Body Composition and the Brain: Investigating Life History Trade-offs in Living Humans

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I, Meghan Shirley, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

The ‘expensive-tissue’ hypothesis of Aiello and Wheeler is well-known in anthropology for positing that an increasingly small gut was a key factor in the evolution of the large hominin brain. The insight that organs and tissues in the body compete for energy resources was also central to the ‘thrifty phenotype’ hypothesis of Hales and Barker, which proposed that nutritional stress in fetal life resulted in differential growth of the brain and pancreas. Both hypotheses are consistent with life history theory, which assumes that energy allocation trade-offs occur in energy-limited environments. The prediction that somatic traits trade off against one another in the context of the body’s fixed energy budget has, however, yet to be rigorously tested in humans. The current thesis project aimed to fill this gap by recruiting 70 healthy young women and obtaining comprehensive, high-quality data on their brain and body composition. This included, specifically, measures of brain gray and white matter volume, fat mass, skeletal muscle mass, and volumes of the heart, liver, kidneys and spleen. Additional outcomes included resting energy expenditure and two proxies of early-life growth: birth weight, a marker of fetal weight gain, and tibia length, a marker of linear growth indexing postnatal experience. With these data, three principal hypotheses were tested: 1) there is variation in the energy expenditure of tissues and organs; 2) trade-offs are observed between brain and body organs/tissues; and 3) trade-off relationships are mediated by early-life growth. Results suggest the metabolic cost of organs and tissues is variable, and that the brain – in particular its gray matter component – trades off against lean tissues in the body (i.e. skeletal muscle, the liver and kidneys), but not fat mass. However, less support was found for the prediction that trade-offs are mediated by fetal and infant growth.

Impact Statement

It is increasingly recognized that a life course approach is essential for gaining a more complete understanding of human variability and health. Integrating several areas of research, the current study demonstrates that such an approach is strengthened by interdisciplinarity. The publication of the work detailed in this thesis will have an impact in anthropology and biomedicine as, for the first time, two highly influential hypotheses proposed by researchers in these fields have been directly tested in living humans using high-quality methods. The two hypotheses concern different points in the life course, so that examining them together allows for a fuller picture, and also draws a line between fields of research which have classically not been closely integrated. For epidemiologists and biomedical researchers, this work highlights the utility of incorporating an evolutionary approach to current problems in population health. For anthropologists, this work demonstrates the utility of body composition and biomedical techniques for addressing important evolutionary questions.

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I dedicate this work to my parents. I love you Mom and Dad – thank you for absolutely everything.

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Abbreviations

^{1}H – Protium

^{2}H – Deuterium

$^{2}H_2O$ – Deuterium oxide

2C model – Two-component model

3C model – Three-component model

3T – 3 Tesla

4C model – Four-component model

ABV – Actual body volume

ADP – Air-displacement plethysmography

AFA – Arm fat area

AGA – Appropriate-for-gestational-age

ALST – Appendicular lean soft tissue

AMA – Arm muscle area

AT – Adipose tissue

ATP – Adenosine triphosphate

BMC – Bone mineral content

BMI – Body mass index

BMR – Basal metabolic rate

CSF – Cerebrospinal fluid

CT – Computed tomography

DOHaD – Developmental origins of health and disease

DXA – Dual-energy X-ray absorptiometry

EQ – Encephalization quotient

ETH – Expensive tissue hypothesis

FM – Fat mass

FMI – Fat mass index

FFM – Fat-free mass

FFMI – Fat-free mass index

FIRST – FMRIB's Integrated Registration and Segmentation Tool

FMRIB – Oxford Centre for Functional MRI of the Brain (Oxford, UK)

FRC – Functional residual capacity

FSL – FMRIB software library

IC – Indirect calorimetry

IPAQ – International Physical Activity Questionnaire

IUGR – Intrauterine growth-restricted

MET – Metabolic equivalent

MRI – Magnetic resonance imaging

NHANES – National Health and Nutrition Examination Survey

PA – Physical activity

RBV – Raw body volume

REE – Resting energy expenditure

RF – Radiofrequency

RLL – Relative limb length

RMR – Resting metabolic rate

ROI – Region of interest

SA – Surface area

SAA – Surface area artefact

SDS – Standard deviation score

SFT – Skinfold thickness

SGA – Small-for-gestational-age

SM – Skeletal muscle

SMR – Tissue-specific metabolic rate, or mass-specific metabolic rate

TBW – Total body water

TE – Echo time

TEE – Total energy expenditure

TEM – Technical error of measurement

TGV – Thoracic gas volume

TIV – Total intracranial volume

TPH – Thrifty phenotype hypothesis

TR – Repetition time

TV – Tidal volume

VAT – Visceral adipose tissue

VSMOW – Vienna Standard Mean Ocean Water

Preface

The ‘expensive-tissue’ hypothesis (ETH) of Aiello and Wheeler (1995) is one of the most well-known in anthropology. Among a number of other theories, it sought to address a longstanding evolutionary problem: how the metabolically expensive hominin brain expanded in size without a concomitant expansion in the energy budget. The ETH suggested a novel answer, specifically that over time and following shifts in dietary quality, energy from a smaller gut was utilized to fund an increasingly large brain. Aiello and Wheeler’s key insight was that the brain and organs in the body are ultimately in competition for finite energy resources.

Around the same time, a similarly famous hypothesis in the biomedical literature had the same insight whilst addressing a different problem. Clinicians and researchers had so far struggled to explain data showing a link between small size at birth and increased adult diabetes risk. Hales and Barker’s (1992) ‘thrifty phenotype’ hypothesis (TPH) proposed that nutritional stress in fetal life resulted in competition amongst tissues; the outcome in human neonates, they argued, was the preservation of the brain at the expense of the pancreas and its blood sugar-regulating functions. This notion of ‘brain-sparing’ echoes the ETH, although the competition is predicted to occur within the life course, specifically in early development, rather than on an evolutionary timescale.

Although not explicitly invoked by either set of authors, both the ETH and the TPH are consistent with evolutionary life history theory, which predicts that finite energy resources are traded off amongst various traits in the living organism. Here, I situate both hypotheses within a life history framework to better integrate their evolutionary and developmental perspectives. Taking a life history approach similarly highlights the persistent dearth of research on physiological tissue/organ trade-offs, which remain understudied relative to functional trade-offs.

The ETH and the TPH have been incredibly influential in their respective fields, and they continue to stimulate theory and research. Although their respective predictions of organ competition in humans are generally accepted, they remain to be tested directly. For this thesis, I gathered comprehensive data on brain and body composition in a sample of adult women to empirically examine whether brain and body tissues are in competition with one another, as predicted by the ETH and TPH. Using the highest quality measurement techniques available *in vivo* I was able as well to extend beyond the whole brain and visceral organs, so that relationships could be tested between the brain and various tissues in the body; among tissues within the brain; and among tissues within the body.

1 Background: the expensive-tissue hypothesis, the thrifty phenotype hypothesis, and life history theory

This chapter reviews the prior work and theory that stimulated the hypotheses of this thesis. Sections 1.1 and 1.2 overview the background to and predictions of the ETH and TPH, respectively. Section 1.3 introduces life history theory in order to place the ETH and TPH within that framework, and reviews previous evidence for competition between somatic traits in non-human animals. In Section 1.4 I return to the developmental perspective of the TPH to expound on the implications of organ/tissue competition for health, and explain how this shaped my decisions in study recruitment.

1.1 The expensive-tissue hypothesis

Questions about human brain evolution have been some of the most persistent in anthropology. Attempts to explain the expansion of the hominin brain have traditionally fallen into two broad camps, one which focuses on benefits and the other on costs. The former camp, as Aiello and Wheeler (1995) noted, is comprised of 'why' questions; namely, why were bigger brains selected in human evolution. Explanations have included, but are not limited to, the potential association of a larger brain with hunting behavior (Washburn and Lancaster, 1968; Laughlin, 1968; Kaplan et al., 2000), foraging (Clutton-Brock and Harvey, 1980; Milton, 1981) or group size and social pressures (Humphrey, 1976, 1986; Byrne and Whiten, 1989; Dunbar, 1992).

Those in the second camp employ an energetics perspective and focus on the costs that would have acted to constrain brain expansion, whatever the potential advantages (i.e. 'how' questions; e.g. Martin, 1981; Foley and Lee, 1991; Leonard and Robertson, 1994; Isler and van Schaik, 2006a,b, 2009). Aiello and Wheeler's ETH emerged from this framework with a novel proposition: the growth of

metabolically 'expensive' brain tissue in humans was funded as another expensive tissue – the gut – decreased in size. The authors framed their argument as the solution to a problem with three main dimensions, which I lay out below: the human brain is large, and it is metabolically expensive, but it is apparently not funded by an increase in the body's energy budget. I then describe Aiello and Wheeler's hypothesis in more detail, as well as an alternative hypothesis suggesting that a reduction in skeletal muscle may have facilitated brain enlargement.

1.1.1 The human brain is relatively large

The human brain is, both relatively and absolutely, unusually large in size (Deacon, 1997). Primates in general have large brains relative to body size in comparison to other mammals (i.e. they are relatively encephalized), but humans in turn have larger brains than the typical primate (Deacon, 1997; Schoenemann, 2006). The encephalization quotient (EQ) is the ratio of observed to expected brain size in relation to species' average body size (Jerison, 1973). Although estimated EQs may differ slightly depending on the parameters used in their calculation, among mammals the values are invariably highest for humans (Schoenemann, 2006).

Using the equation of Martin (1981) to calculate EQ, the human EQ is ~5 whilst the value for anthropoid primates averages ~2. In other words, holding constant for body size, the anthropoid brain is roughly twice the size of the non-primate mammal brain. In turn, the human brain is approximately 3 times the size of the average anthropoid brain, and 5 times the size of the average mammal brain (Aiello and Wheeler, 1995; Schoenemann, 2006).

Indeed, data indicate the hominin brain tripled in size in fewer than 3 million years of evolution (Passingham, 1982; Flinn et al., 2005; Schoenemann, 2006), with a notable increase across the Middle Pleistocene (although a slight decrease in size occurred more recently within *Homo sapiens*; Ruff et al., 1997). Overall, the *Homo* lineage experienced brain volume expansion of ~1000 cubic centimeters over that of *Australopithecus*; the average brain volume in modern humans is ~1400cc

(Aiello and Wheeler, 1995; Potts, 1996). A portion of this enlargement occurred along with increases in body size (Potts, 1996; Deacon, 1997; Aiello and Key, 2002), although brain growth in the period of ~800 to 200 thousand years ago appears to have been independent of body size changes (Antón et al., 2014).

1.1.2 The human brain is metabolically expensive

An energetics approach to questions of brain evolution is driven by the fact that, in addition to being large, the human brain is highly metabolically expensive relative to its percentage of body mass, which is ~2% (Reinmuth et al., 1965; Holliday et al., 1967; Clarke and Sokoloff, 1999). For comparison, the proportional contribution of skeletal muscle to total body mass is approximately 30-40% (Brozek and Grande, 1955; Elia, 1992; Janssen et al., 2000).

The relative expense of an organ is determined by its mass-specific metabolic rate (SMR, kcal/kg/day). The SMR, multiplied by an organ's mass, reflects the number of kilocalories it expends in a 24-hour period. The summed products for all body tissues is equal to whole-body resting metabolic rate (RMR), or resting energy expenditure (REE[1], kcal/day; Brozek and Grande, 1955; Wang et al., 2010). SMRs have been quantified for several tissues, including the brain (Kety and Schmidt, 1945; Drabkin, 1950; Brozek and Grande, 1955; Holliday et al., 1967; Elia, 1992).

The brain in fact does not demonstrate the largest SMR value overall, although it is among the largest. For the brain, the SMR is 240 kcal/kg/day, compared to 440 for the heart and kidneys, and 200 for the liver. Values for skeletal muscle and adipose tissue are relatively small at 13 and 4.5 kcal/kg/day, respectively (Elia,1992; Wang et al., 2010).

[1] RMR and REE (kcal/day) are used interchangeably, both defined as an animal's energetic expenditure measured at rest under thermo-neutral conditions. The term 'basal metabolic rate,' or BMR, is defined in a similar way to RMR and REE, but is typically obtained under more highly controlled measurement conditions (Henry, 2005; although a recent review by Lam and Ravussin (2017) does not distinguish between RMR and BMR). Here, I use BMR only when referencing studies which specifically employed the term.

On the basis of the calculation of SMR with organ mass, adult humans use ~20% of REE to fuel the brain, which is ten times more than predicted based on its weight (Elia, 1992; Clarke and Sokoloff, 1999; Raichle, 2006). Human infants and children may use up to 50% of resting energy expenditure in the brain (Kennedy and Sokoloff, 1957; Elia, 1992). For comparison, the same figure was 2-8% in vertebrates studied by Mink et al. (1981), and 3% and 8% for non-primate mammals and anthropoid primates, respectively, as reported by Leonard and Robertson (1992).

Brain function is sustained day and night, and is highly sensitive to even small interruptions in energy supply. A high rate of O_2 consumption provides energy for "intense physiochemical activity" (Clarke and Sokoloff, 1999, p. 637), very little of which is expended in association with external environmental stimuli (Raichle, 2006). Rather, much of the brain's activity is related to 'intrinsic' demands associated with neuronal signaling, e.g. powering action potentials and maintaining post-synaptic potentials (Clarke and Sokoloff, 1999; Laughlin, 2001; Niven and Laughlin, 2008; Du et al., 2008). According to Attwell and Laughlin (2001), the brain's substantial signaling costs are comparable to the energy used in the leg muscle of a marathon runner.

Oxygen is also consumed to synthesize and metabolize various neurotransmitters, although this represents a much smaller proportion of energy use (Clarke and Sokoloff, 1999). A study in rats further suggested adenosine triphosphate (ATP; cells' 'energy currency') may fund 'housekeeping' functions that serve to maintain the health of brain cells over time (Du et al., 2008).

In their paper, Aiello and Wheeler (1995) calculated that BMR for the observed, large human brain is nearly five times higher than would be expected for a comparably-sized average mammal of expected brain size. They and others recognized that hominins' extra brain tissue would have necessitated extra energy resources.

