig, following maternal nutrient deprivation, an upregulation of deiodinase expression observed coincides with an increase in glucocorticoid levels in these animals (Chan et al., 2005). Cortisol stimulates deiodination of circulating T_4 to T_3 in the fetal sheep (Forhead et al., 2000), and induces expression of type-II iodothyronine 5'-deiodinase in rat brain astrocyte cells (Leonard et al., 1990). In the current study, we observed an increase in salivary cortisol levels in the first 10 days of life. In the fetus, as term approaches (in the last 10 weeks of gestation), fetal tissues including the liver and lung express 11-ketosteroid reductase activity that promotes local conversion of cortisone to cortisol (Fisher, 1998). Plasma cortisol in the fetus increases due to increased ACTH release from the fetal pituitary which acts on the fetal adrenal to increase cortisol secretion (Mastorakos & Ilias, 2003), and this rise in cortisol helps to trigger birth, having an important role in the maturation of several fetal systems critical to extrauterine survival (Challis et al., 2001; Fowden, 1995). This increase in cortisol in late gestation might act to increase deiodinase activity, increasing peripheral conversion of T_4 to T_3, and although the increased salivary cortisol levels do not persist after day 10 postnatally (in the female), the increased conversion to the active form (T_3) appears to persist until at least 36

days. If cortisol continued to increase conversion of T_4 to T_3, we would expect a positive relationship of salivary cortisol and plasma T_3 at later ages. However, at 30 days of age, salivary cortisol is positively correlated with plasma free T_4 measured at day 36 of age, suggesting that by this stage of postnatal life, cortisol is not acting directly on deiodinase activity.

The increase in salivary cortisol levels seen in the first 10 days of life in low birth weight animals is in accordance with previous studies that have shown increased activity of the HPA axis following IUGR in the human (Goland et al., 1993; Jackson et al., 2004) and pig (Poore & Fowden, 2003). In the current study, salivary cortisol in early postnatal life was positively correlated with fractional growth rates of length and abdominal circumference, and tended to associate positively with fractional growth rates of weight. This is in contrast to previous studies, in which cortisol appears to have an inhibitory effect on growth in humans (Cianfarani et al., 2002; Tenhola et al., 2005), and animal species (Gafni et al., 2002). However, in the spontaneously growth restricted pig, hyperactivity of the HPA axis is seen in low birth weight pigs, who exhibit catch-up growth (Poore & Fowden, 2003). In the current study, by 20 days of age, cortisol was inversely related to absolute growth rates of weight, and at 30 days of age inverse correlations were seen with fractional growth rates of head length and head width. It is possible that HPA axis has separate actions in the first days of postnatal life than those after ~ 2 weeks of life in the juvenile guinea pig. Previous studies that have shown an inverse relationship of cortisol and postnatal growth exists at later ages in the human (9 years of age (Cianfarani et al., 2002); 12 years of age (Tenhola et al., 2005).

Although the correlations between salivary cortisol and adipose tissue mass were not consistently significant throughout postnatal life, the relationships seen at days 5 and 15 warrant some comment. Salivary cortisol measured at 5 days of age was negatively correlated with body fat mass at day 36, however by day 15 these correlations were positive. In the growth restricted pig, plasma cortisol measured at 12 months of age are negatively related to fat depths (Poore & Fowden, 2003), however elevated levels of glucocorticoids are associated with abdominal obesity in adult life (Barker et al., 1993; Seckl, 1997). It appears that salivary cortisol in the juvenile guinea pig may also be associated with increased fat mass, yet only in female offspring. The low number of animals studied at day 30 can probably explain the lack of significant correlations between day 30 salivary cortisol and fat mass. In addition, although day 20 cortisol was not associated with body fat mass, it was strongly negatively correlated with fat mass such that increased salivary cortisol predicted a decreased fat mass at day 36 of postnatal life.

In conclusion, LBW guinea pigs have increased salivary cortisol concentrations in the first 10 days of life, but this association only persists in male offspring. This may have persistent actions long-term on the thyroid hormone axis, as highlighted by the increase in plasma free T_3:T_4 at 36 days of age. This appears to be a programming effect that could originate in utero, as cortisol after 20 days of age is not associated with increases in the active form of TH, T_3. Increased salivary cortisol concentrations after day 20 of postnatal life appear to inhibit postnatal growth rates, and inhibit muscle growth. Cortisol measured in the first

10 days after postnatal life is associated with decreased adiposity and increased growth rates. This suggests that cortisol levels after the first two weeks of life have a greater effect on postnatal growth and in determining body composition, than cortisol measured in the immediate 2 weeks after birth. This study also provides evidence for a role for thyroid hormones in postnatal growth and increased muscle mass at 36 days of age. It appears that LBW animals have increased free T_3:T_4, which may in part help to drive postnatal catch-up growth, and suggests that cortisol levels in postnatal life are extremely important in determining postnatal outcomes following IUGR.

Chapter 6

The effect of maternal metyrapone treatment on size at birth and postnatal endocrine state in the juvenile guinea pig

6.1 Introduction

Fetal growth restriction is followed by catch-up growth after birth in the first few months of life in humans, sheep and as shown in Chapter 2, in the guinea pig. The mechanisms responsible for catch-up growth after IUGR may include prenatally induced increased sensitivity to the anabolic actions of IGFs (see Chapter 3), and insulin (De Blasio, 2004), as well as enhanced activation of TH with increased T_3 relative to T_4 (see Chapter 5). The latter may be due in part to increased systemic cortisol present in the low birth weight guinea pig following birth, as glucocorticoids are known to enhance deiodination of T_4. This suggests that intrauterine events associated with or causal of IUGR alter postnatal growth and its endocrine control by affecting the functional development of the major endocrine axes normally regulating infant growth.

While reduced delivery and abundance of oxygen and glucose and other nutrients is often responsible for IUGR (Owens et al., 1986; Owens et al., 1987), the nature of the specific substrate deficits and/or the fetal endocrine and other responses to these, which program altered endocrine control of postnatal growth and contribute to catch-up growth are unknown. One candidate is fetal exposure to increased glucocorticoids in utero in late gestation, which is characteristic of both human (Goland et al., 1993) and experimental IUGR (Robinson & Owens, 1996) in other species, including the guinea pig (Briscoe et al., 2004). Increased glucocorticoid exposure in utero may permanently

program HPAA function, leading to persistent elevations in glucocorticoids postnatally (Challis et al., 2001).

Following placental restriction in the sheep, increased insulin sensitivity is apparent in the neonate (De Blasio, 2004), and in the first 48 hours after birth, human SGA infants are more insulin sensitive compared to control subjects (Bazaes et al., 2003). Increased postnatal growth rate in the first 6 months of life is associated with increased insulin release in response to an injection of glucose (Colle et al., 1976). In addition, infants with the highest weight gain velocity (catch-up growth) and the lowest birth weights, have been shown to be the most insulin sensitive, suggesting that increased insulin sensitivity is associated with catch-up growth (Gray et al., 2002). Cortisol may also play a role in programming of postnatal insulin action and glucose metabolism, as cortisol increases insulin sensitivity of skeletal muscle in the fetal sheep, through increasing expression of GLUT-4, an insulin-sensitive glucose transporter (Li et al., 1998b).

Cortisol may also act to initiate maturational changes in the somatotropic axis. Cortisol infusion increases hepatic IGF-I and GH receptor gene expression in adrenalectomised fetuses and in intact fetuses (Li et al., 1996). Conversely, fetal adrenalectomy prevents the normal decline in IGF-I mRNA abundance in muscle that occurs in late gestation in the fetal sheep, and cortisol infusion lowers muscle IGF-I mRNA compared to saline infused controls (Li et al., 2002), suggesting a tissue-specific role for cortisol in regulating the somatotropic axis. This may explain why fetal cortisol infusion does not affect fetal plasma IGF-I

concentrations (Li et al, 1996). The mechanism of cortisol action on IGF-I is unclear. It is unlikely that cortisol directly acts on the IGF-I gene, as it does not contain any glucocorticoid response elements (GREs) (Dickson et al, 1991). It may be possible that cortisol acts through initiating other endocrine changes, such as increasing deiodination of TH, as discussed in Chapter 5.

Although there are numerous studies on the effect of exogenous glucocorticoid administration prenatally (Matthews, 2002), we were interested in the effect of lowering prenatal glucocorticoids on postnatal outcome. Metyrapone is a competitive inhibitor of the steroidogenic enzyme 11β-hydroxylase (Lye & Challis, 1984), the last enzyme in the biosynthetic pathway for cortisol, which catalyses the formation of cortisol from 11-desoxycortisol. Metyrapone can be used experimentally to inhibit cortisol synthesis, and therefore is useful when studying the actions of cortisol in vivo. Metyrapone was administered to adult guinea pigs for 7 days at a dose of 50mg/kg/day to investigate the role of endogenous cortisol in modulating distal air space liquid clearance (Norlin et al., 1999). At this dose, 2 days of metyrapone treatment reduced plasma cortisol levels by 46% compared with control (Norlin et al., 1999). Recent studies have indicated that metyrapone infusion in the fetal sheep reduces fetal cortisol concentrations during the first 6 hours following infusion, however these levels rise to pre-infusion values by 24 hours post-infusion (Warnes et al., 2003). Fetal body weight and crown-rump length is not altered by metyrapone infusion (Warnes et al., 2003; Warnes et al., 2004), however the relative weights of adrenals, kidneys and liver are increased (Warnes et al., 2003), suggesting that inhibition of cortisol synthesis enhanced growth of some fetal tissues.

In this study, we administered metyrapone at 10mg/kg/day to the mother at day 42 of gestation, and measured outcomes in the offspring. We investigated the effects of inhibiting cortisol on size at birth, postnatal growth and the IGF and TH axes in the juvenile guinea pig, and also looked at their body composition at 40 days of life.

6.2 General Aim

To investigate the effects of maternal metyrapone treatment in late gestation on juvenile guinea pig offspring.

Specific Aim 1:

To investigate the effect of maternal metyrapone treatment in late gestation on size at birth and postnatal growth rates in the first 40 days of life in juvenile offspring, and body composition at 40 days of life

Specific Aim 2:

To investigate the effect of maternal metyrapone treatment in late gestation on insulin sensitivity and circulating concentrations of IGFs and thyroid hormones, and their relationship to postnatal growth and body composition in the juvenile guinea pig.

6.3 Materials and methods

6.3.1 *Mating and housing of guinea pigs*

All guinea pigs (IMVS tri coloured, Gilles Plains Resource Centre, SA) were caged individually in plastic tubs with wire lids, and maintained in a room with a 12:12h light:dark cycle at 25°C, with *ad libitum* access to guinea pig/rabbit ration (Ridley Agriproducts, Australia). Males used for mating were fed lucerne in addition to the guinea pig chow. All guinea pigs also had *ad libitum* access to tap water, containing 400mg/L vitamin C. Females were weighed and checked for oestrus daily, as recognized by an opening of the vaginal orifice associated with a drop in body weight occurring every 15-17 days. They were put into a wire cage with a male for the duration of oestrus. Pregnancy was detected by the presence of a copulatory plug, and/or the absence of oestrus in the next cycle. All pups were weaned at 29 days of age onto normal guinea pig chow and fed *ad libitum*. Previously, it has been shown that litter size can be estimated by weight gain during pregnancy (J.A. Owens, unpublished observations). Therefore, body weight gain was determined at 10, 20, 30 and 40 days gestation to estimate litter size for treatment group allocation. Vehicle and metyrapone treated groups were matched for litter sizes. A description of the total number of animals used in each experiment can be found in Appendix A.