1.1.3 The resting metabolism of humans is not elevated[2]

One potential solution to the problem of funding an increasingly large, expensive organ would be to increase REE. Indeed, early work which approached human brain evolution from an energetics perspective recognized the importance of the brain-metabolism link (e.g. Martin, 1981; Armstrong 1983, 1985). For example, Martin (1981) proposed that adult brain size was shaped within the niche of maternal metabolism during fetal and postnatal development, and is therefore constrained by maternal metabolic turnover. Barton and Capellini (2011) provided more recent support for this hypothesis (although see Pagel and Harvey, 1988).

Several researchers argued that evolutionary changes in foraging efficiency and diet quality (i.e. food digestibility and caloric value) would have been necessary as brain metabolism demanded an increasingly large proportion of the overall energy budget (Milton, 1987, 1988; Foley and Lee, 1991; Leonard and Robertson, 1992, 1994, 1997). An analysis of primate species, including humans, showed that resting metabolism correlated with diet quality, however humans appeared to consume a higher-quality diet than would be predicted, based on their metabolic rate (Leonard and Robertson, 1994). Dietary changes were clearly important, however they were insufficient to fully explain the evolution of the larger human brain (Leonard and Robertson, 1994).

For example, in their paper Aiello and Wheeler (1995) showed that the basal metabolic rates of young adult men and women fit the regression line for similar-sized mammals modelled using the Kleiber equation (which describes the allometric relationship of BMR to body mass; Kleiber, 1961). The figure from their original publication is shown in Figure 1-1.

[2] The title of this section reflects one of the main dimensions of the problem Aiello and Wheeler sought to address with the ETH in 1995. Some recent evidence, described below, indicates human REE may in fact be elevated over that of other apes (Pontzer et al., 2016; but see Simmen et al., 2017).

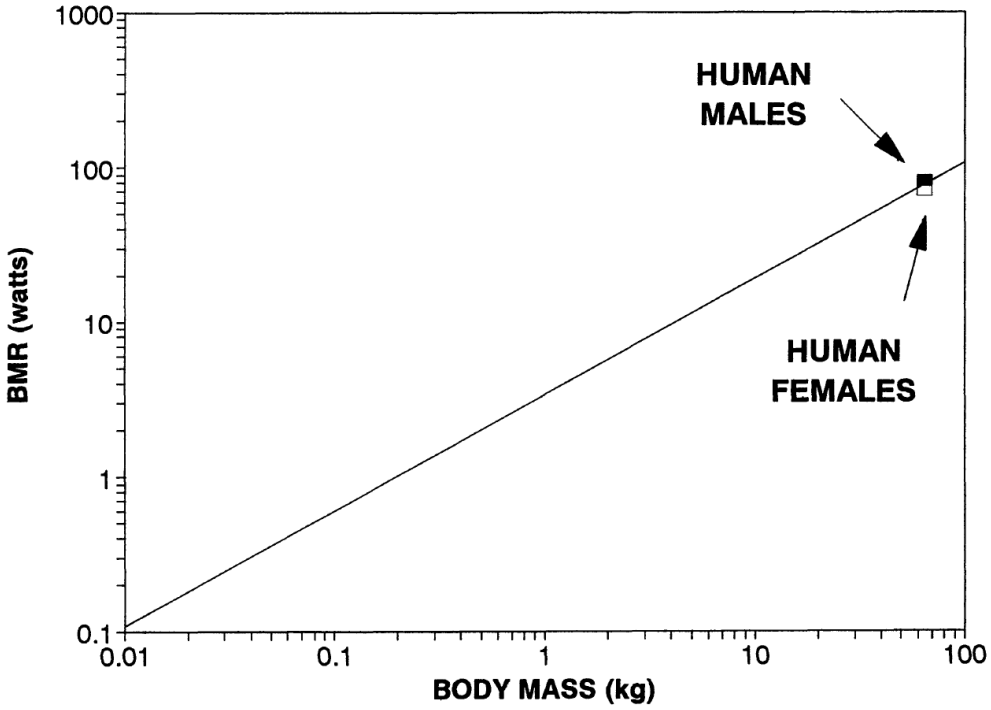


Figure 1-1 BMR against body mass, demonstrating the position of human males and females aged 18-30 in relation to the best-fit line for mammals.

From Aiello and Wheeler, 1995.

Leonard and Robertson (1992, 1994) demonstrated similar results in a larger sample, and also found that humans were outliers in plots of brain size against body weight, and brain size against whole-body resting metabolism (Figure 1-2 below). These results appeared to suggest that humans' relatively large and expensive brains were not funded by a corresponding increase in total resting metabolic rate.

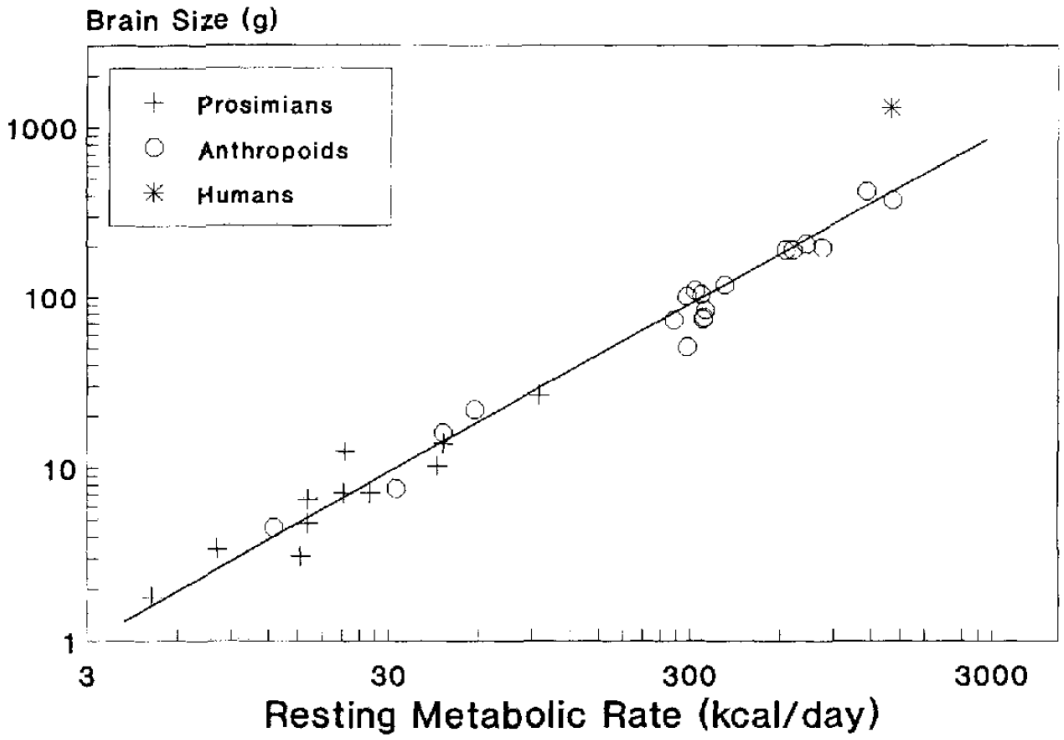


Figure 1-2 Brain size against RMR, demonstrating humans' deviation from the line for brain size, and apparent lack of increase in RMR.

From Leonard and Robertson, 1994.

The paradox of growing a larger brain seemingly without an increase in RMR might be partially explained by a number of factors, each of which could be considered to free up energy from other areas and allow for increased energy allocation to the brain. In addition to dietary changes and, eventually, the adoption of cooking (Wrangham, 2009), energy resources may have been stabilized over time by increased fat stores; increased locomotor efficiency; cooperative care and provisioning; slower, flexible growth patterns; and overall increased behavioral and physiological plasticity (Foley and Lee, 1991; Leonard and Robertson, 1992; Potts, 1996; Kuzawa, 1998; Gurven and Walker, 2006; Sockol et al., 2007; Hrdy, 2009; Wells, 2006a, 2012a, 2016; Isler and van Schaik, 2009, 2012, 2014; Burkart and van Schaik, 2010; Navarette et al., 2011; Antón et al., 2014).

For example, Hrdy (2009) has argued that evolved brain expansion was rendered possible in the context of cooperative breeding, which characterizes humans and some other animals, including primate species, but is not seen in the great apes. Indeed, she posited that alloparental care and provisioning represented the 'pre-existing condition' that must have been present in order for longer lifespans, longer childhoods, and bigger, expensive human brains to evolve (Hrdy, 2009). Isler and van Schaik (2012) have similarly proposed that cooperative breeding was a central factor allowing the hominin brain to enlarge over time, as it would have facilitated a redistribution of energy resources to mothers and their offspring. Importantly, this may have avoided fundamental shifts in the size of the hominin energy budget (Isler and van Schaik, 2012).

A possibility proposed by Aiello and Wells (2002) is that the increased adiposity of humans may in fact be concealing an increase over other primates in resting metabolism per kilogram fat-free mass, which is the more metabolically active component of body composition relative to fat mass (Keys and Brozek, 1953; Garby et al., 1988). More recent evidence supports the idea that human BMR may indeed be elevated relative to that of other apes, which is argued to account for findings of increased total energy expenditure (TEE; kcal/day) in humans compared with chimpanzees, gorillas, bonobos and orangutans (Pontzer et al., 2016). (TEE includes components of energy expenditure beyond that required for metabolism and basic bodily functions at rest, for example physical activity.)

However, based on analyses of wild non-human primates and human subsistence populations, Simmen and colleagues (2017) counter that the TEE of hominins was not inevitably elevated, since subsistence-level human populations lie within the confidence intervals of the general association of size and TEE in primates. Instead, hominins may have reduced costs of digestion by consuming higher-quality food in smaller quantities, and also stabilized energy intake across seasons, thereby allowing brain growth without an increase in energy turnover (Simmen et al., 2017). In either case, such changes in the energetic strategy of

hominins would be predicted not to supplant, but more likely complement the changes proposed above, and those proposed below (Aiello and Wells, 2002; Pontzer et al., 2016).

Aiello and Wheeler (1995) posited that a reorganization of energy allocation among tissues in the body may have been key to the evolution of the human brain. To account for "the missing difference in BMR" (Aiello, 2007, pg. 17), they suggested that energy to fund the brain came from a decrease in the size of the gastrointestinal tract (gut) as diet quality increased.

1.1.4 The brain versus the gut

Key to the ETH was the recognized differential expense of tissues in the body. For example, the internal organs including the heart, liver, and kidneys, along with the brain, comprise roughly 60% of REE in adult humans, despite accounting for only 5-7% of body mass (Brozek and Grande, 1955; Holliday et al., 1967; Elia, 1992; Heymsfield et al., 2012a). Citing data on human organ mass and metabolic rate reported by Aschoff et al. (1971), Aiello and Wheeler (1995) observed that the mass-specific metabolic rate of the gut was also relatively high, with its percentage contribution to total BMR similar to that of the brain.

The considerable contribution of the internal organs to human REE suggested that even small changes in tissue size could have led to a substantial reorganization of the energy budget. Following from that, if a reorganization of the energy budget occurred, it was more likely to have involved these high metabolic rate tissues, rather than larger-in-mass but less expensive skeletal muscle and adipose tissue (Elia, 1992; Aiello and Wheeler, 1995). Aiello and Wheeler's (1995) analyses specifically indicated shifts in energy allocation between gut and brain tissue. First, this was suggested by an analysis based on organ masses in a 'standard' human male and a primate of comparable body size, wherein the human gut was smaller in size than predicted by roughly the same amount that the brain was larger than predicted (Figure 1-3).

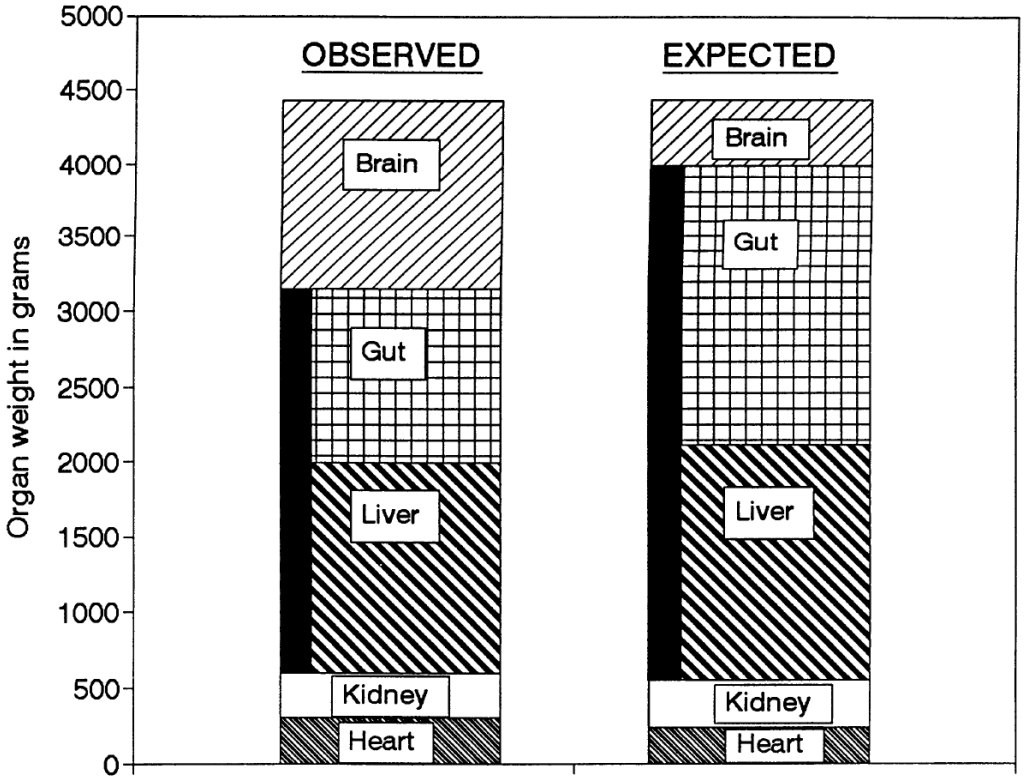


Figure 1-3 Observed vs. expected organ mass for a 'standard' 65kg adult human male.
From Aiello and Wheeler, 1995.