6.3.2 Maternal metyrapone treatment

At 42 days gestation (term = 70 days), an osmotic pump (model 2ML4 Alzet osmotic pump, Alza scientific products, California, USA) was implanted subcutaneously over the dorsal fat pad in the pregnant guinea pig, under general anaesthesia (0.05 mg/kg Atropine s.c., 4 mg/kg Xylazine i.m., and 25 mg/kg Ketamine i.m., Lignocaine locally as required). The pump contained either metyrapone or vehicle in 0.9% sterile saline, and was guaranteed to pump at a rate of 2.5 µl/hour for 28 days delivering metyrapone 10 mg/kg/day, tartaric acid 11 mg/kg/day or vehicle tartaric acid 11 mg/kg/day. Doses were based on maternal weight at 41 days gestation. Animals were maintained at 37^0C from induction of anaesthetic until recovery. After implantation of the pump, animals were housed in paper-lined cages and allowed to deliver naturally at term.

6.3.3 Size at birth and postnatal growth

The total number of animals used in each experiment is summarised in Appendix B, and total number of litters used is summarised in Appendix C. Offspring were weighed at birth, and 4 times per week until post-mortem at 41 ± 0.2 days postnatally. Crown-rump length (CRL), abdominal circumference (AC), head length (HL) and head width (HW) were measured at birth (see Table 1.1). Body mass index was calculated as weight/CRL^2 (kg/cm^2), and ponderal index as weight/CRL^3 (kg/cm^3). Absolute growth rates of weight were calculated for each guinea pig (AGR) by calculating the slope of the regression line of

postnatal age and size, and then expressed in terms of individual birth size to give a measure of catch up growth (fractional growth rate: FGR).

6.3.4 Insertion of vascular catheters and maintenance

At 29 days of age, polypropylene catheters were inserted into the external jugular vein and right common carotid artery under general anaesthetic (0.05 mg/kg Atropine s.c., 6 mg/kg Xylazine i.m., 25 mg/kg Ketamine i.p., Lignocaine locally as required). Catheters were flushed daily with 500 µl of 250 U/ml heparin in saline for 2 days following surgery, and then daily with 500 µl of 50 U/ml heparin in saline.

6.3.5 Hyper-insulinemic-euglycaemic clamp and insulin sensitivity of glucose and FFA metabolism

At 34 ± 1 days of age, insulin sensitivity of glucose metabolism was measured by HEC. Human insulin was infused at a rate of 7.5 mU/kg/min, while glucose (10%) was infused at a variable rate to maintain euglycemia. Blood samples were taken every 5 minutes to assess whole blood glucose levels throughout the HEC. Blood glucose in the arterial blood samples taken during the HEC was measured using a glucometer (Hemocue AB, Sweden). Plasma glucose concentrations were measured prior to and during the final 60 mins of the HEC (t=-20, -10, 0, 60, 75, 80, 85, 90, 95, 105, 120 minutes), using the COBAS MIRA automated sample system, as described in Chapter 3.3.10. Blood was centrifuged and plasma stored at -20^0C for subsequent analysis.

Whole body insulin sensitivity of glucose metabolism was recorded as the steady state glucose infusion rate (SSGIR) averaged during the second hour of the HEC, calculated using a specific algorithm from a previously published version (DeFronzo et al., 1979).

6.3.6 Measurement of plasma IGF-I, IGF-II and IGFBP concentrations

Plasma IGF-I, -II and IGFBPs were separated as described in Chapter 3.3.5. Plasma IGF-I, IGF-II and IGFBP concentrations were measured by specific RIAs, as described in Chapters 3.3.6, 3.3.7 and 3.3.8.

6.3.7 Measurement of free and total thyroid hormones

Free thyroid hormone concentrations were measured in plasma, collected at post-mortem. All thyroid hormones were measured using specific RIAs (Cis-Bio International, France), as described in Chapters 5.3.6.1, 5.3.6.2, 5.3.6.3 and 5.3.6.4.

6.3.8 Post-mortem

Offspring were sacrificed at 41 ± 0.2 days by an intraperitoneal overdose of sodium pentobarbitone (325 mg/ml, Lethabarb, Virbac, Australia). Blood samples were obtained by cardiac puncture immediately following death in heparinised tubes, and plasma obtained after centrifugation at 4°C and frozen at –20°C until assay. All organs, muscles and adipose depots were dissected and weights recorded.

6.3.9 Statistics

All data are presented as mean ± SEM unless otherwise stated. The effect of metyrapone treatment, sex of offspring and their interaction was analysed using 2-way ANOVA (SPSS Inc, Chicago).

6.4 Results

6.4.1 *Metyrapone treatment, size at birth and postnatal growth*

Maternal metyrapone (MET) treatment increased abdominal circumference (p=0.001) and head length (p=0.042) of offspring at birth (Figure 6.1). Weight:length at birth was higher in males (p=0.048) than females. Metyrapone treatment increased AGR_{weight} (p=0.008) (Figure 6.3), and a sex effect was seen, such that males had increased AGR_{weight} compared to females (p=0.001) (Table 6.1). FGR_{weight} was not affected by MET treatment, however tended to be greater in males compared to females (p=0.075) (Table 6.1; Figure 6.2). In addition, FGR_{weight} correlated negatively with birth weight in males and females when treatments were combined (Figure 6.2).

When birth weight was controlled for as a covariate, birth AC, birth HW and AGR_{weight} were increased by maternal metyrapone ($p<0.05$; Table 6.2). In addition, FGR_{weight} was increased by maternal metyrapone after adjustment for birth weight (p=0.007; Figure 6.3). Males still had increased AGR_{weight} compared to females (p=0.001), but also had increased FGR_{weight} after adjusting for birth weight (p=0.001) (Table 6.2).

Table 6.1: The effect of maternal metyrapone treatment on size at birth and postnatal growth in the guinea pig

	Male		Female		ANOVA (p-value)		
	Control (11)	Met (13)	Control (10)	Met (8)	Met	S	MetxS
Size at birth							
Weight (g)	91.7 ± 4.0	95.7 ± 3.9	86.1 ± 3.4	89.6 ± 3.3	ns	0.07	ns
Crown-rump length (mm)	159.9 ± 2.1	161.8 ± 3.5	158.0 ± 2.0	160.0 ± 4.4	ns	ns	ns
Abdominal circumference (mm)	88.8 ± 2.8	101.8 ± 1.7	87.1 ± 3.8	94.6 ± 2.3	0.001	0.055	ns
Head length (mm)	43.5 ± 2.0	47.0 ± 0.6	45.5 ± 0.6	46.3 ± 1.0	0.042	ns	ns
Head width (mm)	25.4 ± 2.1	22.8 ± 0.4	23.0 ± 0.4	22.6 ± 0.7	ns	ns	ns
Weight:length ($g.mm^{-1}$)	5.7 ± 0.2	5.9 ± 0.2	5.4 ± 0.2	5.6 ± 0.1	ns	0.048	ns
Ponderal Index ($g.cm^{-3}$) ($*10^2$)	2.23 ± 0.05	2.28 ± 0.09	2.18 ± 0.05	2.22 ± 0.13	ns	ns	ns
Body Mass Index ($g.cm^{-2}$) ($*10^1$)	3.57 ± 0.09	3.65 ± 0.09	3.44 ± 0.08	3.52 ± 0.11	ns	0.085	ns
Postnatal growth							
AGR of weight (g/day)	8.9 ± 0.3	10.3 ± 0.4	8.2 ± 0.2	8.6 ± 0.3	0.008	0.001	0.095
FGR of weight (%/day) ($*10^2$)	10.0 ± 0.6	11.1 ± 0.8	9.6 ± 0.3	9.6 ± 0.3	ns	0.075	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days.

Table 6.2: The effect of maternal metyrapone treatment on size at birth and postnatal growth in the guinea pig with birth weight as a covariate

	Male		Female		ANOVA (p-value)		
	Control (11)	Met (13)	Control (10)	Met (8)	Met	S	MetxS
Size at birth							
Crown-rump length (mm)	159.6 ± 1.9	158.9 ± 1.7	161.3 ± 2.0	160.1 ± 2.2	ns	ns	ns
Abdominal circumference (mm)	88.6 ± 2.4	100.5 ± 2.3	88.5 ± 2.6	95.0 ± 2.9	0.001	ns	ns
Head length (mm)	43.4 ± 1.1	46.6 ± 1.1	45.9 ± 1.2	46.4 ± 1.3	0.065	ns	ns
Head width (mm)	25.4 ± 1.1	22.2 ± 1.0	23.7 ± 1.1	22.8 ± 1.2	0.036	ns	ns
Weight:length ($g.mm^{-1}$)	5.70 ± 0.07	5.72 ± 0.06	5.64 ± 0.07	5.66 ± 0.08	ns	ns	ns
Ponderal Index ($g.cm^{-3}$) ($*10^2$)	2.23 ± 0.08	2.29 ± 0.08	2.16 ± 0.09	2.22 ± 0.10	ns	ns	ns
Body Mass Index ($g.cm^{-2}$) ($*10^1$)	3.56 ± 0.09	3.60 ± 0.08	3.49 ± 0.09	3.53 ± 0.01	ns	ns	ns
Postnatal growth							
AGR of weight (g/day)	8.9 ± 0.3	10.3 ± 0.3	8.2 ± 0.4	8.6 ± 0.4	0.008	0.001	0.093
FGR of weight (%/day) ($*10^2$)	10.0 ± 0.4	11.6 ± 0.4	8.9 ± 0.4	9.4 ± 0.5	0.007	0.001	0.082

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days.

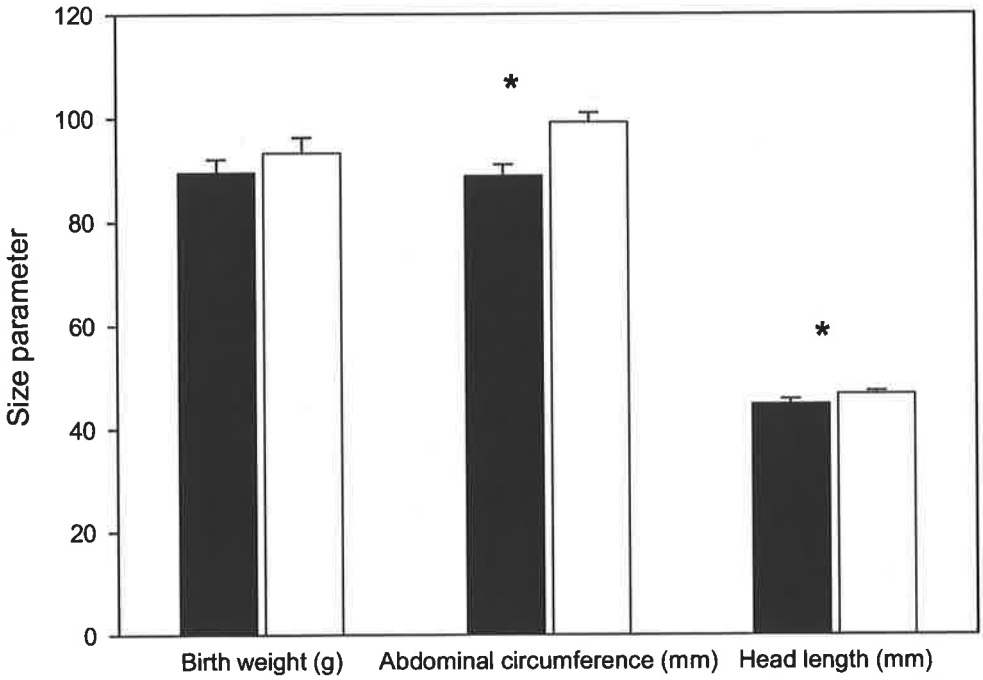

Figure 6.1: The effect of maternal metyrapone treatment on size at birth in the juvenile guinea pig

Maternal vehicle treatment is represented by black, and metyrapone treatment by white bars. Significance at $p<0.05$ *. Males and females are combined. Birth weight was not included as a covariate.