Secondly, a calculation of the estimated energy savings associated with the reduction in gut mass was similarly matched to the estimated metabolic increase associated with brain enlargement. Finally, Aiello and Wheeler showed a negative correlation between relative brain mass and relative gut mass in 18 anthropoid primates, including *Homo sapiens*. A comparison of equations for the relationship of gut mass to body mass in anthropoids and, separately, non-primate mammals suggested that anthropoids had smaller guts relative to body size than the average mammal, in addition to anthropoids' noted larger relative brain size (although Hladik et al. (1999) disagreed with the findings of smaller-than-expected gut size in humans, based on the available data).

Carrying out a similar analysis of relative brain mass against relative gut mass whilst correcting for phylogenetic relationships, Isler and van Schaik (2006) also found a negative correlation between the brain and gut in anthropoids (however in a further analysis in a later paper, the authors reported no association; see Navarette et al., 2011).

The data presented by Aiello and Wheeler appeared convincing, whilst other factors were consistent with their hypothesis; for example, the association between increased brain size and diet quality. Animals that use a larger proportion of their resting metabolism to fuel their brain have higher-quality diets, whilst gut size and diet quality are negatively related (Milton, 1987; Leonard and Robertson, 1994; Aiello and Wheeler, 1995; Snodgrass et al., 2009). For humans, a more readily digestible, nutrient-rich diet may have provided additional nutrients and calories, whilst also facilitating the diversion of energetic resources to the brain as the gut became smaller and less specialized (Aiello and Wheeler, 1995; Kaufman, 2003).

The ETH when published offered little supporting empirical evidence in humans, however it broke new ground with its implicit suggestion that tissues and organs of the body ultimately compete for energy resources. Subsequent studies, which I discuss further in Section 1.3, have provided support for the notion that energy allocation to a given organ or tissue is not independent of allocation to others in the context of a fixed energy budget. Other authors have acknowledged this as well, but have suggested patterns of allocation involving different tissues.

1.1.5 The brain versus skeletal muscle

Leonard, Robertson, Snodgrass and colleagues noted that a reduction in skeletal muscle mass may have been important in the context of hominin brain expansion. They demonstrated that primates overall had less muscle compared to other mammals, and that humans had less muscle than other primates of similar body size (Leonard et al., 2003; Snodgrass et al., 2009). A recent comparative analysis of human and bonobo body composition was consistent, corroborating the finding

that humans are relatively less muscular, and also suggesting that hominins likely experienced a redistribution of muscle tissue between the upper and lower body (Zihlman and Bolter, 2015).

The observed relationships of skeletal muscle relative to body size in humans and other primates may be explained by locomotor patterns. Primates have an arboreal heritage, which evidence suggests would predispose to reduced muscularity relative to terrestrial species (Snodgrass et al., 2009). Humans may have adapted in this way to render bipedal locomotion and physical activity more efficient, or alternatively, lower muscle mass may be related to humans' increased adiposity (Snodgrass et al., 2009; Antón and Snodgrass, 2012). Human males and females – in particular females – are more adipose than would be expected for a mammal with tropical origins (Wells, 2006a, 2010a).

Using estimations of height and weight for hominin species, along with equations developed in modern human populations, Wells (2017) recently derived estimates of fat and lean mass indices for several hominin groups. The indices are derived in the same way body mass index (BMI) is calculated, for example lean mass/height^2. The results indicated a general trend in hominins towards decreased lean mass (which includes skeletal muscle), as shown by a decline in the lean mass index from australopithecines and paranthropines to *Homo* (Wells, 2017). Expending fewer energy resources building muscle may have contributed to freeing up energy for the expensive human brain (Leonard et al., 2003, 2007; Snodgrass et al., 2009; Muchlinski et al., 2012). In turn, reduced muscle and increased fat may have allowed females to more readily fund the growth of large-brained offspring (Wells, 2017).

In their paper, Aiello and Wheeler (1995) argued that a reduction in skeletal muscle could not feasibly balance RMR if brain size increased, due to muscle's lower SMR relative to the SMRs of the internal organs. The authors calculated that nearly three quarters of the body's total muscle mass would need to be replaced by

metabolically inert tissue to cover the costs associated with an increase in brain tissue (Aiello and Wheeler, 1995).

Despite its relatively low metabolic rate, muscle does, however, constitute a considerable proportion of the body's energy budget due to its size (Zurlo et al., 1990; Isler and van Schaik, 2006). As mentioned above, muscle may account for 30-40% of total body mass, in contrast to ~2% for the brain (Figures 1-4A and 1-5A). Elia (1992) puts muscle's relative contribution to BMR at 22% for the reference male, and 16% for the reference female[3] (Snyder et al., 1975). These numbers are not highly dissimilar to those reported for the brain, which are 21% and 20% for females and males, respectively (Elia, 1992; see Figures 1-4B and 1-5B). (In Elia's model, 'Miscellaneous' comprises all remaining body tissues after those explicitly quantified (heart, liver, brain, etc.) are accounted for; this includes gut tissue).

It should be noted, however, that the reference female and male are comparatively fat in relation to the average forager, at 33% and 21% fat, respectively. For example, a recent study by Pontzer and colleagues (2012) reported the average percentage fat of Hadza women to be 21%, whilst Hadza men demonstrated 13.5% fat on average.

[3] Researchers in 1975 described the body size and composition of the reference male and female to aid in calibrating appropriate radiation doses for occupational, public and medical settings (Snyder et al., 1975). Average body composition has since changed (Later et al., 2010), however the data as reported by Elia remains generally illustrative of the proportional contributions of tissues to body mass and metabolic rate.

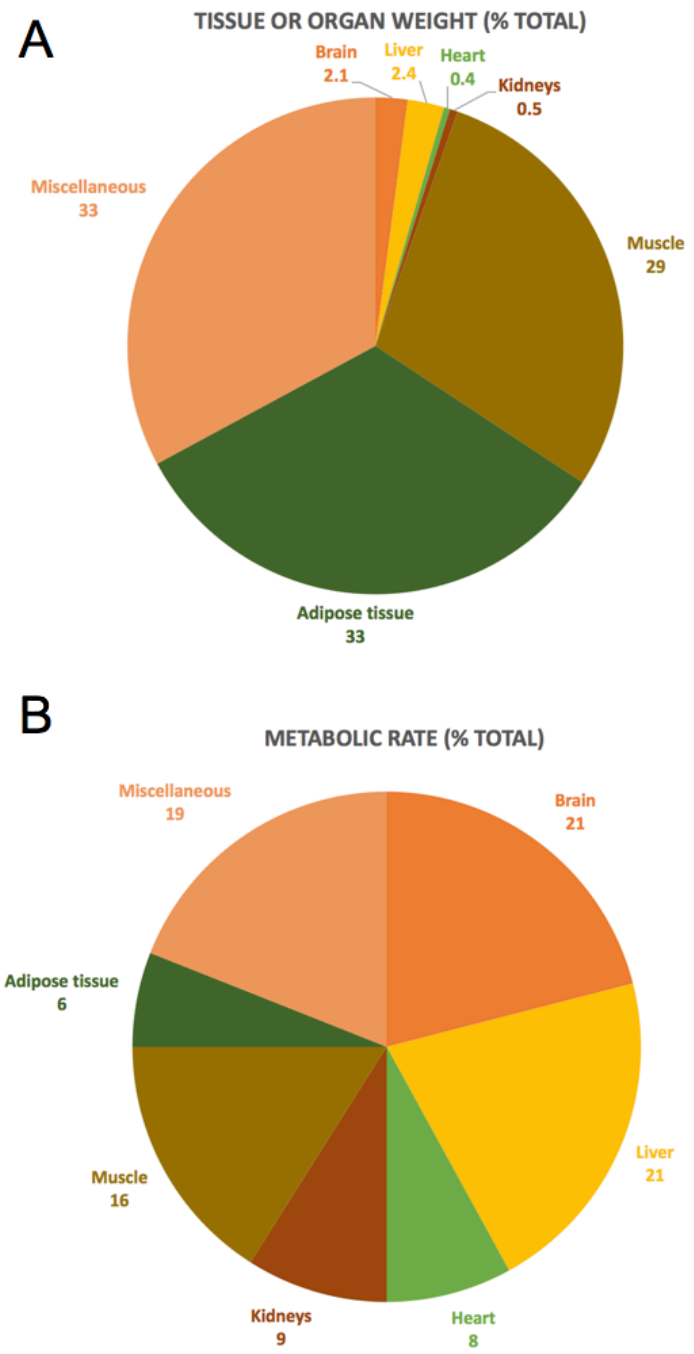


Figure 1-4 Percentage contribution of organs and tissues to body weight (A) and of organ/tissue metabolism to the overall energy budget (B) in the reference female.

Data from Elia, 1992.

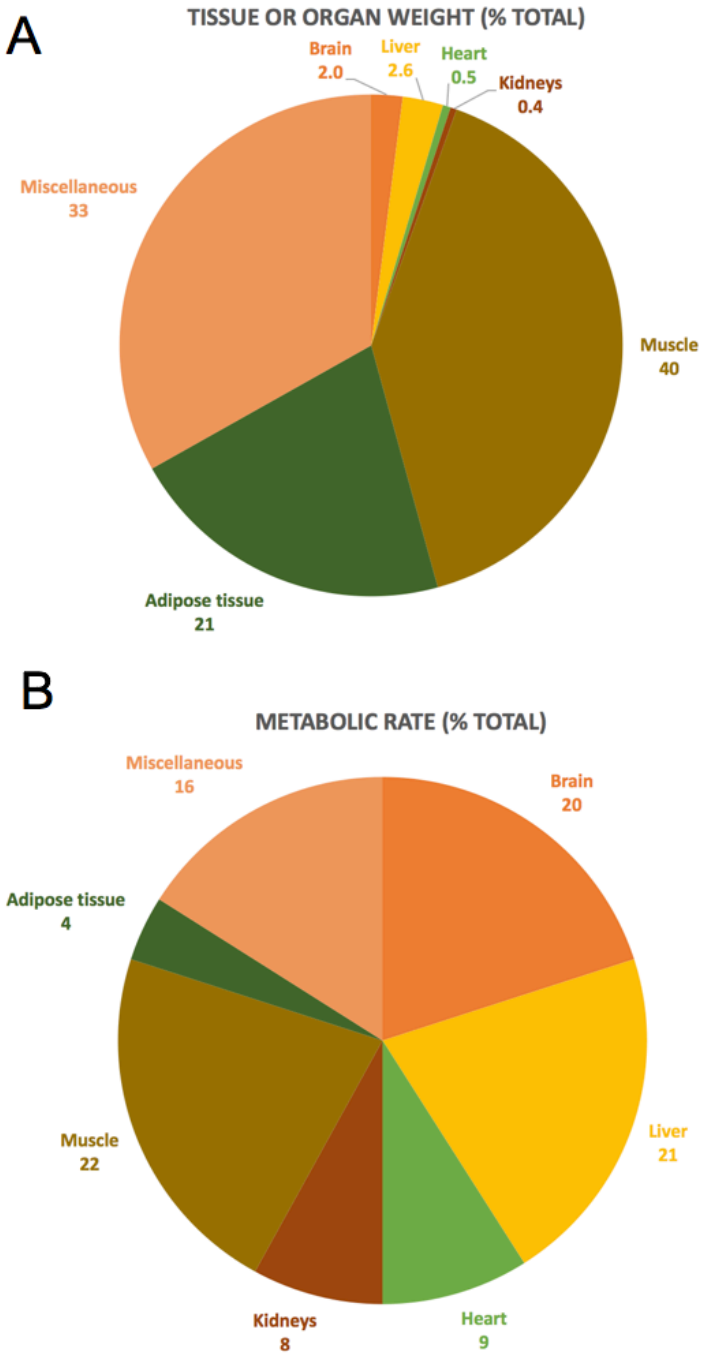


Figure 1-5 Percentage contribution of organs and tissues to body weight (A) and of organ/tissue metabolism to the overall energy budget (B) in the reference male.

Data from Elia, 1992.

Beyond its contribution to REE, the metabolism of skeletal muscle is elevated during physical activity, which is defined as “any bodily movement produced by skeletal muscle that results in energy expenditure” (Caspersen et al., 1985, pg. 126). Muscle metabolism may increase by a factor of 100 during exercise (Snodgrass et al., 2009; Muchlinski et al., 2012), whilst consistent, less rigorous activity is likely to have been an important factor shaping the allocation of energy within the hominin budget as well.

The hypotheses of Aiello and Wheeler and Leonard, Snodgrass and coworkers are both plausible, and are not mutually exclusive. It is possible that reductions both in the hominin gut, and in the level of skeletal muscle mass over time served to ‘release’ energy that then became available to support the growing brain. Both proposals concern what would have been long-term physiological changes in the hominin lineage, and certainly involved genetic adaptation. They highlight how competition amongst tissues may have occurred over an evolutionary timescale.

Remarkably, a very similar idea was put forward in a very different context, this time to help understand the effects of energy constraint in early life on the life course development of the human body. This was the purview of Hales and Barker’s TPH, which is described in the next section.

1.2 Tissue competition in development: the thrifty phenotype hypothesis

In line with Aiello and Wheeler’s ETH, Hales and Barker’s TPH predicted that energetic constraints on resource allocation would engender competition amongst tissues in the body. A key difference is that the TPH predicted this would occur in fetal life and infancy, when organs and tissues are initially developed. The hypothesis has made a substantial contribution within a framework which seeks to elucidate the early-life etiology of adult chronic disease risk: the developmental origins of health and disease, or DOHaD.

1.2.1 Evidence linking size at birth with adult chronic disease risk

The DOHaD framework employs a life course approach to understand how variability in early development may differentially predispose to chronic, non-communicable diseases, which include type II diabetes, cardiovascular disease, obesity and the metabolic syndrome (e.g. Barker, 1990, 1995; Cameron and Demerath, 2002; Gluckman and Hanson, 2006; Gluckman et al., 2007, 2008; Criscuolo et al., 2008; Kuzawa and Quinn, 2009; Wells, 2009, 2014).

For some time, the etiology of these diseases was attributed to genetics and lifestyle factors in adulthood, such as diet, smoking, and exercise patterns (Barker, 2007; Wells, 2016). One prominent hypothesis along these lines was the 'thrifty genotype' hypothesis of Neel (1962), which argued that population variability in the predisposition to type II diabetes was rooted in differential historical exposure to periods of 'feast and famine.' Neel predicted that genes which were selected to buffer poor conditions via effects on insulin production and the promotion of fat storage were now detrimental in industrialized nutritional milieus (Neel, 1962).