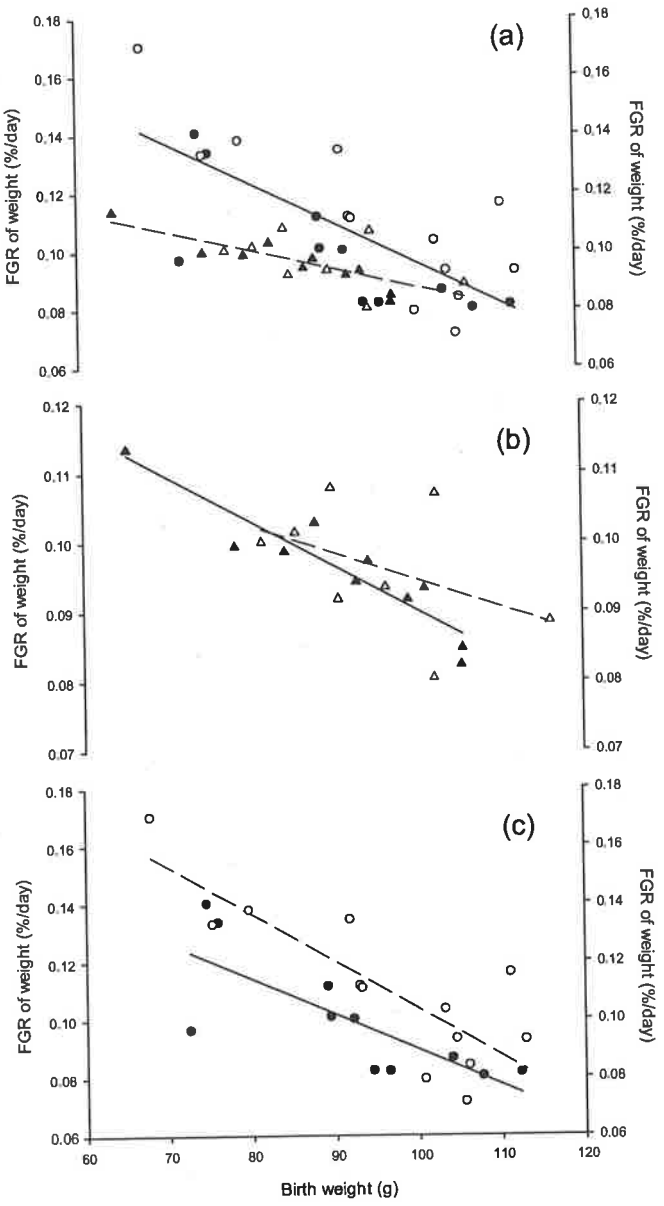

Figure 6.2: The association between birth weight and fractional growth rate of weight in response to metyrapone treatment

Males: vehicle = closed circles; metyrapone = open circles. Females: vehicle = closed triangles; female metyrapone = open triangles (a): Male regression line is solid; female is dashed. Males: weight correlated negatively with FGR_{weight} (r=-0.74; p=0.0005**; regression equation $y=0.23x - 1.4e^{-3}$). Females: weight correlated negatively with FGR_{weight} (r=-0.71; p=0.001**; regression equation $y=0.15x - 6.4e^{-4}$). (b): Female vehicle (regression solid line): weight correlated negatively with FGR_{weight} (r=-0.87); female metyrapone (regression dashed line): weight correlated negatively with FGR_{weight} (r=-0.64). (c) Male vehicle (regression solid line): weight correlated negatively with FGR_{weight} (r=--0.68); male metyrapone (regression dashed line): birth weight correlated negatively with FGR_{weight} (r=-0.78) ($p<0.05$ for all).

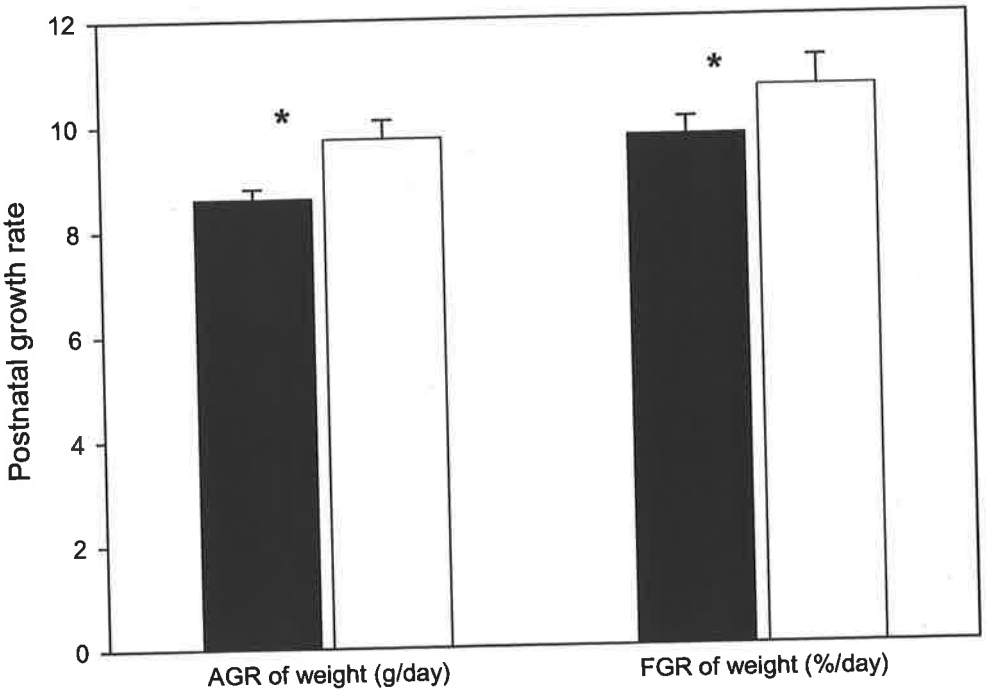

Figure 6.3: The effect of maternal metyrapone treatment on postnatal growth rate in the juvenile guinea pig

Maternal vehicle treatment is represented by black, and metyrapone treatment by white bars. Significance at $p<0.05$ *. Males and females are combined; birth weight was included as a covariate.

6.4.2 The effect of maternal metyrapone treatment on organ size in the juvenile guinea pig

Metyrapone increased body weight (+8%; p=0.022) and tended to increase body length (+3.5%; p=0.06) in the juvenile guinea pig (Figure 6.4). Body weight (p=0.001) and length (p=0.02) also differed with sex, such that males were larger (Table 6.3). Metyrapone increasing body length in females to a greater extent than in males (p=0.038; Table 6.3).

Metyrapone increased weights of kidney (p=0.03), GIT (p=0.027), liver (p=0.001), and heart (p=0.008) (Figure 6.5), and also the relative weight of the heart (p=0.04). However, the relative weight of the brain was decreased by maternal metyrapone treatment (p=0.017) (Figure 6.5). Adrenal weights, and the relative weight of the brain were higher in females compared to males ($p<0.05$) (Table 6.3). In contrast, kidney, brain, liver and thyroid weights were all lower in females compared to males ($p<0.05$) (Table 6.3). Maternal metyrapone tended to reduce adrenal weight in male offspring, but increased it in females (p=0.078; Table 6.3). Maternal metyrapone also altered the relative weight of the kidney different with sex, increasing it in males, but decreasing it in females (p=0.07; Table 6.3).

After adjusting for birth weight, body length in the juvenile guinea pig was no longer altered by maternal metyrapone, but body weight was still increased (p=0.043; Table 6.4). Maternal metyrapone also altered body weight (p=0.015) and body length (p=0.023) differently with sex, increasing both to a greater

extent in female offspring (+ 9.9% and + 7.7% respectively) than in males (+ 2.3% and + 0% respectively) (Table 6.4). Maternal metyrapone also increased weights of the kidney, GIT, liver and heart, and the relative weights of liver and heart, after adjusting for birth weight ($p<0.05$ for all; Table 6.4). Maternal metyrapone decreased the relative weights of the brain, pancreas and thyroid after adjusting for birth weight ($p<0.05$; Table 6.4). Males had increased kidney, brain and liver weights after adjusting for birth weight, however had decreased adrenal weights and decreased relative adrenal, pancreas; GIT, and brain weights compared to females ($p<0.05$; Table 6.4).

Table 6.3: The effect of maternal metyrapone treatment on organ size in the juvenile guinea pig

	MALES		FEMALES		ANOVA p-value		
Organ	*Control (8)*	*Met (12)*	*Control (8)*	*Met (8)*	*Met*	*Sex*	*Met x Sex*
Body weight (g)	391 ± 12	409 ± 12	315 ± 13	353 ± 15	0.022	0.001	ns
Body length (mm)	254 ± 4	253 ± 5	233 ± 6	251 ± 6	0.06	0.02	0.038
Adrenal (g)	0.155 ± 0.009	0.14 ± 0.005	0.158 ± 0.009	0.17 ± 0.009	ns	0.035	0.078
(%BW)	0.04 ± 0.003	0.04 ± 0.001	0.05 ± 0.003	0.04 ± 0.003	0.077	0.001	ns
Kidney (g)	3.69 ± 0.12	4.13 ± 0.18	3.36 ± 0.18	3.59 ± 0.17	0.03	0.008	ns
(%BW)	0.95 ± 0.03	1.01 ± 0.03	1.12 ± 0.08	1.00 ± 0.02	0.09	ns	0.07
Pancreas (g)	1.25 ± 0.08	1.11 ± 0.12	1.12 ± 0.04	1.22 ± 0.14	ns	ns	ns
(%BW)	0.32 ± 0.02	0.27 ± 0.02	0.36 ± 0.01	0.34 ± 0.04	ns	ns	ns
GIT (g)	61.0 ± 1.7	68.9 ± 4.9	58.2 ± 3.1	67.5 ± 4.5	0.027	ns	ns
(%BW)	16.0 ± 0.4	17.0 ± 1.3	18.9 ± 0.9	18.6 ± 0.9	ns	0.02	ns
Brain (g)	3.66 ± 0.10	3.64 ± 0.06	3.42 ± 0.06	3.55 ± 0.06	ns	0.011	ns
(%BW)	0.96 ± 0.03	0.90 ± 0.02	1.09 ± 0.03	1.02 ± 0.04	0.017	0.001	ns
Liver (g)	16.1 ± 0.6	17.7 ± 0.7	12.6 ± 0.3	15.1 ± 0.6	0.001	0.001	ns
(%BW)	4.12 ± 0.07	4.35 ± 0.15	4.02 ± 0.11	4.26 ± 0.14	0.08	ns	ns
Lungs (g)	3.2 ± 0.2	3.74 ± 0.3	4.02 ± 0.1	3.09 ± 0.4	ns	ns	ns
(%BW)	0.82 ± 0.06	0.93 ± 0.09	0.99 ± 0.12	0.88 ± 0.10	ns	ns	ns
Heart (g)	1.57 ± 0.12	2.08 ± 0.23	1.35 ± 0.09	1.90 ± 0.27	0.008	ns	ns
(%BW)	0.40 ± 0.03	0.50 ± 0.05	0.43 ± 0.03	0.51 ± 0.09	0.04	ns	ns
Thyroid (g)	0.08 ± 0.003	0.07 ± 0.01	0.06 ± 0.003	0.06 ± 0.008	ns	0.03	ns
(%BW)	0.019 ± 0.001	0.017 ± 0.003	0.019 ± 0.001	0.016 ± 0.002	0.092	ns	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days.

Table 6.4: The effect of maternal metyrapone treatment on organ size in the juvenile guinea pig using birth weight as a covariate

	MALES		FEMALES		ANOVA p-value		
Organ	***Control (8)***	***Met (12)***	***Control (8)***	***Met (8)***	***Met***	***Sex***	***Met x Sex***
Body weight (g)	393 ± 12	402 ± 10	322 ± 12	354 ± 13	0.043	ns	0.015
Body length (mm)	254 ± 4	253 ± 5	233 ± 6	251 ± 6	ns	ns	0.023
Adrenal (g)	0.155 ± 0.009	0.143 ± 0.007	0.157 ± 0.008	0.169 ± 0.009	ns	0.048	0.071
(%BW)	0.04 ± 0.003	0.036 ± 0.002	0.049 ± 0.003	0.047 ± 0.003	ns	0.001	ns
Kidney (g)	3.69 ± 0.18	4.12 ± 0.15	3.37 ± 0.19	3.59 ± 0.18	0.038	0.012	ns
(%BW)	0.94 ± 0.05	1.03 ± 0.04	1.06 ± 0.05	1.00 ± 0.05	ns	ns	0.055
Pancreas (g)	1.26 ± 0.10	1.06 ± 0.08	1.17 ± 0.10	1.23 ± 0.10	ns	ns	0.081
(%BW)	0.33 ± 0.03	0.26 ± 0.02	0.36 ± 0.03	0.34 ± 0.03	0.036	0.012	ns
GIT (g)	61.0 ± 4.5	69.1 ± 3.8	58.0 ± 4.9	67.5 ± 4.2	0.03	ns	ns
(%BW)	15.9 ± 1.0	17.3 ± 0.8	18.4 ± 1.1	18.6 ± 1.0	ns	0.03	ns
Brain (g)	3.67 ± 0.07	3.62 ± 0.06	3.44 ± 0.07	3.56 ± 0.07	ns	0.02	ns
(%BW)	0.96 ± 0.03	0.91 ± 0.02	1.08 ± 0.03	1.02 ± 0.03	0.037	0.001	ns
Liver (g)	16.1 ± 0.6	17.6 ± 0.5	12.7 ± 0.6	15.1 ± 0.6	0.002	0.001	ns
(%BW)	4.10 ± 0.12	4.41 ± 0.11	3.96 ± 0.13	4.26 ± 0.14	0.018	ns	ns
Lungs (g)	3.16 ± 0.31	3.85 ± 0.26	2.97 ± 0.32	3.07 ± 0.31	ns	0.059	ns
(%BW)	0.81 ± 0.09	0.97 ± 0.08	0.95 ± 0.09	0.88 ± 0.10	ns	ns	0.095
Heart (g)	1.57 ± 0.22	2.08 ± 0.19	1.37 ± 0.22	1.90 ± 0.22	0.012	ns	ns
(%BW)	0.40 ± 0.05	0.51 ± 0.04	0.43 ± 0.05	0.51 ± 0.05	0.031	ns	ns
Thyroid (g)	0.08 ± 0.007	0.06 ± 0.008	0.06 ± 0.008	0.06 ± 0.007	ns	ns	ns
(%BW)	0.02 ± 0.002	0.015 ± 0.002	0.02 ± 0.002	0.017 ± 0.002	0.026	ns	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days.

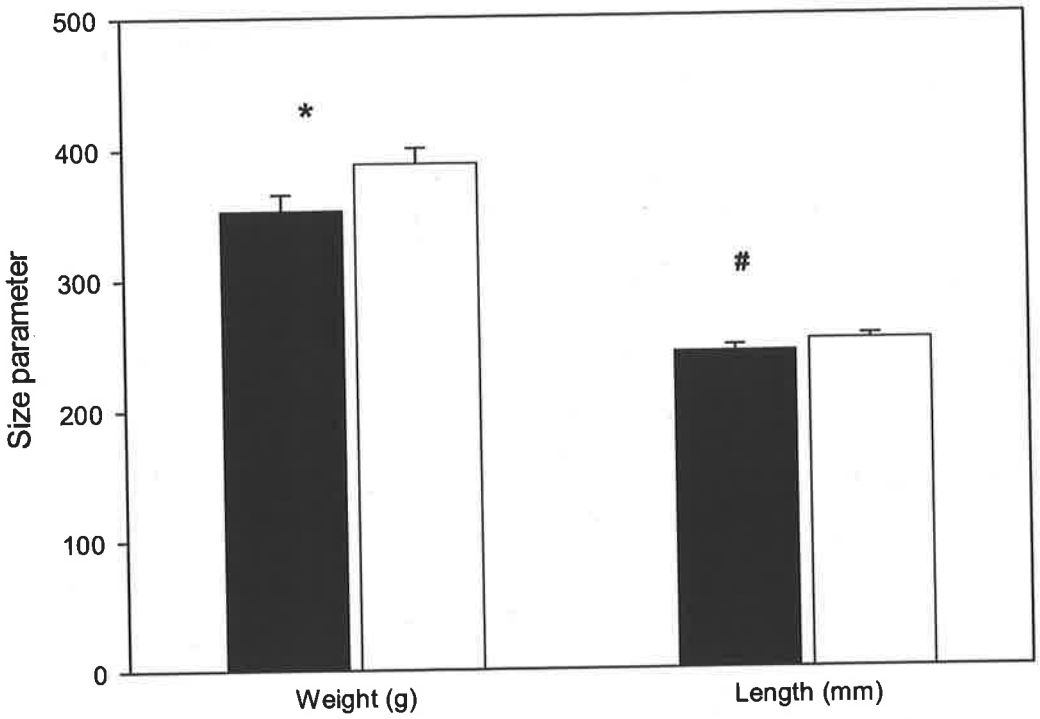

Figure 6.4: The effect of metyrapone treatment on body weight and length at 40 days of age

Vehicle treatment is represented by black, and metyrapone treatment by white bars. * denotes significance at $p<0.05$, # $p<0.1$. Males and females are combined.

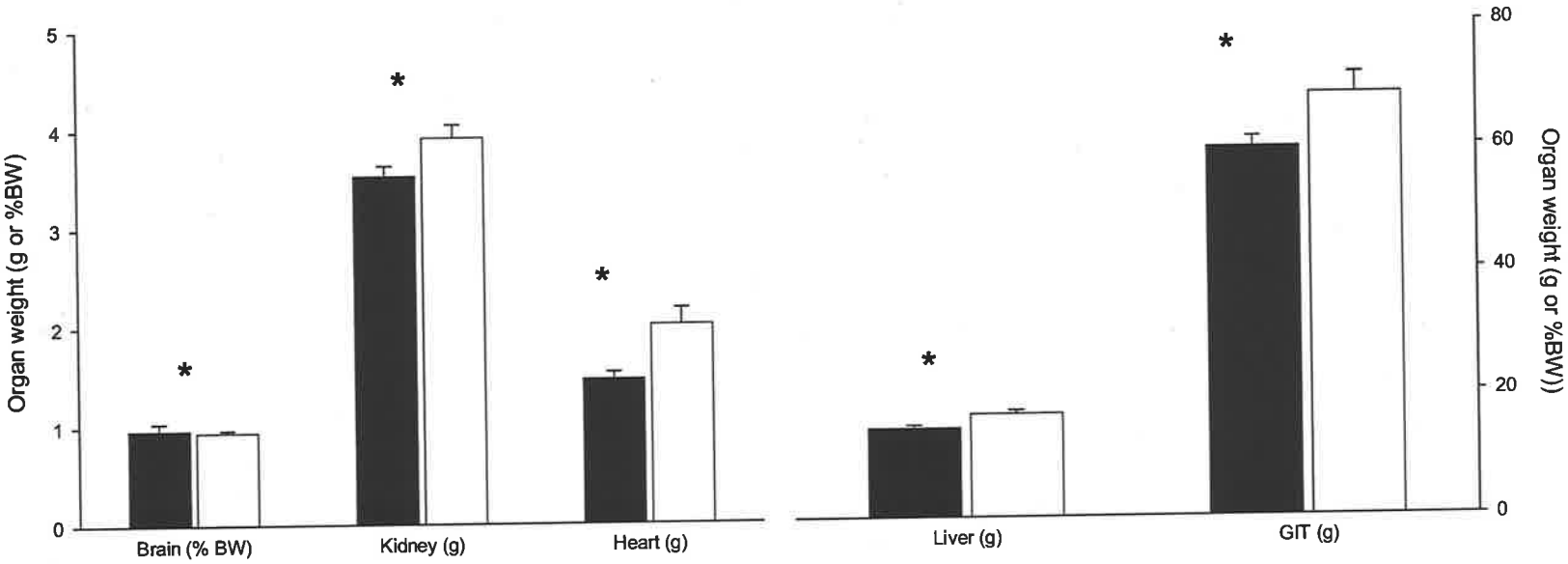

Figure 6.5: The effect of metyrapone treatment on organ size at 40 days of age

Vehicle treatment is represented by black, and metyrapone treatment by white bars. * denotes significance at $p<0.05$. Males and females are combined

6.4.3 Maternal metyrapone treatment and body composition in the juvenile guinea pig

Maternal metyrapone increased summed muscle weight (p=0.031), and the individual weights of gastrocnemious (p=0.03), semitendinosus (p=0.025) and biceps femoris (p=0.002) (Table 6.5; Figure 6.6). Males had increased summed muscle mass (p=0.002), and individual weights of gastrocnemious, semitendinosus, vastus lateralis, biceps brachii and diaphragm muscles compared to females ($p<0.05$ for all) (Table 6.5). Maternal metyrapone altered absolute (p=0.02) and relative weights (p=0.048) of the semitendinosus muscle, with both decreasing in males, but increasing in females (Table 6.5).

After adjusting for birth weight, maternal metyrapone still increased summed muscle weight (p=0.048), and that of biceps femoris (p=0.049) and gastrocnemious (p=0.03) (Table 6.6). Males also had increased summed muscle weight, and weights of gastrocnemious, semitendinosus, vastus lateralis, biceps brachii, diaphragm, and biceps femoris muscles compared to females, after adjusting for birth weight ($p<0.05$ for all; Table 6.6). Maternal metyrapone altered the absolute (p=0.02) and relative (p=0.04) weights of the semitendinosus muscle with sex, decreasing it in males but increasing it in females (Table 6.6).

Maternal metyrapone tended to increase summed fat weight (p=0.051; Table 6.7), and weights of dorsal and neck fat, as well as relative dorsal fat weight ($p<0.05$) (Table 6.7). Males had more summed fat than females (p=0.034; Table 6.7). Males had more dorsal fat, retroperitoneal fat and perirenal fat weight, as

well as increased retroperitoneal and perirenal fat relative to body weight ($p<0.05$ for all). However, males had less neck fat and groin fat than females ($p<0.05$) (Table 6.7).