However, such explanations could not adequately account for the variation researchers were observing in the incidence and geographical distribution of chronic diseases (Barker, 2007). The importance of early-life conditions was increasingly recognized, as more and more evidence emerged linking poor fetal and infant growth with increased susceptibility to non-communicable conditions in adulthood (see McMillen and Robinson, 2005 for an extensive review).

For example, a classic study found that men exposed early in their gestation to undernutrition associated with the Dutch Famine of 1944-45 were more likely to develop obesity (Ravelli et al., 1976). In an English cohort, men in the lowest birth weight category were more likely to die of heart disease (Barker et al., 1989), while similarly, heart disease prevalence was higher in Indian men and women born relatively small (Stein et al., 1996). In a follow-up study of the Hertfordshire,

England cohort studied by Barker et al. (1989), men who were born small and who were smaller in infancy were more likely to have impaired glucose tolerance or type II diabetes as adults (Hales et al., 1991; Figure 1-6).

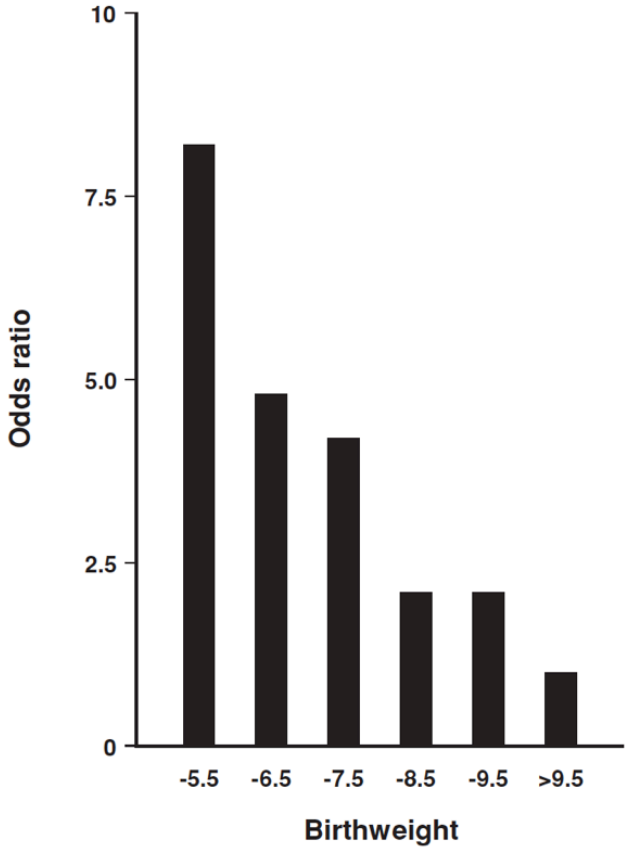


Fig. 1 Odds ratio for impaired glucose tolerance or type 2 diabetes according to birth weight among 370 men aged 64 years born in Hertfordshire (adjusted for adult body mass index).

Figure 1-6 An inverse association between birth weight and later risk of impaired glucose tolerance or type II diabetes.
From Hales and Barker, 2001.

The high prevalence of obesity and diabetes in the Pima Indians of Arizona in the United States has been attributed to genetics (e.g. Knowler et al., 1983), however a separate study found that Pimas born at lower birth weight later demonstrated greater insulin resistance for their size (Dabalea et al., 1999). Birth weight was also

negatively correlated with type II diabetes risk in Chinese adults (Tian et al., 2006), in women in the Nurses' Health Study in the US (Rich-Edwards et al., 1999) and in a cross-population systematic review (Whincup et al., 2008).

A crucial factor in the link between early development and later ill-health is the existence of 'critical windows' of plasticity. I describe this in the next section, before going on to introduce Hales and Barker's TPH.

1.2.2 Plasticity in critical periods of early life

The term 'plasticity' reflects the capacity of a single genotype to produce different phenotypes in different environments (Bradshaw, 1965; West-Eberhard, 1989; Bateson et al., 2004; Gabriel et al., 2005). Plasticity can be demonstrated in a variety of ways throughout the life course in association with environmental conditions. For example, humans adapt to current circumstances through changes in their behavior, as studied by human behavioral ecologists (Nettle et al., 2013). It is also well-established that components of the skeleton remain plastic throughout life and demonstrate variability in size and shape in association with mechanical loading patterns (Stock and Pfeiffer, 2004; Ruff et al., 2006).

In early life, humans and other animals demonstrate morphological and physiological plasticity in development (West-Eberhard, 2003), which may allow for a better 'match' between the phenotype and prevailing ecological conditions (Bateson et al., 2004; Barker, 2007). In particular, plasticity in the growth of body tissues and organs is substantial *in utero* and in early infancy, within the so-called 'critical periods' or 'windows' of development (Barker, 2007; Cameron and Demerath, 2002; Gluckman et al., 2008; Wells, 2016). These periods are described as such because they ultimately close, after which tissues become relatively canalized (less sensitive to environmental stimuli), and any deficits in growth track on into adulthood (Widdowson and McCance, 1963; Lucas, 1991; Godfrey and Barker, 2001; Fowden et al., 2006; Wells, 2016). This is less true for fat/adipose tissue, which remains relatively plastic after infancy and across the life

course (Bhargava et al., 2004; Ezzahir et al., 2005; Kensara et al., 2005; Wells et al., 2007; Wells, 2011).

Early critical windows are of major consequence because a specific type of organ/tissue growth occurs within them. This is hyperplasia, which is characterized by cell multiplication, and thus a progressive increase in cell number. It can be contrasted with hypertrophy, where existing cells enlarge (Enesco and Leblond, 1962; Winick and Noble, 1965; Allen et al., 1979; Owens et al., 1993). Both contribute to the mass of an organ or tissue, however hyperplasia is largely confined to the periods of increased plasticity in fetal and infant life, wherein cells rapidly divide (Barker, 1998, 2004). Studies in humans have indicated that cells divide just five times after birth, in contrast to 42 times *in utero*, although there is variation amongst specific tissues and organs (Hales and Barker, 1992; Owens et al., 1993; Barker, 1995).

For example, nearly all of a mammal's skeletal muscle fibers develop prenatally through hyperplasic growth (Allen et al., 1979; Owens et al., 1993). This is also true for nephrons in the kidney, and a study by Hinchliffe and coworkers (1992) found nephron numbers to be reduced in intrauterine growth-restricted (IUGR) infants compared to a control group. Crucially, they found no evidence for a compensatory increase in nephron number following birth (Hinchliffe et al., 1992). Perturbed pancreatic Beta cell development has similarly been found to persist in mature rats following early-life protein or caloric restriction (Reusens and Remacle, 2006), although Beta cell proliferation continues postnatally (Kassem et al., 2000), and thus is sensitive to environmental conditions in infancy as well (Barker, 1992; Barker and Fall, 1993).

Once sensitive developmental windows close and hyperplasic growth ceases, tissues may still enlarge through hypertrophic growth, and the body of course continues to grow following infancy. However, the persistence in adult phenotype of early-determined aspects of organ/tissue size and composition may have

implications for adult health. Hales and Barker (1992) recognized this in the context of the mounting evidence for a link between poor early growth and later chronic disease. This led them to propose the TPH.

1.2.3 The thrifty phenotype hypothesis

Critical windows of developmental plasticity occur within the niche of maternal phenotype (Barker, 1990; Wells, 2007a, 2014). Among other factors, the nutritional condition of the mother – shaped by her own development, and also current circumstances – shapes the growth of fetal tissues and organs (Wells, 2007a; Gluckman et al., 2008; Barker, 2012). With the TPH, Hales and Barker (1992) proposed that undernutrition during the sensitive periods of fetal life and infancy may be associated with deficits in specific organs, which would in turn predispose to adult chronic diseases like type II diabetes.

They argued that in the nutritionally-stressed human fetus, competition between the brain and pancreas would manifest, wherein the brain would be relatively buffered from growth disruption at the expense of poor pancreatic development. This is referred to as 'brain-sparing' growth, whereby the brain is relatively protected over other organs (Barker, 2004; Giussani, 2011; although protection is not always complete, see Hediger et al., 1998; Antonow-Schlorke et al., 2011; Pomeroy et al., 2012). With poor nutrition imposing "mechanisms of nutritional thrift upon the growing individual," Hales and Barker hypothesized that pancreatic Beta cell development would be adversely impacted (Hales and Barker, 1992, pg. 595). Beta cells produce insulin and thus have a vital role in glucose homeostasis. A defect in Beta cell function was therefore proposed as one potentially important link between poor early growth and adult type II diabetes susceptibility (Hales and Barker, 1992).

As Aiello and Wheeler recognized for earlier hominins, the pattern of energy allocation among organs and tissues may vary within the constraints of an organism's energy budget. If sufficiently constrained, the fetus may be forced to

'choose' to invest in some tissues over others. As Barker elaborated in a recent lecture, the developing individual has "a hierarchy of priorities" (Barker, 2012, pg. 186; also Wells, 2013) that are shaped by its genes and ecological conditions. For humans and many other animals, buffering growth of the brain is a central priority, and energy used to do so cannot then be used to fund the growth of tissues positioned lower in the hierarchy (Barker et al., 1993; Barker, 2012). This may affect the pancreas, as the original TPH predicted, or possibly other organs including the liver, kidneys and spleen.

The TPH of Hales and Barker was immediately distinguished from the 'thrifty genotype' hypothesis proposed by Neel (1962), which was briefly introduced above. Both held that something was interacting with aspects of adult lifestyle in the industrialized niche to promote chronic disease, however they diverged with respect to what the key factor was. Genetics are not unimportant, as Hales and Barker acknowledged, however limited evidence has been found to support the contention that specific genes have substantial effects on variability in metabolism and disease risk (Wells and Stock, 2011; Barker and Lampl, 2013). In contrast, there is compelling support for the TPH, as suggested by lines of evidence from several studies.

1.2.4 Supporting evidence for the TPH

In 1993 Barker and colleagues assessed cholesterol profiles in men and women who had their abdominal circumference taken at birth. The circumference measure, which served as an index of liver size, was found to be negatively related to total and LDL cholesterol concentrations. In line with the central predictions of the TPH, decreased liver size may have resulted from brain-sparing growth, which ultimately impacted cholesterol metabolism (Barker et al., 1993). This indicated that the growth of internal organs other than the pancreas could be forfeited in the face of early nutritional insufficiency.

Brain preservation at the expense of the liver had been noted previously, and head-to-abdominal circumference ratios were found to be higher in a sample of small-for-gestational-age (SGA) fetuses relative to those exhibiting normal growth (Campbell and Thoms, 1977). In another study on the effects of the Dutch Famine, an increased head-to-birth weight ratio in exposed individuals likewise suggested brain-sparing (Ravelli et al., 1998). Indeed, the predictions of the TPH manifest in what is referred to as asymmetric IUGR, which represents approximately 70-80% of IUGR incidence (Brodsky and Christou, 2004). The asymmetric pattern is characterized by a reduction in the size of the abdomen relative to the head, and "is attributed to the ability of the fetus to adapt, redistributing its cardiac output to the spleen, adrenal, coronary and cerebral circulations" (Brodsky and Christou, 2004, pg. 307). An investigation by Latini and colleagues (2004) assessed differential organ growth in SGA and appropriate-for-gestational-age (AGA) newborns in more detail using ultrasonography. Although brain or head size was not measured, the authors were able to compare volumes of the kidneys, liver and spleen between SGA and AGA infants, and found that organs were smaller in the former group.

Several studies have observed differential organ growth in rats under nutritional stress. For example, rats given a restricted diet after weaning demonstrated lower organ weights than controls; after refeeding, just the brain and lungs appeared to recover normal weight (Winick and Noble, 1966). Similar experimental results saw reductions in the pancreas, spleen, muscle and liver of rats in response to nutritional insufficiency, whilst the brain and lungs decreased less in weight (Desai et al., 1996; Petry et al., 1997). An earlier study showed that rats which first grew quickly and were then subject to undernutrition had heavier testes and stomachs, but lighter spleens, livers and small intestines (Widdowson and McCance, 1963).

Findings have also been reported which support the broader formulation of the TPH, but suggest tissues and organs may be forced into competition under the influence of environmental exposures that extend beyond nutritional stress per se.

For example, a study by Alexander (2003) showed that the experimentally induced disruption of uteroplacental perfusion in pregnant rats induced IUGR and low birth weight, and predisposed the affected animals to hypertension. Elsewhere, rats exposed to hypoxia and anoxia demonstrated increased cerebral blood flow, whilst perfusion to the intestines was reduced (Barbiro-Michaely et al., 2007). An experiment using chick embryos further indicated that variation in oxygen availability may impact organ or tissue growth. If a hypoxic condition was induced, chick embryos were found to largely maintain the size of the brain and heart, as determined by comparison with a control group, however liver size was more adversely affected (McCutcheon et al., 1982).

Tissues may be affected beyond the brain and internal organs. As noted in the title of their 2012 paper, Pomeroy and colleagues were able to extend the thrifty phenotype hypothesis to limb proportions in a test of two groups of Peruvian children. Limb length (femur + tibia) is known to demonstrate greater plasticity than head-trunk height (i.e. sitting height), and the former, like birth weight, has been negatively associated with adult chronic disease risk linked to adverse environmental impacts (Gunnell et al., 2002; Bogin and Varela-Silva, 2010; Whitley et al., 2012). Children living in the Andean highlands faced several environmental stressors including hypoxia, cold temperature, poverty, and reduced access to healthcare and adequate nutrition. Children in the second, lowland population faced environmental stress as well, but apparently to a lesser degree, as suggested by the lowland group's relatively low rates of stunting and wasting (Pomeroy et al., 2012).

Using anthropometry, measurements were obtained of highland and lowland children's head-trunk height, upper and lower limb lengths, and also limb segments including zeugopod (ulna/radius or tibia/fibula) and autopod (hand or foot) segments. Head circumference was measured as well, and was found to demonstrate the smallest difference of all the measurements between the populations. Comparing the two groups of children with adjustment for head size,

the authors found that differences were greatest in full limb and zeugopod segment lengths, relative to head-trunk height or the lengths of the autopod elements (Pomeroy et al., 2012, Figure 1-7). The results are consistent with brain-sparing, whilst also suggesting that the organ-containing trunk is preserved relative to growth of the limbs. A novel finding was the apparent difference in preservation of specific aspects of the limbs: autopod segments may be relatively protected due to their functional importance in manual object manipulation and locomotion (Pomeroy et al., 2012).