After adjusting for birth weight, maternal metyrapone still increased dorsal fat and neck fat weights ($p<0.05$), but no longer increased relative dorsal fat weight (Table 6.8). The difference in summed fat weights between males and females appeared to be dependent on birth weight, as summed fat ($p=0.06$) and dorsal fat ($p=0.052$) weights only tended to be increased in males ($p=0.06$) compared to females. Retroperitoneal and perirenal absolute and relative fat weights were still increased in males, and neck fat was reduced in males ($p<0.05$) compared to females after adjusting for birth weight (Table 6.8).

Table 6.5: The effect of maternal metyrapone treatment on skeletal muscle mass in the juvenile guinea pig

	MALES		FEMALES		ANOVA p-value		
Muscle	***Control (8)***	***Met (12)***	***Control (8)***	***Met (8)***	***Met***	***Sex***	***Met x Sex***
Plantaris (g)	0.13 ± 0.008	0.16 ± 0.03	0.16 ± 0.005	0.14 ± 0.02	ns	ns	0.08
(%BW)	0.03 ± 0.002	0.04 ± 0.007	0.05 ± 0.002	0.04 ± 0.005	ns	0.09	0.06
Gastrocnemious (g)	2.19 ± 0.08	2.39 ± 0.12	1.77 ± 0.08	1.98 ± 0.09	0.03	0.001	ns
(%BW)	0.56 ± 0.02	0.58 ± 0.02	0.56 ± 0.01	0.56 ± 0.02	ns	ns	ns
Tibialis (g)	0.45 ± 0.06	0.49 ± 0.04	0.38 ± 0.03	0.43 ± 0.05	ns	0.08	ns
(%BW)	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	ns	ns	ns
Semitendinosus (g)	1.79 ± 0.07	1.72 ± 0.11	1.32 ± 0.10	1.73 ± 0.13	0.025	0.07	0.02
(%BW)	0.46 ± 0.02	0.42 ± 0.03	0.42 ± 0.03	0.47 ± 0.02	ns	ns	0.048
Biceps Femoris (g)	5.01 ± 0.22	5.4 ± 0.3	3.9 ± 0.28	4.8 ± 0.3	0.002	0.001	ns
(%BW)	1.28 ± 0.04	1.32 ± 0.04	1.24 ± 0.06	1.34 ± 0.04	0.085	ns	ns
Vastus Lateralis (g)	1.59 ± 0.05	1.60 ± 0.05	1.29 ± 0.07	1.47 ± 0.08	0.07	0.001	ns
(%BW)	0.41 ± 0.01	0.39 ± 0.01	0.41 ± 0.02	0.42 ± 0.02	ns	ns	ns
EDL (g)	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.02	ns	ns	ns
(%BW)	0.042 ± 0.002	0.043 ± 0.002	0.053 ± 0.004	0.046 ± 0.006	ns	0.051	ns
Biceps Brachii (g)	0.51 ± 0.01	0.51 ± 0.02	0.42 ± 0.02	0.46 ± 0.02	ns	0.001	ns
(%BW)	0.13 ± 0.003	0.13 ± 0.004	0.13 ± 0.004	0.13 ± 0.002	ns	ns	ns
Diaphragm (g)	1.39 ± 0.02	1.49 ± 0.07	1.20 ± 0.08	1.36 ± 0.11	0.06	0.024	ns
(%BW)	0.36 ± 0.01	0.36 ± 0.01	0.38 ± 0.02	0.38 ± 0.03	ns	ns	ns
Summed Muscle (g)	13.0 ± 0.4	13.7 ± 0.5	11.0 ± 0.4	12.4 ± 0.7	0.031	0.002	ns
(%BW)	3.4 ± 0.08	3.4 ± 0.08	3.4 ± 0.07	3.5 ± 0.1	ns	ns	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days.

Table 6.6: The effect of maternal metyrapone treatment on muscle mass in the juvenile guinea pig adjusted for birth weight

	MALES		FEMALES		ANOVA p-value		
Muscle	***Control (8)***	***Met (12)***	***Control (8)***	***Met (8)***	***Met***	***Sex***	***Met x Sex***
Plantaris (g)	0.13 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.14 ± 0.02	ns	ns	ns
(%BW)	0.03 ± 0.006	0.04 ± 0.005	0.05 ± 0.006	0.04 ± 0.006	ns	0.072	ns
Gastrocnemious (g)	2.20 ± 0.11	2.37 ± 0.09	1.79 ± 0.11	1.98 ± 0.11	0.049	0.001	ns
(%BW)	0.56 ± 0.02	0.59 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	ns	ns	ns
Tibialis (g)	0.45 ± 0.05	0.50 ± 0.04	0.38 ± 0.05	0.43 ± 0.05	ns	0.085	ns
(%BW)	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	ns	ns	ns
Semitendinosus (g)	1.80 ± 0.11	1.68 ± 0.10	1.36 ± 0.12	1.74 ± 0.11	ns	0.04	0.02
(%BW)	0.46 ± 0.03	0.42 ± 0.02	0.42 ± 0.03	0.47 ± 0.03	ns	ns	0.04
Biceps Femoris (g)	5.05 ± 0.25	5.27 ± 0.21	4.06 ± 0.26	4.83 ± 0.25	0.03	0.003	ns
(%BW)	1.29 ± 0.05	1.31 ± 0.04	1.25 ± 0.05	1.34 ± 0.05	ns	ns	ns
Vastus Lateralis (g)	1.60 ± 0.07	1.58 ± 0.06	1.31 ± 0.07	1.47 ± 0.07	ns	0.002	0.09
(%BW)	0.41 ± 0.02	0.40 ± 0.01	0.41 ± 0.02	0.42 ± 0.02	ns	ns	ns
EDL (g)	0.17 ± 0.02	0.18 ± 0.01	0.17 ± 0.02	0.16 ± 0.02	ns	ns	ns
(%BW)	0.042 ± 0.004	0.043 ± 0.003	0.053 ± 0.004	0.046 ± 0.004	ns	0.043	ns
Biceps Brachii (g)	0.51 ± 0.02	0.50 ± 0.01	0.43 ± 0.02	0.46 ± 0.02	ns	0.001	0.09
(%BW)	0.13 ± 0.004	0.13 ± 0.003	0.13 ± 0.004	0.13 ± 0.004	ns	ns	ns
Diaphragm (g)	1.41 ± 0.08	1.45 ± 0.06	1.23 ± 0.08	1.37 ± 0.08	ns	0.045	ns
(%BW)	0.36 ± 0.02	0.36 ± 0.02	0.38 ± 0.02	0.38 ± 0.02	ns	ns	ns
Summed Muscle (g)	13.2 ± 0.5	13.6 ± 0.4	11.1 ± 0.6	12.5 ± 0.5	0.048	0.002	ns
(%BW)	3.35 ± 0.09	3.36 ± 0.07	3.41 ± 0.09	3.48 ± 0.09	ns	ns	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days

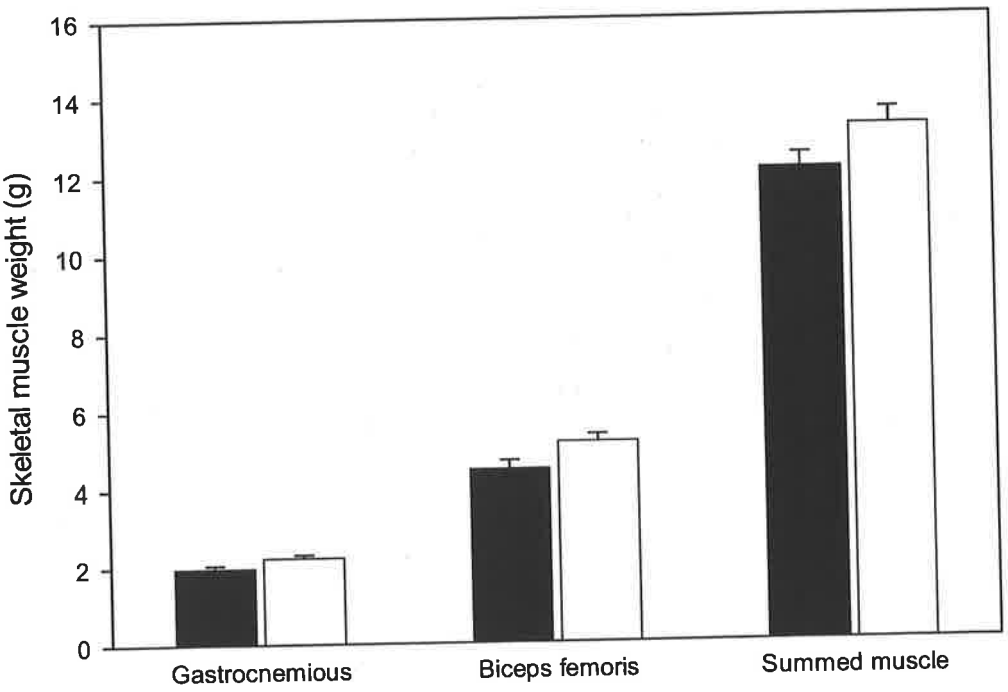

Figure 6.6: The effect of metyrapone treatment on muscle size at 40 days of age

Vehicle treatment is represented by black, and metyrapone treatment by white bars. * denotes significance at $p<0.05$, # $p<0.1$. Males and females are combined.

Table 6.7: The effect of metyrapone treatment on adiposity in the juvenile guinea pig

	MALES		FEMALES		ANOVA p-value		
Adipose tissue	***Control (8)***	***Met (12)***	***Control (8)***	***Met (8)***	***Met***	***Sex***	***Met x Sex***
Dorsal Fat (g)	3.26 ± 0.22	3.66 ± 0.29	2.35 ± 0.16	3.39 ± 0.45	0.01	0.03	ns
(%BW)	0.83 ± 0.05	0.89 ± 0.05	0.74 ± 0.04	0.89 ± 0.09	0.045	ns	ns
Shoulder Fat (g)	2.50 ± 0.21	2.37 ± 0.22	2.03 ± 0.16	2.43 ± 0.26	ns	ns	ns
(%BW)	0.63 ± 0.04	0.58 ± 0.05	0.64 ± 0.04	0.66 ± 0.05	ns	ns	ns
Neck Fat (g)	0.38 ± 0.07	0.44 ± 0.04	0.35 ± 0.03	0.51 ± 0.08	0.03	ns	ns
(%BW)	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.07	0.048	ns
Retroperitoneal Fat (g)	2.15 ± 0.28	2.44 ± 0.29	1.15 ± 0.14	1.80 ± 0.38	0.06	0.005	ns
(%BW)	0.55 ± 0.07	0.58 ± 0.06	0.36 ± 0.04	0.46 ± 0.09	ns	0.012	ns
Perirenal Fat (g)	1.78 ± 0.21	2.07 ± 0.27	1.13 ± 0.08	1.42 ± 0.28	ns	0.006	ns
(%BW)	0.45 ± 0.05	0.49 ± 0.05	0.36 ± 0.02	0.36 ± 0.06	ns	0.02	ns
Groin Fat (g)	2.22 ± 0.35	2.19 ± 0.23	1.90 ± 0.25	2.48 ± 0.25	ns	ns	ns
(%BW)	0.56 ± 0.09	0.53 ± 0.05	0.60 ± 0.08	0.68 ± 0.06	ns	0.04	ns
Summed Fat (g)	11.7 ± 1.2	13.2 ± 1.1	8.9 ± 0.7	12.0 ± 1.6	0.051	0.034	ns
(%BW)	3.0 ± 0.3	3.2 ± 0.2	2.8 ± 0.2	3.2 ± 0.3	ns	ns	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days

Table 6.8: The effect of metyrapone treatment on adiposity in the juvenile guinea pig after adjusting for birth weight

	MALES		FEMALES		ANOVA p-value		
Adipose tissue	***Control (8)***	***Met (12)***	***Control (8)***	***Met (8)***	***Met***	***Sex***	***Met x Sex***
Dorsal Fat (g)	3.29 ± 0.31	3.56 ± 0.26	2.44 ± 0.32	3.41 ± 0.31	0.03	0.052	ns
(%BW)	0.84 ± 0.06	0.88 ± 0.05	0.75 ± 0.06	0.89 ± 0.06	ns	0.07	ns
Shoulder Fat (g)	2.53 ± 0.22	2.27 ± 0.18	2.13 ± 0.22	2.45 ± 0.22	ns	ns	0.09
(%BW)	0.64 ± 0.05	0.57 ± 0.04	0.66 ± 0.05	0.67 ± 0.05	ns	ns	ns
Neck Fat (g)	0.38 ± 0.06	0.43 ± 0.05	0.36 ± 0.06	0.51 ± 0.06	0.047	ns	ns
(%BW)	0.097 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.09	0.045	ns
Retroperitoneal Fat (g)	2.18 ± 0.31	2.36 ± 0.26	1.22 ± 0.31	1.82 ± 0.31	ns	0.01	ns
(%BW)	0.55 ± 0.07	0.57 ± 0.06	0.38 ± 0.07	0.46 ± 0.07	ns	0.02	ns
Perirenal Fat (g)	1.80 ± 0.25	2.00 ± 0.21	1.20 ± 0.26	1.44 ± 0.25	ns	0.012	ns
(%BW)	0.46 ± 0.05	0.48 ± 0.04	0.37 ± 0.05	0.36 ± 0.06	ns	0.024	ns
Groin Fat (g)	2.25 ± 0.27	2.08 ± 0.23	2.01 ± 0.27	2.51 ± 0.27	ns	ns	ns
(%BW)	0.57 ± 0.07	0.51 ± 0.06	0.62 ± 0.07	0.68 ± 0.08	ns	0.06	ns
Summed Fat (g)	12.4 ± 1.2	12.7 ± 1.0	9.4 ± 1.2	12.1 ± 1.2	0.09	0.06	ns
(%BW)	3.16 ± 0.24	3.11 ± 0.20	2.89 ± 0.24	3.19 ± 0.25	ns	ns	ns

** Denotes significance at $p<0.001$; * $p<0.05$; # $p<0.1$. Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days

6.4.4 The effect of maternal metyrapone treatment on the endocrine status of the juvenile guinea pig

Maternal metyrapone reduced plasma free T_3 (p=0.022) (Figure 6.6), but did not alter plasma total T_3 or T_4 or free T_4 (Table 6.9). Plasma free T_3:T_4 tended to be lower in offspring of metyrapone treated mothers (p=0.07). Males had higher plasma free T_4 (p=0.008) compared to females (Table 6.9). Maternal metyrapone tended to reduce plasma IGF-II (p=0.084), and males had greater plasma IGF-II levels than females (p=0.014) (Table 6.9). Males also had greater plasma IGF-I levels (p=0.001) and IGF-I:IGFBP (p=0.025) compared to females (Table 6.9).

Maternal metyrapone did not alter insulin sensitivity of glucose metabolism, but males had greater insulin sensitivity than females (p=0.003) (Table 6.9).

Table 6.9: The effect of maternal metyrapone treatment on endocrine axes in the juvenile guinea pig

	MALES		FEMALES		ANOVA p-value		
	Control (4)	*Met (4)*	*Control (6)*	*Met (6)*	*Met*	*Sex*	*Met x Sex*
Endocrine measure							
Plasma IGF-I ($ng.ml^{-1}$)	683 ± 73	729 ± 84	311 ± 59	287 ± 65	ns	0.001	ns
Plasma IGF-II ($ng.ml^{-1}$)	753 ± 115	490 ± 103	390 ± 94	352 ± 103	0.084	0.014	ns
Plasma IGFBP	756 ± 86	569 ± 150	626 ± 79	538 ± 75	ns	ns	ns
IGF-I:IGFBP	0.89 ± 0.10	0.74 ± 0.18	0.46 ± 0.09	0.61 ± 0.09	ns	0.025	ns
IGF-II:IGFBP	1.16 ± 0.26	0.74 ± 0.45	0.76 ± 0.23	0.88 ± 0.23	ns	ns	ns
Plasma free T3 (pmol/L)	2.55 ± 0.43	1.88 ± 0.34	2.50 ± 0.36	1.50 ± 0.45	0.026	ns	ns
Plasma free T4	35.9 ± 3.9	29.1 ± 2.9	23.9 ± 3.2	23.3 ± 3.5	ns	0.008	ns
Plasma total T3 (nmol/L)	0.13 ± 0.09	0.14 ± 0.09	0.23 ± 0.08	0.17 ± 0.09	ns	ns	ns
Plasma total T4	27.4 ± 6.9	19.1 ± 4.5	12.6 ± 4.9	15.7 ± 4.9	ns	0.053	ns
Free T3:T4 (pmol/L)	0.073 ± 0.021	0.069 ± 0.017	0.125 ± 0.017	0.070 ± 0.021	0.07	0.09	0.092
Total T3:T4 (nmol/L)	0.009 ± 0.010	0.008 ± 0.007	0.024 ± 0.007	0.010 ± 0.007	ns	ns	ns
Glucose sensitivity							
Insulin SSGIR (μU/min/kg)	12.5 ± 1.5 (4)	11.1 ± 1.5 (4)	6.3 ± 1.8 (3)	4.6 ± 2.2 (2)	ns	0.003	ns

Met refers to maternal metyrapone treatment (10mg/kg/day, tartaric acid 11mg/kg/day), and control to maternal vehicle (tartaric acid 11mg/kg/day), administered at 42 days gestation for 28 days

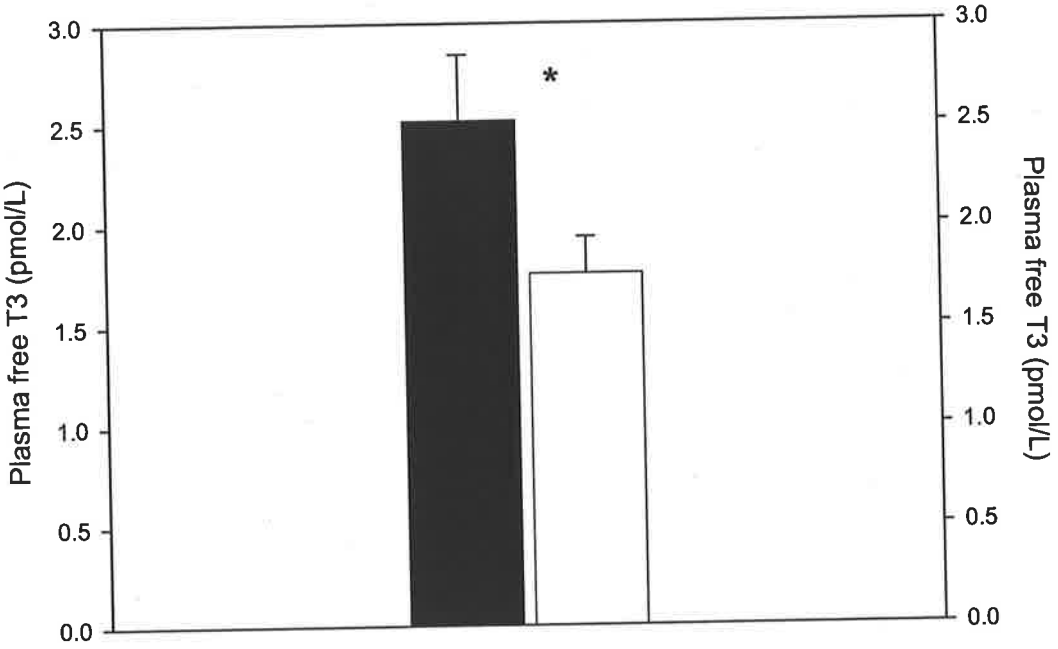

Figure 6.7: The effect of maternal metyrapone on plasma free T_3 in the juvenile guinea pig

Vehicle treatment is represented by black, and metyrapone treatment by white bars. * denotes significance at $p<0.05$. Males and females are combined

6.5 Discussion

This study is the first to investigate the effect of maternal metyrapone on postnatal outcomes in the juvenile guinea pig. We have shown that maternal metyrapone, which should reduce cortisol production and circulating cortisol in the mother and possibly the fetus, does not alter birth weight, however increases abdominal circumference and head length at birth. In the juvenile guinea pig, at 40 days of age, visceral organ weights (liver, GIT) are increased. In addition maternal metyrapone increased muscle mass in the juvenile guinea pig. Maternal metyrapone treatment tended to reduce plasma IGF-II, and reduced plasma free T_3.

Metyrapone inhibits the action of the steroidogenic enzyme 11β-hydroxylase (Lye & Challis, 1984), thereby inhibiting cortisol synthesis. Birth weight was not increased in offspring of mothers treated with metyrapone, but offspring were larger in terms of abdominal circumference and head length at birth. Similarly, maternal metyrapone treatment in the sheep does not alter fetal body weight or crown-rump length (Warnes et al., 2003; Warnes et al., 2004). These offspring had increased visceral organ weights at 40 days of age, but whether the increased abdominal circumference seen at birth persisted until 40 days of age, and can be linked to the increased visceral organ size, is unknown. In this manner, metyrapone treatment in the fetal sheep during late gestation increases the relative weights of kidneys and liver (Warnes et al., 2003).

In the current study, although females had smaller organ sizes than males at 40 days of age, the effect of maternal metyrapone was still apparent in both sexes. However, maternal metyrapone treatment did appear to have sex specific effects on the organ size in the juvenile guinea pig, in particular in females where adrenal weight was increased, but that of males weight decreased. However, the relative weight of the adrenals was decreased in females and unchanged in males following maternal metyrapone. Other studies have also shown longer-term sex-specific effects of maternal HPAA axis manipulation on organ size postnatally in the guinea pig (Liu et al., 2001; McCabe et al., 2001), and maternal dexamethasone treatment in the guinea pig has been shown to increase the relative weight of the adrenals in females and decrease the relative weight in males (Liu et al., 2001).

Increases in size of various organs and tissues in juvenile offspring of maternal metyrapone treated animals can partially explain the increased juvenile body weight, but in addition these animals had increased muscle mass, and appeared to have more fat mass, although this result did not quite reach statistical significance (Table 6.7). Although males were heavier and had increased muscle mass and fat mass compared to females as juveniles, the sex difference appeared to be unaffected by maternal metyrapone, with the exception of the semitendinosus muscle, which increased in female offspring with metyrapone treated mothers, but decreased in males. Juvenile offspring of metyrapone treated mothers were also larger in body size compared to vehicle-treated animals. Due to the lack of difference in birth weight between offspring of metyrapone and vehicle treated mothers, the maternal metyrapone offspring

must have grown faster in terms of weight during the postnatal period. Indeed, the latter had increased absolute growth rates, and had increased fractional growth rates after adjusting for birth weight. Betamethasone treatment of pregnant sheep reduces fetal postnatal growth rates (Moss 2002), indicating that increased fetal cortisol levels may inhibit postnatal as well as fetal growth (Fowden et al., 1996). We therefore suggest that decreased exposure to cortisol during gestation, induced by maternal metyrapone treatment, programs endocrine and other factors that affect postnatal growth such that increased growth postnatally occurs.