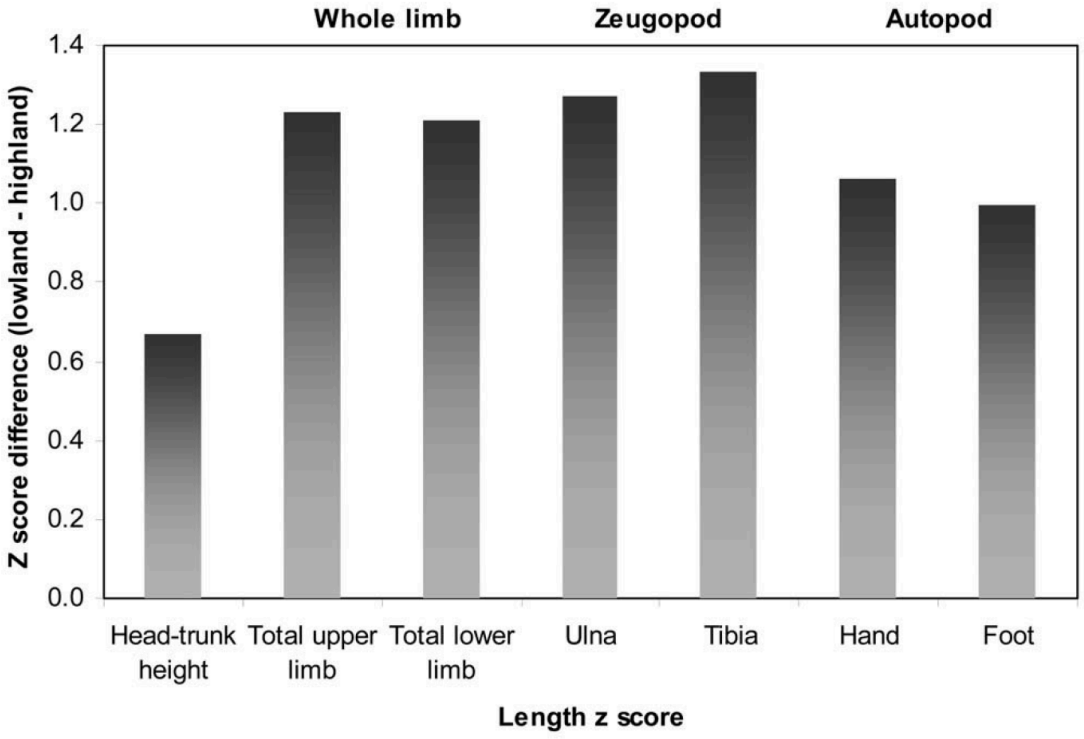


Figure 1-7 Differences in head-trunk and limb segment z-scores (reflecting number of standard deviations from the mean) for lowland versus highland children.
From Pomeroy et al., 2012.

In another recent article, Baker and coworkers (2010) developed an allometric model to test the predictions of the TPH, which they framed as a test of 'brains

versus brawn.' Using data collected from US children in 1998 (the third National Health and Nutrition Examination Survey (NHANES III)), they asked whether individuals born at lower birth weight could be seen to have 'sacrificed' muscle mass to protect the brain, and if the rates of brain and skeletal muscle growth were inversely related. To test their hypotheses, the authors utilized head circumference as a proxy for brain mass, and a proxy for muscle mass was derived from an upper-arm circumference measurement. This approach relies on less robust methods than are used in the current study (this is discussed further in Chapter 3). Nevertheless, Baker et al. (2010) found support for the predictions of Hales and Barker's TPH: relative growth of the head was not different between low and normal birth weight groups of children, whilst muscle growth appeared to be restricted in low birth weight children (<2.5kg), relative to those in the normative birth weight range.

As the above studies highlight, all organisms – human and non-human – are expected to face competition amongst organs and tissues as they build their bodies during development. In humans, this contributes to variability in body composition within populations, and also shapes differential chronic disease risk, as Hales and Barker (1992) and others showed. I return to expand on this in Section 1.4 and discuss its relevance to the selection of my study cohort. First, I introduce life history theory, which provides a valuable theoretical framework within which to integrate the ETH and TPH more systematically and situate my own study hypotheses.

1.3 Life history theory

Life history theory explains how local environments shape the manner in which organisms utilize energy resources, and this in turn shapes species' diversity. It is a robust framework, with its central predictions supported by a wealth of data. Neither Aiello and Wheeler (1995) nor Hales and Barker (1992) explicitly invoked life history theory in their publications, however its central tenet – the trade-off – is what they predicted must occur among components of the body in the face of finite

energy resources. Their approach was groundbreaking, and remains rare from a life history perspective; the majority of the literature focuses on trade-offs among biological functions such as growth and reproduction, rather than individual somatic traits.

In this section I introduce the central components of life history theory and discuss supporting evidence for functional trade-offs in order to highlight the theory's explanatory power (although the review is not exhaustive). Building on that discussed in the previous section, I then overview further evidence for tissue trade-offs, some of which has been reported from studies testing the predictions of Aiello and Wheeler's ETH.

1.3.1 The principle of allocation

The notion of competition features prominently in Charles Darwin's theory of evolution by natural selection; specifically, competition amongst individuals for the resources necessary to survive and reproduce. Life history theorists in diverse fields including biology, ecology and anthropology have long-recognized that competition also occurs at an intra-individual level, between a number of essential functions that require energetic input in the living animal (Cody, 1966; Gadgil and Bossert, 1970; Hill, 1993). Both inter- and intra-individual competition occur in environments where energy resources are finite.

The 'principle of allocation' reflects the notion that with resources fundamentally limited, energy used to fund one function is unavailable to fund others (Cody, 1966; Hill, 1993). Thus, investment in one function necessarily comes at the cost of another, or is 'traded off' (van Noordwijk and de Jong, 1986; Stearns, 1989; Roff and Fairbairn, 2006). Organisms are predicted to trade off resources to various essential functions in a manner that will promote their survival and reproduction, given constraints of their biology and environment (Gadgil and Bossert, 1970; Horn, 1978; Case, 1978; Stearns, 1992; Hill, 1993; Tracer, 2002).

Growth and reproduction are essential functions at the center of many energetic trade-offs (Stearns, 1992; Hill, 1993). Energy is also required for maintenance and repair of the body, and to defend against pathogens via the immune response (Stearns, 1992; Hill, 1993; Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; McDade, 2003). Strategizing allocation among these areas to ultimately promote reproduction and enhance fitness is a delicate balancing act. Fitness may suffer if growth or maintenance is funded at the expense of reproduction (although this ultimately depends on environmental circumstances; see below). At the same time, however, maintaining the soma increases the chance an animal will survive to reproductive age, and investment in growth may further increase reproductive capacity (Gadgil and Bossert, 1970).

An organism's full life cycle, positioned by Bonner (1965) as "the central unit in biology" (pg. 488), is thus a series of investment decisions which are part of an overarching life history strategy to pass on one's genes (Ellison, 2003; Speakman, 2008; Wells, 2016). The overarching strategy is that of the species, shaped in part through long-term genetic adaptation, whilst plasticity allows for flexibility in the magnitude and direction of allocation depending on prevailing conditions (Wells, 2016). As introduced in the previous section, plasticity operates on a much shorter timescale than genetic adaptation.

Mortality risk and environmental quality profoundly shape species-level life history strategies. These factors also influence organisms' energy allocation 'decisions' within the life course in response to more acute circumstances. This is described further below.

1.3.2 The impact of environmental circumstances

Patterns of energy investment across the life course are associated with observable life history traits including an animal's growth trajectory, age at maturity, adult body size, age at death, and the number and quality of its offspring (Stearns, 1992; Hill and Kaplan, 1999). Investment strategies and the

characteristics of these key traits are largely determined by levels of extrinsic mortality risk, or the probability of dying at a given point in the life course due to predation, violence, disease, starvation, or climatic factors (Promislow and Harvey, 1990; Gurven and Kaplan, 2007).

Mortality risk, for example, affects the calculus regarding investment in growth and body size, as it signals the likelihood of receiving returns on such an investment. As noted above, larger adult body size may promote greater reproductive potential (Gadgil and Bossert, 1970; Stearns and Koella, 1986; Charnov and Berrigan, 1993; Stearns, 2000). However, any such benefits would be outweighed by the costs if an organism missed out on reproduction altogether. If mortality risk is high, organisms are thus predicted to grow less and reach maturity more quickly so as to start reproducing sooner (Promislow and Harvey, 1990). This has been demonstrated with experiments in the lab and in nature.

Stearns and colleagues (2000) demonstrated variable investment in the growth and reproductive effort of fruit flies through the manipulation of their mortality rates in a laboratory experiment. Specifically, adult flies in the higher mortality group were smaller and demonstrated higher fecundity, whilst the opposite traits were observed in the low mortality group (Stearns, 2000). In the field, groups of guppy fish were observed to grow at different rates and mature earlier or later, apparently in association with the level of predation in their area of the stream. To test this, researchers transplanted the fish so they were exposed to more or less predated areas; over generations, guppies newly exposed to greater mortality risk matured earlier, whilst those moved to a safer environment grew relatively slowly and matured later (Reznick and Ghalambor, 2005).

Beyond predation levels, the quality of the environment may also impact the allocation of resources to life history functions in association with energy availability (Lambers and Poorter, 1992; Ellison, 2003; French et al., 2007; Wells, 2016). As explored in Section 1.2 above, this is a key factor shaping energy

allocation to organs and tissues in the body in early-life development. Similarly, the capacity of an organism to allocate energy to key functions such as reproduction may be constrained in an environment characterized by inadequate nutrition and/or exposure to infection and disease (Lochmiller and Deerenberg, 2000; Tracer, 2002; Walker et al., 2006; Gurven et al., 2016).

For a human fetus facing undernutrition, the prospect of reproduction remains far in the future; the immediate drive is to allocate available energy so as to promote survival, and thereby increase the chance of reaching reproductive age. For organisms which develop, reproduce and die much more quickly than humans, the calculus is predicted to be different. For example, when young adult male fruit flies were injured and nutrient-deprived through experimental parasitization, they responded by increasing their reproductive effort (Polak and Starmer, 1998; Figure 1-8 below). This demonstrates how in certain organisms and at certain points in the life course, constraints due to low resource availability may be overridden if the potential to lose out on reproduction altogether is high (Stearns and Koella, 1986; Charnov and Berrigan, 1993; Walker et al., 2006).

Signals of risk and/or poor environmental quality may favor reproduction early in adolescent human females, promoting earlier maturation. High rates of mortality have been inversely associated with age at first birth in populations from rural Dominica (Quinlan, 2010) and Sub-Saharan Africa (Gant et al., 2009). In the United States and England, an earlier average onset of reproduction was identified in disadvantaged neighborhoods characterized by low life expectancy (Wilson and Daly, 1997; Nettle, 2010).

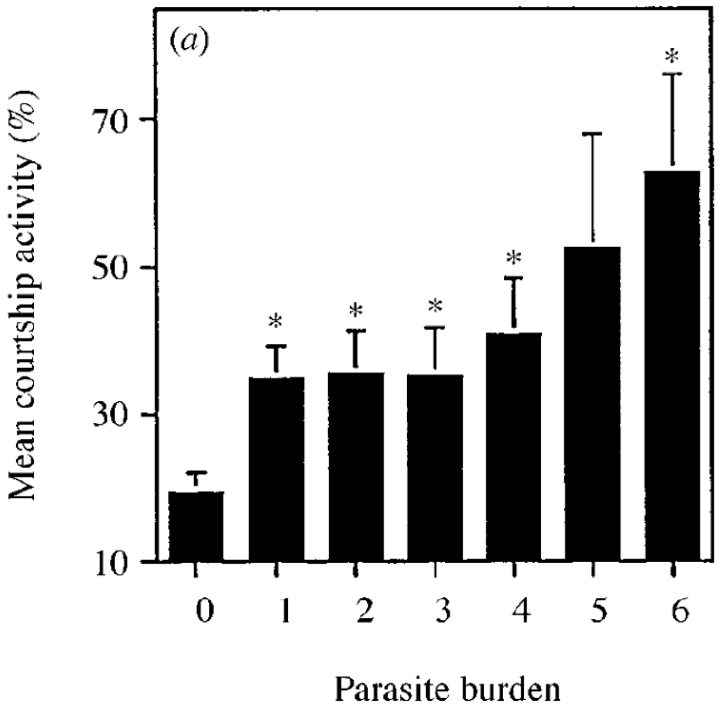


Figure 1-8 Proportion of time expended on courtship activity in relation to level of parasite burden in male *Drosophila* flies.
From Polak and Starmer, 1998.

A recent study suggests that an energy allocation strategy leading to earlier maturation in humans may be set in motion in response to conditions *in utero*, and demonstrates how earlier maturation may in turn affect growth. In South Asian women in the UK, birth weight was taken as a proxy for maternal investment, which reflects environmental and maternal condition (Wells et al., 2016a). Those born with low birth weight reached menarche earlier and were relatively shorter. The results suggest that signals of suboptimal conditions via decreased maternal investment in utero favored a 'faster' life history strategy (Wells et al., 2016a).

Risk levels over time shape growth patterns, final body size, and other traits at a species level, which explains trait diversity amongst organisms in nature (Stearns, 1992; Hill, 1993). Species in riskier environments are typically placed on the 'fast'

end of a slow-fast life history continuum, due to their faster/earlier maturation, smaller adult size, production of more, low-quality offspring, and shorter lifespan (Stearns, 1983; Read and Harvey, 1989; Promislow and Harvey, 1990). Overall, the life course is shorter and more quickly traversed, in contrast to 'slow' species, which generally demonstrate the opposite traits.