In general, adjusting for birth weight did not change the effect of maternal metyrapone treatment on body composition in the juvenile guinea pig, suggesting that the increases in tissue size and alterations in body composition result from consequences additional to being 'bigger' at birth. We can therefore assume that a programming effect occurred in utero, and this effect continued after birth. This suggests a role for in utero cortisol exposure on adult outcomes, and may explain in part why elevated levels of glucocorticoids in the fetus are linked to abdominal obesity, hypertension and glucose tolerance later in life (Barker 1993, Newnham 1998).

Maternal metyrapone treatment greatly reduced plasma free T_3 levels in the juvenile guinea pig, and tended to reduce plasma free T_3:T_4. In the current study, the effect of maternal metyrapone on plasma cortisol in the juvenile guinea pig was not determined. However, low birth weight guinea pigs, especially males, have increased salivary cortisol as juveniles, which correlate

with increased T_3 and T_3:T_4. Such changes may be due in part to increased cortisol in utero in the low birth weight animal. Therefore we predict that lowering fetal cortisol by maternal metyrapone would possibly lower postnatal cortisol, and hence T_3 and T_3:T_4. Although we have no measure of plasma cortisol or deiodinase activity in these offspring, we would expect that maternal metyrapone treatment would decrease plasma cortisol and deiodinase expression.

Maternal metyrapone treatment tended to reduce plasma IGF-II, but did not affect plasma IGF-I levels in the juvenile guinea pig. Cortisol decreases IGF-II mRNA expression in liver and muscle of fetal sheep (Li et al., 1998b; Li et al., 1993), and it is probable that it does so postnatally also. A tissue-specific role has been suggested for cortisol in the regulation of IGF-I, with cortisol infusion lowering muscle IGF-I mRNA expression in fetal sheep (Li 2002), but increasing IGF-I mRNA in the liver (Li 1996). The effect of maternal metyrapone treatment on TH and IGF levels, however, raise the question of how maternal metyrapone increases size at birth and postnatal growth rates, if not through increasing action of these hormones, which are associated with increased postnatal growth.

Maternal metyrapone did not affect insulin sensitivity of glucose metabolism in the juvenile guinea pig in the current study, although numbers were low. Although cortisol infusion increases insulin sensitivity of skeletal muscle in the fetal sheep through increasing GLUT-4 expression, an insulin-sensitive glucose transporter (Li et al., 1998a), dexamethasone treatment to adult humans

decreases insulin sensitivity, resulting in a dramatic increase in plasma insulin concentrations (Besse et al., 2005). In addition, increased insulin sensitivity is apparent in the placentally restricted lamb (De Blasio, 2004), and in the first 48 hours after birth human SGA infants are more insulin sensitive than AGA controls (Bazaes et al., 2003). We suggest that these results are due to the low number of animals having insulin clamps, which reduced the statistical power in this study. Glucose infusion rates did appear to be lower in maternal metyrapone offspring (Table 6.9).

In conclusion, inhibition of maternal cortisol synthesis through metyrapone treatment increases abdominal circumference at birth and increases postnatal growth rates in the juvenile guinea pig. Maternal metyrapone treatment also increases organ and tissue growth postnatally, independent of birth weight, suggesting a role for in utero cortisol exposure on determining body composition in later life. The effect of maternal metyrapone on postnatal growth is probably not through the upregulation of the synthesis of other hormones, as circulating plasma free T_3 and IGF-II in offspring appear to be reduced. The effects of metyrapone treatment on postnatal outcomes of offspring are most likely due to a programming effect on the HPA axis in utero, which persists after birth, leading to lower postnatal cortisol levels.

Chapter 7

General Discussion

General Discussion

Intrauterine growth restriction, and subsequent postnatal catch-up growth, are major risk factors for subsequent adult diseases, such as hypertension, obesity, type-2 diabetes and cardiovascular disease (Barker et al., 1993; Newnham, 1998). Understanding the metabolic and endocrine adaptations that occur in the fetus in response to intrauterine insults is an important step in determining the causes of such adverse outcomes, and to develop health strategies to combat the development of adult diseases.

Perturbations in numerous endocrine axes have been implicated in the development of subsequent adult disease, and these axes may be programmed in utero such that postnatally, their actions are altered from what would normally occur (Barker, 1991; Barker et al., 1993; Hales & Ozanne, 2003; Newnham, 1998). These include the glucose-insulin axis, the HPT axis (thyroid hormones), the HPA axis (in particular, glucocorticoids), and the somatotropic axes (including the insulin-like growth factors IGF-I and IGF-II and their binding proteins, and growth hormone). This study was designed to determine what role these axes play in postnatal catch-up growth following spontaneous fetal growth restriction in the juvenile guinea pig, and how they impact on each other during this period. In addition, the effects of fetal growth restriction on juvenile body composition were investigated to determine whether at an early stage of development, adverse changes in body composition following intrauterine challenge are already apparent, and which could lead to adverse adult outcomes.

The current study has shown that spontaneous fetal growth restriction in the juvenile guinea pig, due to increased litter size, lowers weight, crown-rump length, abdominal circumference, head length and head width in the offspring at birth. The animals smallest at birth have increased fractional growth rates in the first 36 days of life compared to high birth weight animals, indicating that catch-up growth occurs in this species. However, juvenile guinea pigs do not completely catch-up in size to their high birth weight counterparts, as they are still smaller at 36 days of age in terms of weight and crown-rump length.

We have found evidence to support a role for increased sensitivity of IGF-I as a major factor in driving this postnatal catch-up growth, albeit in the presence of reduced plasma IGFs. This reduction in plasma IGFs is exacerbated by decreased ratios of IGF to IGFBP, indicating that LBW animals have greater amounts of binding protein in comparison to IGFs, as well as lower total IGFs, indicating substantially reduced bioavailable IGFs in circulation during the catch-up growth period. It is possible, however, that increased sensitivity to IGFs reflects increased IGF receptors/binding to receptors, and uptake and clearance from blood, which leads to low circulating IGFs. Measurement of tissue expression of IGF-I and –II is necessary to determine if this is the case and whether production is actually reduced or not.

Interestingly, LBW juvenile guinea pigs with decreased circulating plasma IGFs had smaller kidneys, suggesting a role for inhibition of kidney growth in the presence of low IGF bioavailability. Alternatively, nephron number is complete

by this age, and is reduced in LBW guinea pigs, and may limit the extent of postnatal catch-up growth. The long-term consequences of altered kidney development are far-reaching, leading to hypertension and cardiovascular disease (Hinchliffe et al., 1992; Huxley et al., 2000), whereas an IGF-I stimulated increase in kidney weight has been linked to the development of diabetic glomerular disease (Rabkin & Schaefer, 2004).

Increased plasma IGFs in juvenile guinea pigs were also predictive of increased muscle and adipose tissue weights, however as HBW animals had increased muscle and adipose tissue masses, and had increased IGFs, this is probably a consequence of increased size of the animal, in contrast to a direct effect of IGFs. However, due to the stimulatory action of IGFs on muscle (Oksbjerg et al., 2004) and adipose tissue (Rogers & Group., 2003; Soret et al., 1999), the increased plasma IGFs seen in HBW animals may be the driving factor behind their increased size. Or, in other words, a decrease in plasma IGFs in LBW animals may lead to a decreased muscle and adipose tissue mass in later life. The decreased muscle mass in LBW animals lends support to the theory that small size at birth results in decreased lean muscle mass in later life (Hediger et al., 1998; Li et al., 2003; Singhal et al., 2003), however most studies also report an increase in relative fatness in IUGR subjects (Bavdekar et al., 1999; Yajnik et al., 2002). We did not observe increased adipose tissue mass in absolute or relative terms in our juvenile guinea pigs at 36 days of age, however, previous studies in our laboratory have indicated that LBW animals develop obesity and components of the metabolic syndrome only in late adulthood (400 days; Thavaneswaran *et al*, results unpublished). Young adult guinea pigs of low birth

weight are not obese, and therefore the animals in the current study may be far too immature to show signs of prenatally induced obesity despite having undergone catch-up growth.

Low birth weight guinea pigs that catch-up also have increased plasma free T_3:T_4 at 36 days of age, indicating an increased circulating plasma level of T_3 may also be a factor in driving postnatal catch-up growth. This increase in plasma free T_3:T_4 may in part be due to increased plasma cortisol levels in the first 10 days of life, stimulating deiodination of T_4 to T_3, which may persist throughout juvenile life of the guinea pig. Increased postnatal salivary cortisol is associated with low birth weight throughout the first 30 days of life in males, but only in the first 10 days of life in females, suggesting sex specific differences in response to prenatal growth restriction. In addition, when mothers are treated with metyrapone, an inhibitor of the action of the steroidogenic enzyme 11β-hydroxylase (Lye & Challis 1984), size at birth in terms of head measures and abdominal circumference are increased in offspring. These animals also had increased postnatal growth rates, suggesting that decreased exposure to cortisol in utero programs the fetus to grow faster postnatally. These results are consistent with cortisol inhibiting growth (Fowden, 1995), and fetal growth restriction and catch-up growth being associated with increased cortisol concentrations in human IUGR infants (Cianfarani et al., 2002; Jackson et al., 2004; Tenhola et al., 2005). Maternal metyrapone treatment also reduced plasma free T_3 and IGF-II levels, suggesting that the increase in size and growth in their offspring is not due to an increase in these anabolic hormones. We have not measured cortisol in the offspring of metyrapone treated mothers,

and therefore cannot be sure whether the effects seen are due decreased fetal cortisol, or decreased maternal cortisol. Therefore, an important step in the future is to determine how maternal metyrapone treatment affects fetal cortisol levels.

The major limitation of this experiment was the low number of animals in some studies. Although 22 animals were subjected to hyper-IGF-I-euglycemic clamp, only 14 were completed, due to blocking of catheters, and the nature of the juvenile guinea pigs to become excitable and dislodge their catheters. The low number inhibited the opportunity to determine sex differences, and changes in some parameters, as only 4 animals were low birth weight, and all were males. In addition, part of the designed protocol was to subject the animals to a hyper-insulinemic-euglycemic clamp at 36 days of age, immediately prior to post-mortem. However, only 2 animals had working catheters up to this stage, and therefore we could not include these results. The determination of the insulin sensitivity of these animals would prove insightful, as we would then have measures of metabolic sensitivity to two major anabolic hormones following fetal growth restriction, and we would be able to determine whether changes in one axis are mirrored or opposed in the other. It must also be stated that the associations seen in this study require subsequent studies to directly test the existence and causal nature of the implied links, due to the majority of statistical analyses involving simple multiple correlations.