A key avenue by which extrinsic risk shapes the lifespan is through its impact on senescence, which refers to the gradual wearing down of an organism's cells and tissues and their functions with age (Kirkwood and Austad, 2000). One prominent explanation for this phenomenon invokes life history theory; namely, the body wears down because investment in its maintenance (e.g. through DNA repair and antioxidant defense) is traded off against other functions, such as reproduction (Kirkwood, 1977; Kirkwood and Rose, 1991). Increased somatic maintenance is favored, however, in lower-risk environments, so that organisms in such conditions age more slowly and live longer.

Consistent with the reduced adult mortality of human foragers relative to chimpanzees and similarly-sized mammals (Hill, 1993; Hill et al., 2001; Robson and Wood, 2008), humans live exceptionally long lives (Robine and Allard, 1998; Gurven and Kaplan, 2007). The human species is thus habitually placed at the 'slow' end of the life history continuum, although some 'faster' life history traits including early infant weaning and short inter-birth intervals complicate this (Hawkes et al., 1998; Kennedy, 2005; Kuzawa and Bragg, 2012; Wells 2012a, 2016). Nevertheless, humans grow incredibly slowly and thus take longer than other primates to reach maturity, and they invest considerable resources in large bodies and brains (Potts, 1996; Aiello and Wheeler, 1995; Aiello and Key, 2002; Gurven and Walker, 2006; Robson and Wood, 2008).

In the section below, I describe a range of studies that have supported the predictions of life history theory, by showing how ecological circumstances shape energy allocation to different functions. This results in organisms residing at

different points along the slow-fast life history continuum, both across and within species.

1.3.3 Evidence for functional trade-offs

As introduced above, the 'disposable soma' theory of Kirkwood and colleagues predicts that signals of extrinsic mortality risk will ultimately determine how much an animal invests in maintenance, and thereby shape the senescence rate and the length of the animal's lifespan (Kirkwood and Rose, 1991; Kirkwood and Austad, 2000). These predictions have been supported by several lines of evidence. For example, female Virginia opossums living on less-predated islands experience lower mortality, age slowly relative to their counterparts on the more heavily predated mainland, and produce fewer offspring per litter (Austad, 1993). Similarly, reduced predation may explain the longer lifespans identified in arboreal relative to terrestrial mammals (Shattuck and Williams, 2010). In general, bats and birds are found to demonstrate greater longevity compared to eutherian non-flying mammals of similar body size, which has also been interpreted as an effect related to reduced predation risk (Austad and Fischer, 1991; Wilkinson and South, 2002; Munshi-South and Wilkinson, 2009).

Evidence for the disposable soma model has likewise come from lab experiments in organisms such as *D. melanogaster* and *C. elegans* (Kirkwood and Austad, 2000). Zwaan and colleagues (1995) demonstrated that selection for increased longevity leads to reduced reproductive output in female *Drosophila* flies, which is consistent with the prediction that maintenance of the body trades off against reproductive effort. With this in mind, the queens of eusocial insect species are an interesting case, as they live much longer than solitary insects or their own workers, whilst also reproducing prodigiously to maintain the viability of the colony. Keller and Genoud (1997) suggested that the longevity of queens is attributable to their low mortality risk in heavily protected nests, and their exhibited pattern of increasing age-dependent fecundity, which may slow ageing.

I described the idea and offered examples in the previous section of ecological conditions shaping the scheduling of reproduction through earlier or later maturity. As suggested already through some of the examples, age at maturation is likely to trade off with somatic growth. In mammals, growth in body size generally ceases once reproduction commences (Charnov and Berrigan, 1993). Human pygmies are illustrative of the growth-reproduction trade-off, as shown by Migliano and colleagues (2007). Rather than direct selective pressures explaining pygmies' small body size, the authors argue that it has arisen via the strategy of ceasing growth and reaching reproductive maturity earlier. This may be the most viable reproductive strategy in environments which are characterized by significant mortality in both young and older individuals (Migliano et al., 2007). Similar findings have also been reported for small-scale societies in Venezuela and the Philippines (Walker et al., 2006).

On one hand, expanding the period of growth so as to achieve greater height may increase fitness, however at the same time, fitness may also be augmented by increasing the number of years during which offspring can be produced. Taller height is associated with increased survival and fecundity, and the number of surviving offspring is a primary marker of evolutionary success; however, a trade-off is expected due to the energetically demanding nature of investment in these areas (Sear et al., 2004). Sear and colleagues modelled such a trade-off among females in the Gambia, finding that women who began reproducing earlier were shorter than those whose age at first birth was later (Sear et al., 2004; Allal et al., 2004). Their optimality model indicated that reproductive success in the context of the local environment would be maximized for women reaching puberty at age 18, as at this point, time spent growing would be balanced by the benefits that additional height conferred through offspring mortality reduction (Allal et al., 2004).

Energetic costs of activating the immune response are also recognized to engender trade-offs with growth and reproduction, and have further been shown to alter the length of the lifespan. For example, endemic helminth infections are

associated with stunted growth, evidenced in part by the fact that growth outcomes are seen to improve in children treated with anthelminthic drugs (Crompton and Nesheim, 2002). With respect to reproduction, researchers who manipulated the clutch size of female common eider birds (*Somateria mollissima)* observed that those with larger clutches demonstrated markers of decreased immune function (Hanssen et al., 2005). Similar findings have been reported in collared flycatchers (*Ficedula albicollis)*, where increased brood size correlated with the rate of parasite infection (Gustafsson et al., 1994).

With respect to immune trade-offs impacting longevity, Crimmins and Finch (2006) have linked reduced early-life mortality with increased longevity in European cohorts born before 1900. The authors posit longer lifespans were related to a reduction in the exposure of individuals to inflammation and infection as children, which when previously encountered may have diverted resources from growth and maintenance and increased the rate of senescence. At the same time, those exposed to infection in early life may survive, but eventually pay a penalty that is reflected in their earlier mortality. In adult cohorts from England and Wales, maternal mortality rates at the time cohort participants were born significantly predicted their age of mortality (Barker and Osmond, 1987). Similarly, exposure to airborne infectious disease in the first year of life increased mortality among individuals aged 55-80 in Sweden (Bengtsson and Lindström, 2003).

The examples offered above and in the previous section represent just a small number of studies offering evidence for trade-offs among life history functions, but hopefully serve to highlight the robustness of the theory for explaining energy allocation patterns under various environmental circumstances. Below, I describe evidence for trade-offs among somatic traits, largely identified in non-human animals. Although substantially fewer studies have taken a life history perspective to test tissue trade-offs, as I noted above, several have emerged since the publication of the ETH, in a number of cases to test its predictions.

1.3.4 Evidence for tissue trade-offs in non-human animals

Aiello and Wheeler's ETH and Hales and Barker's TPH both predicted competition – or trade-offs – between the brain and organs of the body in humans. Looking to evidence in non-human animals, the tissue trade-offs observed are more mixed. This is not hugely surprising; as highlighted above, there is considerable variability in the strategies animals pursue to adapt to local ecologies.

For example, ground squirrels are animals that require prodigious fat stores for hibernation. Thus, when they are experimentally trapped, restrained, and unable to forage, they are seen to forfeit lean mass and retain fat, as measured against the body composition of control animals (Dark, 2005).

In another study, researchers examined potential developmental trade-offs between head horns, a sexually-selected trait, and genitalia of male horned scarab beetles (*Onthophagus taurus*; Moczek and Nijhout, 2004). Their experiment involved removing the genital precursor tissues, referred to as disks, during development to test for impacts on horn growth. They found that beetles who had the disks removed grew markedly larger head horns than those who had received a sham disk-removal operation. Interestingly, one of the hypotheses these authors sought to test was whether structures located at opposite ends of the body, rather than those situated adjacent or very close, would be seen to compete for resources. They concluded that structures draw from the same resource pool and will thus compete for those resources, irrespective of their specific location on the body (Moczek and Nijhout, 2004).

Nijhout and colleagues found comparable results in earlier studies of the butterfly (*Precis coenia*; Nijhout and Emlen, 1998; Klingenberg and Nijhout, 1998*)*. Like the structures of the beetles described above, the butterfly's wings develop from disks. When these were experimentally removed from the hindwings of butterflies in the larval stage, forewings after metamorphosis were relatively increased in size. The thorax and forelegs were also seen to increase in size, however measures of the

head and abdomen were not statistically different between experimental and control groups (Nijhout and Emlen, 1998).

In order to test for a functional trade-off between maintenance and immune defense in laboratory mice, Ksiazek and Konarzewski (2012) artificially selected for higher or lower BMR in two mouse lines and then subjected them to dietary restriction and an immune challenge. Their results were consistent with a trade-off between maintenance and immune function under nutritional stress (specifically, the immune response appeared to be compromised in both mouse lines), however they also noted effects on the size of organs: high BMR-selected mice lost more spleen and lymph node mass, whilst the low BMR line lost more thymus mass. Both lines saw reductions in internal organs over the course of dietary restriction (Ksiazek and Konarzewski, 2012).

With respect to the ETH, several apparently corroborative findings have come from studies in fish and amphibians. In 2013, Kotrschal and colleagues carried out an artificial selection experiment in guppies (*Poecilia reticulata*), selecting in different lines for either smaller or larger brain size relative to body length. They found a concomitant decrease in gut size in the larger-brained fish, demonstrating a reorganization of energy investment within the body in line with Aiello and Wheeler's hypothesis.

Further evidence of a gut-brain trade-off was found in Lake Tanganyika cichlid fish (Tsuboi et al., 2015), and in 30 species of frogs and toads, where brain mass was negatively correlated with the length of the digestive tract (Liao et al., 2016). A similar negative correlation was found in the African freshwater fish *Gnathonemus petersii*, which, similar to humans, uses a substantial proportion of its resting energy budget to fuel its brain, and consumes an energy-rich diet (Nilsson, 1996; Kaufman, 2003).

In contrast, a test of a negative correlation between brain mass and gut mass was not supported in 313 bat species by Jones and MacLarnon (2004), although a separate study in bats found an apparent trade-off between brain and testes size (Pitnick et al., 2006).

Two notable studies have been carried out by Isler and van Schaik and coworkers. In 2006, they conducted a test of the ETH in bird species, wherein they also tested for relationships among other body tissues, including visceral organs and skeletal muscle. They found that the brain did not trade off against the gut or other expensive tissues like the heart, liver, kidneys or lungs. Brain mass instead demonstrated a negative relationship with pectoral muscle mass, which suggests a trade-off in birds between locomotion and brain size and/or cognitive capacity (Isler and van Schaik, 2006).

In a more recent study, the authors performed necropsies in 100 mammal species, including 23 non-human primate specimens (Navarette et al., 2011). The brain volume of the measured mammals was not correlated with the digestive tract, or other organs including the heart, lungs, kidneys, liver and spleen. In this sample, Navarette et al. (2011) did however report evidence of a trade-off between brain size and adipose tissue, controlling for fat-free mass (primarily in the non-primate portion of the sample; Figure 1-9). They suggested that increased adiposity and increased brain size may represent alternative strategies for buffering starvation, and that the mammals under study tended to invest in one at the expense of the other (Navarette et al., 2011).

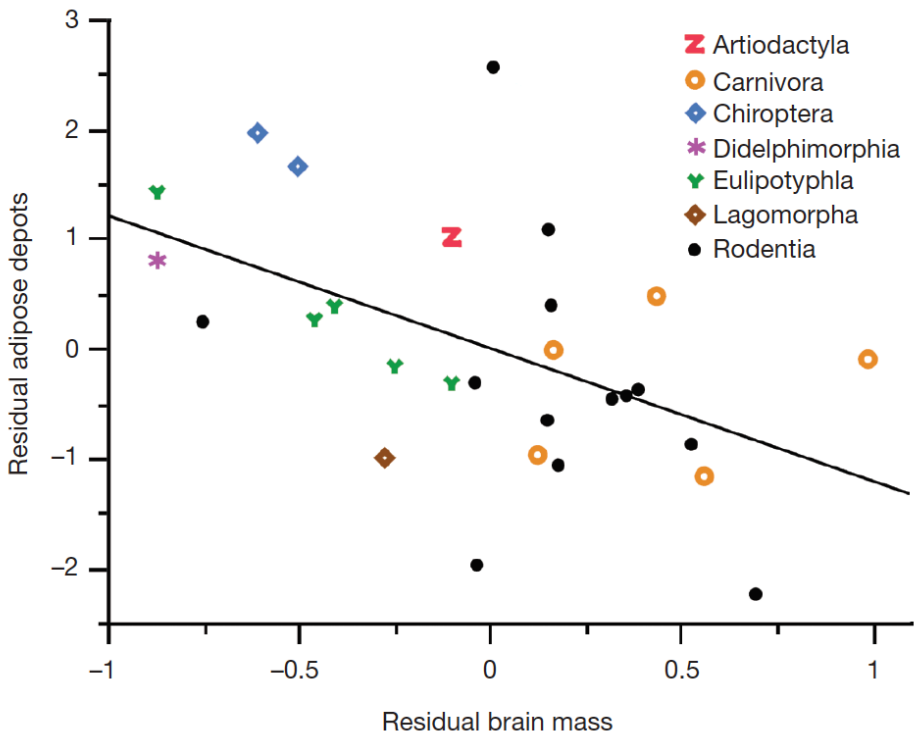


Figure 1-9 A negative association between brain mass and adipose tissue mass in wild-caught female mammals.
From Navarette et al., 2011.

Wells (2010a, 2012b, 2016) has developed the idea of the brain and fat stores as two interrelated 'risk management systems' in the body, which have the capacity to buffer a range of environmental stressors. The brain allows the animal to respond to shorter-term signals through behavioral responses, whilst adipose tissue is an important regulator of energy use within the body and its allocation amongst life history functions. In this way, adipose tissue both stores fuel and plays an essential role in determining how it is used in the context of shorter and longer-term ecological pressures. Humans demonstrate both relatively large fat stores and brains, which suggests that hominin evolution occurred in highly stressful environments (Wells, 2016).

Heldstab et al. (2016) further tested the brain-fat trade-off in non-primate mammal and primate species, finding a negative relationship between brain size and seasonal variability in body mass. The latter served as a marker of animals' propensity to store fat. Specifically, the negative correlation was seen in arboreal, but not terrestrial species. The authors posited that the costs of transporting extra fat on the body may be reduced in terrestrial animals, so that maintaining both increased brain and fat mass is viable. They further suggested this may have allowed large brains and fat stores to evolve in terrestrial hominins. A potential brain-fat trade-off in living humans remains to be tested, however the results presented by Heldstab and coworkers (2016) suggest these body components are less likely to demonstrate a negative relationship in humans. Indeed, a recent imaging study reported that fat-free body mass was correlated with decreased gray matter volume in the adult human brain, but fat mass demonstrated no such relationship (Weise et al., 2013).

1.3.5 The ETH and TPH in a life history framework

Evidence given above strongly supports the notion that competition for energy resources exists among functional areas and somatic traits, and such competition results in trade-offs when energy is constrained. Drawing from thermodynamic principles, life history theory holds that constraints on energy resources will be present in all environments (Hill, 1993), however the magnitude will vary in relation to external factors and the condition of the organism. The examples I have described highlight that costs and benefits associated with trade-offs occur over variable timescales and may involve genetic changes or shorter-term plastic responses in the service of augmenting survival and reproductive fitness.

The evolutionary ETH and developmental TPH can thus both be situated within a life history framework. Aiello and Wheeler (1995) largely focused on the energetic constraints of hominin encephalization, rather than why the brain was selected to grow bigger. Nevertheless, if the authors' hypothesis is correct, a trade-off between the brain and gut was incorporated over time into the hominin life history strategy

through genetic adaptation. It has long been recognized that investment in body size is an important component of the human strategy (Potts, 1996; Aiello and Key, 2002), however Aiello and Wheeler (1995) highlighted that tissues and organs of the body may be equally subject to allocation trade-offs, and should be considered as well. 'Why' and 'how' questions are both important for increasing our understanding of human brain evolution; a life history perspective underscores the importance of metabolic cost questions by highlighting that no tissue can be understood in isolation if the energy budget is fixed. This was also noted by Aiello and Wheeler (1995).

The capacity to demonstrate substantial plasticity in response to variable life course conditions is increasingly recognized as another key life history trait in humans (Potts, 1996, Wells and Stock, 2007, 2011; Kuzawa and Bragg, 2012; Wells, 2012a, 2016; Antón et al., 2014). This allows individuals to alter their life course strategy through shorter-term flexibility in the allocation of resources, as Hales and Barker (1992) predicted to occur in early life development in the face of undernutrition. The 'brain-sparing' nature of the trade-off predicted by the TPH indicates this may be the most viable 'decision' for the human fetus in poor circumstances, allowing it to survive and potentially reproduce later in life. Ultimately, as evidence outlined in Section 1.2 suggests, shorter-term benefits may be traded off against long-term costs in health and/or survival. In contemporary populations, the costs are expected to be higher for those encountering an obesogenic environment following infancy (Hales and Barker, 1992; Wells, 2016).

In the chapter's final section below I return to the TPH and discuss further how brain-sparing growth may shape adult body composition and the risk of ill-health. There is evidence that the 'thrifty phenotype' operates at the population level in modern humans, for example following long-term exposure to malnutrition. This

scenario is seen in contemporary South Asian[4] populations, wherein the characteristic body composition phenotype suggests developmental trade-offs between the brain and lean body mass.

1.4 The thrifty phenotype in South Asia

At the species level, humans are less variable genetically than other hominoid taxa (Gagneux et al., 1999), and greater variation is observed within human populations than among them (Rosenberg et al., 2002). Beyond genetics, geographical variability in modern humans has been and continues to be shaped by phenotypic plasticity, although differentiating between these two sources of variability is not always straightforward (Wells and Stock, 2007; Wells, 2017).

Diverse environmental factors and stresses including those related to climate, disease and nutrition have had long-term differential impacts on the world's populations. One consequence is that body composition varies in relation to regional and ethnic background (Ortiz et al., 1992; Rush et al., 2004; Yajnik, 2004; Wells, 2012c). Bergmann's (1947) and Allen's (1877) rules are well-known examples in anthropology, where local temperature is predicted to shape average body mass and proportions. More recently, it was recognized that populations differ on average in the percentage contributions of fat and lean tissues to overall body mass (Wang et al., 1994; Rush et al., 2007; Lim et al., 2011). For example, Asians from Indonesia, Singapore and China were found to have a higher percentage body fat compared to Europeans with the same BMI (Deurenberg et al., 2002).

This pattern is especially marked in South Asian populations: much evidence suggests they have, on average, decreased skeletal muscle mass and increased central fat mass relative to other populations (Banerji et al., 1999; Raji et al., 2001;

[4] In using the terms 'South Asia' or 'South Asian' I am referring collectively to the countries on the Indian subcontinent; otherwise, I refer to the specific country within South Asia, for example India or Bangladesh.

Rush et al., 2007; Unni et al., 2009; Wulan et al., 2010). Both aspects of phenotype are associated with higher metabolic risk, and indeed severe epidemics of type II diabetes and cardiovascular disease are occurring in South Asian countries (McKeigue et al., 1991; Shelgikar et al., 1991; Yajnik, 2004; Yusuf et al., 2005; Jayawardena et al., 2012). In a sample of children and adolescents in the UK, those with South Asian ethnicity had lower lean mass-for-height than individuals identifying as black or white (Haroun et al., 2010). Measured by computed tomography (CT), Indian men had more adipose tissue, less lean mass, and less leg muscle than Swedish men matched for body size and age (Chowdhury et al., 1996). There is also evidence that South Asians have smaller organs compared to Europeans of the same height (Wells et al., 2016b).

Even before specific aspects of body composition are considered, the TPH appears more likely to manifest in the life course development of South Asian individuals than in many other groups. South Asian populations demonstrate some of the lowest averages for stature worldwide, and it appears some countries are struggling to reverse these deficits; for example, adult height in Bangladesh and India appears to have plateaued below trends in East Asia (NCD Risk Factor Collaboration, 2016).

Low stature is in turn associated with low birth weight (Deshmukh et al., 1998; Bisai, 2010). Data show that South Asian countries indeed experience high rates of low birth weight (UNICEF/WHO, 2004), and also considerable child malnutrition (FAO, 2015), although the specific incidence varies by country. Data from the Millennium Cohort Study in the UK showed that Indian, Pakistani and Bangladeshi infants were smaller and more likely to demonstrate low birth weight than infants of European ethnicity (Kelly et al., 2008). The recognized link between low birth weight and later chronic disease risk was, as described above, a central stimulus for Hales' and Barker's (1992) TPH, which hypothesized trade-offs in organ investment would be necessary in the face of undernutrition in fetal life.

In 2003, Yajnik and colleagues reported findings from a study in which they used anthropometric techniques (e.g. circumferences and skinfold thickness measurements) to look beyond birth size and test whether body composition differed between babies born in India and the UK. Indian babies were found to be smaller and their abdominal circumference suggested decreased visceral organ size, although head circumference was similar between the two groups. Indian babies also appeared to have less muscle, but demonstrated a "fat-preserving tendency," especially those of smaller body size (Yajnik et al., 2003, pg. 177). The authors referred to this as the 'thin-fat' phenotype, and proposed, consistent with the TPH, that brain growth was buffered at the expense of muscle and visceral organs (Yajnik et al., 2003; Yajnik, 2004). Fat may have been preserved to promote survival and further support the growing brain.

Yajnik et al. (2003), like Hales and Barker (1992), recognized the important impact of maternal nutrition and intra-uterine energy supply on body composition development. They showed that the thin-fat phenotype, already recognized in Indian adults, actually emerged in fetal life, and subsequent data have supported this. For example, a magnetic resonance imaging (MRI) study comparing body composition in Indian and UK newborns found increased central adiposity in the Indian babies (Modi et al., 2009). Stanfield and colleagues (2012) further found that South Asian infants had more fat and less lean mass than those of European descent. The early-life presentation of this characteristic phenotype suggests the action of inter-generational maternal and/or epigenetic effects in populations that have been chronically undernourished (Hardikar et al., 2015), although genetic effects on birth size and body composition are also plausible (Hales and Barker, 1992; Dunger et al., 1998; Yajnik, 2004; Stanfield et al., 2012; Wells et al., 2013).

Hardikar and coworkers (2015) recently found support for the epigenetic/environmental scenario in a rat model, where they specifically sought to mimic the chronic undernutrition faced by humans in developing countries. Rats undernourished over 50 generations had lower birth weight, more centrally-

deposited fat, and less muscle than control rats, although the average size of the brain was similar between the groups. Results suggested the outcomes were driven by epigenetic, rather than genetic, effects. Undernourished rats were 'recuperated' for two generations in which they ate a normal diet, however the observed epigenetic modifications persisted (Hardikar et al., 2015).

The available evidence is suggestive of a thrifty phenotype effect at the population level, whereby chronic nutritional stress over generations has driven the thin-fat phenotype's manifestation in contemporary South Asians. Wells and colleagues (2016b) developed this argument in a recent paper based on a comprehensive review of archaeological and historical evidence extending back 10,000 years. They posit that a long-term negative secular trend in height and changes in muscularity and fatness are associated with persistent ecological stressors related to diet and infectious disease, and a number of severe famines (Wells et al., 2016b). Again, however, the relative contribution of plasticity and genetics has yet to be elucidated.

As described in Section 1.2, low birth weight and the underdevelopment of organs has been linked in many studies with increased chronic disease risk in adulthood. Low lean mass and increased central adiposity are also associated with cardiovascular disease and type II diabetes, and the high prevalence of these conditions in South Asian countries has been linked to South Asian body composition, as noted above (Banerji et al., 1999; Yajnik, 2004; Mohan et al., 2007; Unni et al., 2009; Jayawardena et al., 2012; Wells et al., 2016b; Wells, 2016).

Crucially, detrimental effects of sub-optimal organ/tissue growth are not limited to individuals who, for example, were severely malnourished in early life, experienced very low birth weight, became obese and diabetic, or would be described as generally 'unhealthy' in adulthood. In other words, associations between birth weight, differential organ growth and later health are not limited to extremes of early nutrition/growth and adult condition (Wells, 2016). In his capacity-load model

which expands on the insights of Hale's and Barker's TPH, Wells (Wells, 2010b, 2011, 2016, 2017; Grijalva-Eternod et al., 2013) identifies that there is a continuous, negative correlation between birth weight and the risk of later developing chronic conditions (e.g. Hales et al., 1991; Rich-Edwards et al., 1997). The link furthermore depends on adult body size and additional markers of 'metabolic load' (e.g. smoking, poor diet, lack of physical activity) interacting with 'metabolic capacity,' represented by traits including muscle mass and the structure and function of organs.

In this way, even those characterized by normative birth weight and organ development may experience differential chronic disease risk. Individuals in South Asian countries who fall within the normal range of birth size may be at increased risk relative to Europeans even before factoring in later metabolic load, as reduced metabolic capacity appears to persist at the population level for South Asians, as described (Yajnik et al., 2003; Wells et al., 2016b).

In the sections above I have introduced the ETH and TPH, and discussed their similar predictions of competition between somatic traits in the face of finite energy resources. As I have argued here, both these hypotheses are consistent with life history theory, and theoretically strengthened by being situated within its framework. However, gaps in the literature are recognized, as relationships between organs and tissues have been inadequately tested in humans, and appealing to life history theory highlights the dearth of studies on somatic, rather than functional, trade-offs. For the current study, I aimed to recruit healthy individuals of South Asian ethnicity to test for somatic trade-offs. The decreased skeletal muscle mass and potentially decreased visceral organ mass recognized in the 'thin-fat' phenotype is suggestive of somatic trade-offs, and therefore evidence of competition between tissues may be more readily observable in a South Asian cohort. At the same time, the current study will add to the literature on

variability in South Asian body composition, which is recognized to contribute to their heightened chronic disease susceptibility.

2 Research questions and hypotheses

As discussed in Chapter 1, two incredibly influential hypotheses in different fields arrived at a similar answer to problems concerning the evolution of the human brain and the etiology of adult chronic disease risk. Namely, Aiello and Wheeler and Hales and Barker both predicted the brain and visceral organs of the body would trade off against one another. As I showed, there is support for the prediction that organs and tissues compete for energy resources, both in humans and non-human animals, from lab experiments and studies in the field. At the same time, the predictions of life history theory, which are well-supported across species, closely match the predictions of the ETH and TPH.

However, a more direct test of the predictions of these two hypotheses in humans remains a gap in the literature of anthropology and biomedicine. A robust way to address this would be to gather detailed data on body and brain composition in a human cohort and test for relationships among organs and tissues. Prior evidence where brain and body tissues were directly measured largely comes from non-human animals (e.g. Moczek and Nijhout, 2004; Isler and van Schaik, 2006; Navarette et al., 2011; Kotrschal et al., 2013). Studies which have found evidence for tissue competition in humans have mostly done so using proxies for the brain, visceral organs or skeletal muscle size, such as head, abdominal and arm circumferences (e.g. Yajnik et al., 2003; Baker et al., 2010; Pomeroy et al., 2012); or, they have examined fat or organs using MRI or ultrasonography, but not measured the brain (e.g. Latini et al., 2004; Modi et al., 2009).

Also as yet unexplored is the question of whether specific components of the brain (e.g. gray matter, white matter, the cerebellum) can be seen to compete with organs or tissues in the body. Previous studies have focused on the whole brain. A more nuanced analysis could be done using high-resolution MRI data, which allows for the partitioning of the brain into its gray and white matter components.

Beyond central predictions regarding tissue competition, the ETH and TPH highlight two additional questions that can be tested. First, both hypotheses posit trade-offs between metabolically expensive organs in the body; indeed, Aiello and Wheeler invoked this in naming the 'expensive-tissue' hypothesis. At the same time, others have proposed the brain may trade off against less expensive tissues, such as skeletal muscle (Leonard et al., 2003; Snodgrass et al., 2009) or fat (Navarette et al., 2011; Heldstab et al., 2016). As introduced in Chapter 1, it is well-established that tissues and organs demonstrate specific metabolic rates, which are indicative of their relative expense and appear to be relatively conserved in humans across development and among individuals (Elia, 1992; Javed et al., 2010; Wang et al., 2001). However, with the collection of data on REE, it would be possible to test the differential expense of organs and tissues in the same cohort being investigated for tissue trade-offs.