Reductions in IGF-I receptor affinity have been described in IUGR children (Ducos et al., 2001), and reduced IGF-IR expression in IUGR rat fetuses in

response to uterine bilateral ligation, in the presence of normal insulin receptor gene expression (Reid et al., 2002). In the current study, preliminary work was undertaken to isolate IGF-I receptor and insulin receptor mRNA from guinea pig tissues, however the two receptors are highly homologous (Zapf et al., 1999), and although a transcript for the IGF-IR was successfully amplified using polymerase chain reaction, we could not amplify the IR gene (results not shown). Therefore, a future direction for this work would be to determine receptor expression in guinea pig tissues, and also examine the gene and protein expression of the IGFs in tissues, to determine whether the low plasma IGFs observed in low birth weight animals equate to low tissue mRNA expression.

There is a lack of studies to date on the expression of thyroid hormones and their receptors during catch-up growth, and in particular no-one has developed a procedure to test for TH sensitivity. It would be of great benefit to design a clamp, similar to the HEC and HIEC used in the present study, to examine the sensitivity to the actions of TH in the IUGR animal. This may also be a future direction for this work.

In conclusion, spontaneous fetal growth restriction in the guinea pig reduces size at birth, but increases postnatal growth rates in the first 36 days of life. An increased IGF-I sensitivity of glucose metabolism appears to be a major factor in driving this postnatal catch-up growth, however the abundant plasma IGFs do not play a role in postnatal growth. An increased cortisol level in the first 2 weeks of life may affect the thyroid hormone axis, by increasing deiodinase

activity and increasing the T_3:T_4 ratio, and the increased active thyroid hormone may also play a role in postnatal catch-up growth of these animals (Figure 7.1). Whether or not these endocrine axes perturbations persist long-term, and the subsequent outcomes on health and longevity are still to be determined. This study has helped to elucidate the factors involved in postnatal catch-up growth, and this knowledge may be used to design and improve therapies for the human IUGR infant.

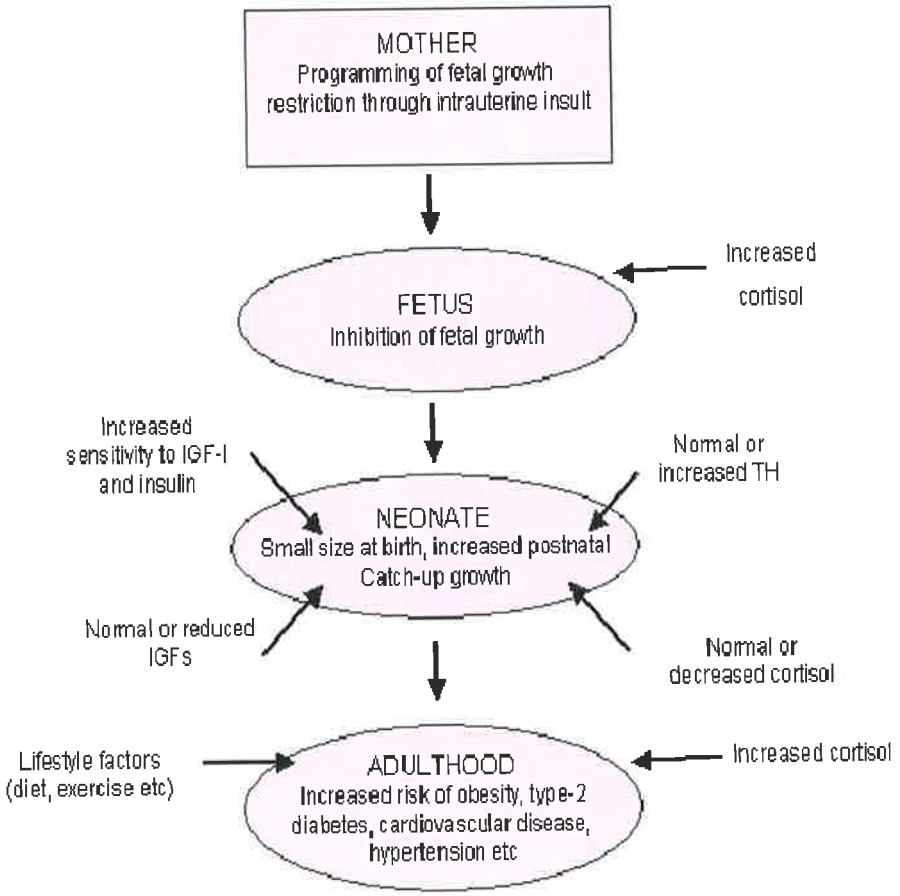

Figure 7.1: Flow diagram of IUGR, catch-up growth and adult outcomes

Chapter 8

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Chapter 9

Appendices

9.1 Appendix A

Experiment/measurement	Total number of animals	Low birth weight	High birth weight
Size at birth (weight, CRL, AC, HL and HW)	29	Male n=8, female n=4	Male n=6, female n=11
Absolute and fractional growth rates of weight, CRL, AC, HL and HW	21	Male n=5, female n=3	Male n=6, female n=7
Post-mortem	22	Male n=8, female n=5	Male n=5, female n=9
Insertion of vascular catheters, HIEC, plasma IGFs during HIEC, plasma glucose and FFA	14	Male n=4, female n=0	Male n=5, female n=5
IGFs measured in post-mortem plasma	21	Male n=5, female n=2	Male n=5, female n=9
Salivary cortisol at postnatal days 5, 10, 15 and 20	19	Male n=4, female n=3	Male n=5, female n=7
Salivary cortisol at postnatal day 30	10	Male n=2, female n=2	Male n=2, female n=4
Plasma free T_3, free T_4 and total T_4	21	Male n=5, female n=2	Male n=5, female n=9
Plasma total T_3	15	Male n=4, female n=3	Male n=5, female n=3

9.2 Appendix B

Experiment/measurement	Total number of animals	
	Vehicle	Metyrapone
Size at birth (weight, CRL, AC, HL and HW)	Male n=11, female n=10	Male n=13, female n=8
Absolute and fractional growth rates of weight	Male n=11, female n=10	Male n=13, female n=8
Post-mortem	Male n=11, female n=10	Male n=13, female n=8
Insertion of vascular catheters, HEC	Male n=4, female n=3	Male n=4, female n=2
Plasma IGFs measured in post-mortem plasma	Male n=4, female n=6	Male n=4, female n=8
Plasma free T_3, free T_4, total T_3 and total T_4	Male n=4, female n=6	Male n=4, female n=8

9.3 Appendix C

Litter size	Pregnant guinea pigs	No. offspring used in study
Chapters 2, 3, 4 and 5		
2	4	8
3	6	13
4	3	6
5	2	3
Chapter 6		
Vehicle		
3	2	6
4	4	14
Metyrapone		
3	4	8
4	2	13

THESIS CORRECTIONS – Natasha Campbell, PhD candidate

EXAMINER 1:

1. *Chapter 2:*

Animals were weighed within 12 hours of birth, by placing them in a plastic tub attached to electronic scales. (NO CHANGE TO THESIS)

Section 2.3.3: Growth of 21 out of the 29 animals born was measured but not for 8 other animals, due to lack of manpower for several weeks. The latter had birth weights in both the low and high birth weight categories, and the absence of growth data would have little impact on analysis of outcomes. (NO CHANGE TO THESIS)

Section 2.4.1: The current study lacked sufficient numbers and therefore power to detect sex differences in size at birth. We have previously detected such differences in the guinea pig and other species, but only with larger numbers of animals, as has been described by others. (NO CHANGE TO THESIS).

Table 2.4: For birth measures, only ponderal index and BMI are reported, as these are commonly used to assess growth of soft tissues Vs linear growth at birth in a range of species. Weight to length was calculated only for juvenile animals later in life as an additional index of fatness, hence was included only in tables where juvenile parameters were reported or used. (NO CHANGE TO THESIS).

Discussion: This approach has been used previously in descriptive and epidemiological studies in humans (Cianfarani et al., 2002), and in experimental studies in non-human species (Fowden et al., 2001; Poore et al., 2002), to identify potential links between early life exposures and outcomes, function and homeostasis in later life. The associations examined in this thesis were selected to provide additional tests of the hypotheses based on previous findings, including those revealed in the above studies. While there is an increased chance of false positive finding with increasing numbers of correlations examined, this is countered by examining only those that are hypothesis driven and justified by previous reports. As outlined in the general discussion, the candidate is well aware these associations require subsequent studies to directly test the existence and causal nature of the implied links. As such, no additional comment has been added to the discussion.

2. *Chapter 3*:

Page 105: The examiner has misinterpreted the methodological basis for using IGFBP interference in the radioimmunoassay for IGF-I as an index of IGFBP activity in plasma. To clarify this, the following has been inserted on p105: An index of apparent IGFBP levels and activity in plasma can be obtained by collecting the fraction of eluent containing the IGFBPs following molecular sieving fractionation of plasma at low pH to obtain a free IGF fraction for subsequent assay. The fraction containing the IGFBPs is assayed by IGF-I radioimmunoassay, where they are detected as apparent IGF-I, as a result of their competition for radioiodinated ligand with the antisera. The greater the levels of IGFBPs, the greater the interference and apparent IGF-I levels. This is a quantitative index that reflects the combined levels of IGFBPs present.

Discussion: The following paragraph was added to Chapter 3, page 130: In the current study, infusion of IGF-I to weanling guinea pigs resulted in no change to plasma free fatty acid concentrations. Similarly, infusion of IGF-I to healthy human

volunteers does not change plasma free fatty acid concentration (Russell-Jones et al., 1995), and plasma FFA levels remain unchanged when IGF-I is infused into the awake fasted rat (Jacob et al., 1989). In addition, no association between size at birth, catch-up growth and free fatty acid utilisation was seen.

3. *Chapter 5*:

Page 173: Saliva was only taken from 10 animals at day 30 because if surgery was carried out within 24 hours of this measurement, saliva samples were not collected. (NO CHANGE TO THESIS)

Plasma free T3, free T4 and total T4 were measured in 22 animals, whereas plasma total T3 only in 15 animals. This was due to a low volume of plasma being collected in 7 animals, and total T3 could not be measured. (NO CHANGE TO THESIS)

Table 5.6: An independent t-test was performed because the number of animals differed between groups, and therefore a repeated measured ANOVA could not be carried out. (NO CHANGE TO THESIS)

MINOR CORRECTIONS: All minor corrections were made in the thesis text.

EXAMINER 2:

1. An appendix was added into the thesis outlining the numbers of litters used in the experiments, and a brief description of these litters (see Chapter 9 Appendices).

2. A table has been inserted as an Appendix outlining the amount of animals used in each experiment, and a sentence into each Materials and Methods section to refer to this appendix for numbers of animals (see Chapter 9 appendices).

Figure 4.3(f): this is incorrect, both males and females are included in the figure. The significance value of $p<0.1$ has been left in the tables to indicate trends for possible future followup. These results have not been referred to as significant in the figures or in the text. (NO CHANGE TO THESIS)

3. Plasma cortisol was not measured in these animals as only a limited amount of plasma was available, and this was used to measure other blood hormones, such as the IGFs and thyroid hormones. (NO CHANGE TO THESIS)

4. No assessment of HPA activity was measured in these animals, as they were a separate cohort of animals that did not have saliva collected. Therefore unfortunately salivary cortisol levels are not available for this cohort. (NO CHANGE TO THESIS)

5. This is the same comment as made by Examiner 1, therefore see response to discussion, Chapter 2.

6. Postnatal food intakes were not measured. GIT weight was measured without contents. (NO CHANGE TO THESIS)

MINOR CORRECTIONS: All minor corrections were made in the thesis text.

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