Second, the TPH and supporting studies suggest that fetal life and infancy are essential developmental periods when competition amongst organs and tissues is predicted to occur. Furthermore, evidence suggests the potential outcome of this competition – differential organ/tissue growth – persists into later life and is incorporated into adult phenotype. This appears to be the case in particular for organs and skeletal muscle, but less so for fat. Birth weight (Hales and Barker, 1992; Wells et al., 2007; Victora et al., 2008) and relative leg length (leg length/height) or tibia length (Pomeroy et al., 2012; Wells et al., 2016c) are commonly used as proxy measures of fetal and infant developmental experience. Thus, collecting information on birth weight and tibia length would allow investigation of developmental mediation on any organ/tissue trade-offs that might be observed.

Overall, testing questions of tissue expense, tissue competition, and the potential influence of early developmental periods on tissue trade-offs would be novel if carried out in a South Asian cohort. Relatively few studies to date have investigated body or brain composition with gold-standard techniques in South

Asians. I am unaware of any study which has combined a range of such techniques with the assessment of REE in a South Asian cohort, despite the relevance of body composition and metabolism to the etiology of chronic disease in South Asian populations, as described in Chapter 1.

The aim of the current study was therefore to collect comprehensive data of the best possible quality on body and brain composition; REE; and birth weight and tibia length in young adult South Asians. With these data, I aimed to address the following questions (given in the order in which they are discussed in later chapters, with the exception of part (iv) of Question 2, which is addressed in Chapter 5):

Research Question 1: Can I detect variation in the energy expenditure of organs and tissues in my cohort using data on REE and organ/tissue masses? If so, are the results consistent with previous studies undertaken in non-South Asian cohorts?

Research Question 2: Using data on the volumes or masses of the brain, internal organs, skeletal muscle and fat, (i) can I detect evidence for trade-offs (negative statistical relationships) among brain and expensive body components, as predicted by the ETH and TPH? Or (ii) can I detect evidence for trade-offs among less-expensive components such as skeletal muscle and fat mass? Expanding further, (iii) can specific components of the brain be seen to trade off against body organs/tissues?

Alternatively, (iv) do brain and body tissues grow in proportion to one another, and thus demonstrate positive relationships?

Research Question 3: Using birth weight and tibia length as markers of fetal and infant nutritional experience, respectively, can I detect evidence of developmental mediation on any observed brain-body component trade-offs?

To address these questions, I test the following hypotheses:

Hypothesis No. 1 – Organs and tissues in the body will demonstrate variation in specific metabolic rate (kcal/kg/day)

I predict I will find evidence of differential organ/tissue expense, a notion that is well-supported in the literature (e.g. Kety and Schmidt, 1945; Drabkin, 1950; Brozek and Grande, 1955; Holliday et al., 1967; Elia, 1991, 1992), and which has been demonstrated statistically with similar data in a more recent paper (Wang et al., 2010). I further hypothesize that I will find tissue-specific metabolic rate values similar to those reported by previous researchers in non-South Asian cohorts; as noted above, these values appear to be relatively conserved across individuals (Elia, 1992; Javed et al., 2010; Wang et al., 2010).

Hypothesis No. 2 – Both positive and negative relationships will be observed amongst components of the brain and body

Specifically, I hypothesize that:

(i) negative associations (indicating trade-offs) will be observed among the brain and 'expensive' internal organs, consistent with the predictions of the ETH and TPH, and evidence that South Asians sacrifice internal organs as the brain is protected (Yajnik et al., 2003; Wells, 2016)

(ii) a negative association will be observed between the brain and less-expensive skeletal muscle; this would be consistent with suggestions the brain may trade off against muscle (e.g. Leonard et al., 2003), and also evidence that skeletal muscle is reduced on average in South Asians (e.g. Rush et al., 2007; Unni et al., 2009; Stanfield et al., 2012); however, because humans have both large brains and fat stores, I predict I will not

find the negative correlation between brain size and fat mass that Navarette et al. (2011) identified in non-human mammals

(iii) gray matter in the brain will be seen to trade off against organs and/or skeletal muscle, whilst white matter will show no such association; this is suggested by evidence that gray matter has a higher energy expenditure than white matter (Hofman, 1983; Karbowski, 2007; Zhu et al., 2012), and thus may be more likely to trade off against other tissues

(iv) positive relationships will be observed amongst tissues within the body (e.g. organs, fat and skeletal muscle) and positive relationships will be observed amongst brain components; in contrast, positive relationships between brain and body components will be fewer and weaker (this has been demonstrated previously, e.g. Gallagher et al., 1998; Illner et al., 2000; Heymsfield et al., 2012a,b)

How do I expect to find both positive and negative associations among some of the same brain and body components? Nijhout and Emlen (1998) discuss how failing to control for overall body size may render differences in the relative size of specific bodily traits or components difficult to detect unless the effects are extreme. In flies, for example, “the correlation of traits with overall body size (wings and legs of large individuals are larger than wings and legs of small individuals) may [mask] subtler relations among the individual traits” (Nijhout and Emlen, 1998, pg. 3688). Following their argument, I expect that the absolute sizes of various tissues will correlate positively with one another in my sample, but ‘subtler relations among the individual traits’ (i.e. negative trade-offs, if they exist) will be seen once body, and also head/skull size when considering the brain, are controlled for.

Hypothesis No. 3 – Observed trade-offs between the brain and organs or skeletal muscle will be associated with markers of growth variability in fetal life and/or infancy

Based on evidence that fetal and infant developmental experience shapes adult body composition, in particular organ and muscle components (see Chapter 1, Sections 1.2 and 1.4), I hypothesize that birth weight and tibia length will prove to be mediating factors in any statistical models where trade-offs among brain and visceral organs or skeletal muscle are found.

3 Selection of study methods

To test the hypotheses set out in Chapter 2, I sought to collect data on the following outcomes: the brain and its component volumes; visceral organs; skeletal muscle; fat mass; tibia length; and resting energy expenditure. (I also required information on birth weight, but this was collected by recall from study subjects, not measured.) For each outcome, my aim was to obtain data of the highest available quality.

Cadaver analysis is the true gold-standard for measuring body and brain composition, however it is largely impractical for research. Several methods have thus been developed which predict body composition *in vivo.* This chapter describes methods which are of varying quality for the purposes of testing my hypotheses. It is not an exhaustive review of every possible method, but rather a summary of available techniques from which there was an opportunity to choose. A number of techniques were recognized not to provide adequate accuracy in measuring brain and body tissues, and were thus discarded.

The first section below introduces the five-level body composition model of Wang and colleagues (1992), which is used to organize the subsequent sections. Two of the levels are not relevant to the current thesis, therefore Sections 3.2 – 3.4 of this chapter are Level V, Level IV and Level II, respectively. I proceed in this order because Level V is associated with simpler techniques, whilst Levels IV and II are more complex. Section 3.5 describes the method used to collect data on REE.

3.1 The five-level body composition model

In vivo body composition techniques are largely based on indirect measures of body components used in conjunction with established theoretical assumptions (Wells, 2006b). Their accuracy varies in relation to the assumptions used and variation in methodological error (Wells and Fewtrell, 2006).

A model proposed by Wang and coworkers (1992; Figure 3-1) distinguishes five levels at which body composition can be assessed. The levels, defined by the outcomes measured, increase in complexity from atomic (I) to molecular (II), cellular (III), tissue-system (IV), and finally whole body (V; Wang et al., 1992; Heymsfield et al., 1996, 1997). The most complex level (V) is the most easily measured, but offers less information and is less accurate for quantifying body composition outcomes such as fat, skeletal muscle and organs. Summing the components at a given level is equal to body weight (Heymsfield et al., 1996).

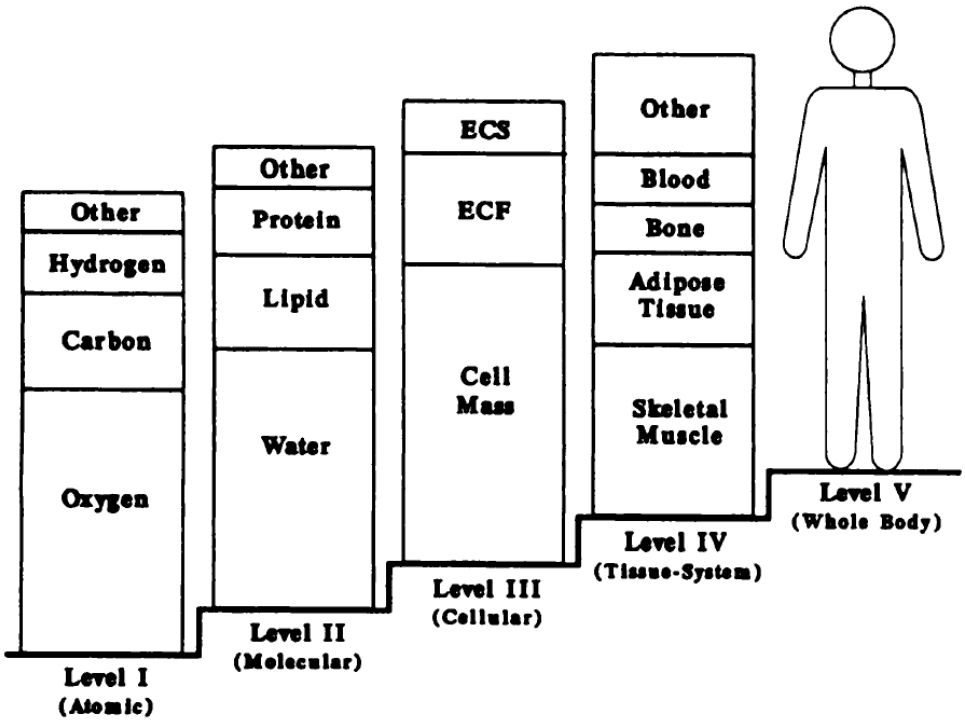


Figure 3-1 The five-level body composition model of Wang and colleagues.
From Wang et al., 1992.

Various aspects of body size, shape and length can be measured at Level V. These include height; weight; limb segment lengths; circumferences/girths (e.g. of the waist, hip, arm, head) and skinfold thicknesses (Wang et al., 1992). BMI can be calculated using weight and height. As only a tape measure, stadiometer, scale

or pair of handheld calipers are required, such data remain the most readily obtainable proxies of body composition, particularly for very large studies and field situations. These are referred to as anthropometric techniques.

Skeletal muscle (SM), visceral organs, the brain, and adipose tissue (AT) are included at Level IV, the tissue-system level (Wang et al., 1992; Heymsfield et al., 1997). Available methods are more limited for accurately quantifying organ size and other tissues *in vivo*, but I discuss two below: CT and MRI. I also describe how body organ and brain volumes can be extracted from imaging data. Whole-body SM can be assessed using dual-energy X-ray absorptiometry (DXA), discussed in Section 3.4.

Across the five levels, a main aim of body composition research is to quantify fat mass (FM) and fat-free mass (FFM). (In Chapter 1, I referred to 'lean mass;' I use this term and FFM synonymously in this thesis. Both are distinguished from FM, and include SM and visceral organs). Whole-body weight is comprised of FM and FFM. Obtaining comprehensive data on the body composition of my study subjects, therefore, requires both components. As noted, SM and visceral organs are part of FFM, as they are largely non-fat tissues; the same is true for the brain, which is mostly water. I thus aim to quantify FFM in order to achieve a broad measure of lean body mass, and also independently measure organs and SM. As described below, measurements at Level IV and V can be used to predict whole-body FM and FFM, however Level II methods are more accurate for this purpose.

Level II, following Wang and coworkers (1992), is the molecular level. Typically, four main molecular components are measured to assess FM and FFM: water, protein, lipid, and mineral. Depending on how many of the four are measured, two-component (2C), three-component (3C) or four-component (4C) body composition models can be constructed. All model FM and FFM, but they vary in their assumptions and accuracy. In Section 3.4, I describe the 2C, 3C and 4C models further, and measurement techniques which can be used to build them.

Each section below discusses body composition outcomes, and techniques available to measure those outcomes. As I proceed through the sections I discuss the uses and limitations of the various methods, and indicate where techniques were used for the current project or discarded.

3.2 Level V (whole body) measurement outcomes and techniques

3.2.1 Height, weight and BMI

Anthropologists interested in population phenotypic variation have long explored associations among height, body mass and local ecology (Bergmann, 1847; Allen, 1877; Roberts, 1953; Ruff, 1994; Katzmarzyk and Leonard, 1998; Wells, 2012c). Height is likewise an important marker of individual and population growth and health (Steckel, 1979; Eveleth and Tanner, 1976; Cole, 2000; Deaton, 2008; NCD Risk Factor Collaboration, 2016). Body weight can similarly serve as an index of health and nutritional status (McWhirter and Pennington, 1994; Norgan, 2005; Racette et al., 2005; Wu et al., 2009).

As established by Quetelet (1994), weight is often reported in relation to height, where weight in kilograms divided by height in meters squared is an individual's BMI. BMI is widely used to assess relative weight, and identify categories of underweight, overweight and obesity (Nuttall, 2015), following its original application to the assessment of obesity by Garrow and Webster (1985). These categories are further related to health outcomes including type II diabetes and the metabolic syndrome. Measuring BMI is practical and economical, thus its continued use for very large studies and the monitoring of secular trends in populations has been advocated (Hall and Cole, 2006).

However, links between body fatness, lean mass and BMI are not straightforward. BMI is often interpreted as a marker of FM, rather than FFM, however it is associated with both, as well as other body composition measures (Nuttall, 2015).

For a given BMI, individuals differing by sex, age, ethnicity, average physical activity, and health may differ in the relative proportion of FM to FFM (Prentice and Jebb, 2001; Hall and Cole, 2006; Wells, 2006). Two healthy adult males may have an identical BMI, but differ considerably with respect to their percentage fat (%fat; see Yajnik and Yudkin, 2004). The BMI measurement does not have the resolution to elucidate the FM/FFM ratio.

The limitations of BMI for assessing relative FM and FFM are visualized with the Hattori chart (Hattori et al., 1997, 2004), which simultaneously incorporates data on fat mass index (FMI), fat-free mass index (FFMI), %fat, and BMI. FMI and FFMI are calculated as FM/height^2 and FFM/height^2, respectively (Hattori et al., 1997). A graph from Wells et al. (2007) is shown in Figure 3-2 below. Green and red circles indicate two children with similar BMI, but quite different FMI and FFMI; green and blue circles highlight two children with similar FMI, but very different BMI and FFMI (Wells et al., 2007).

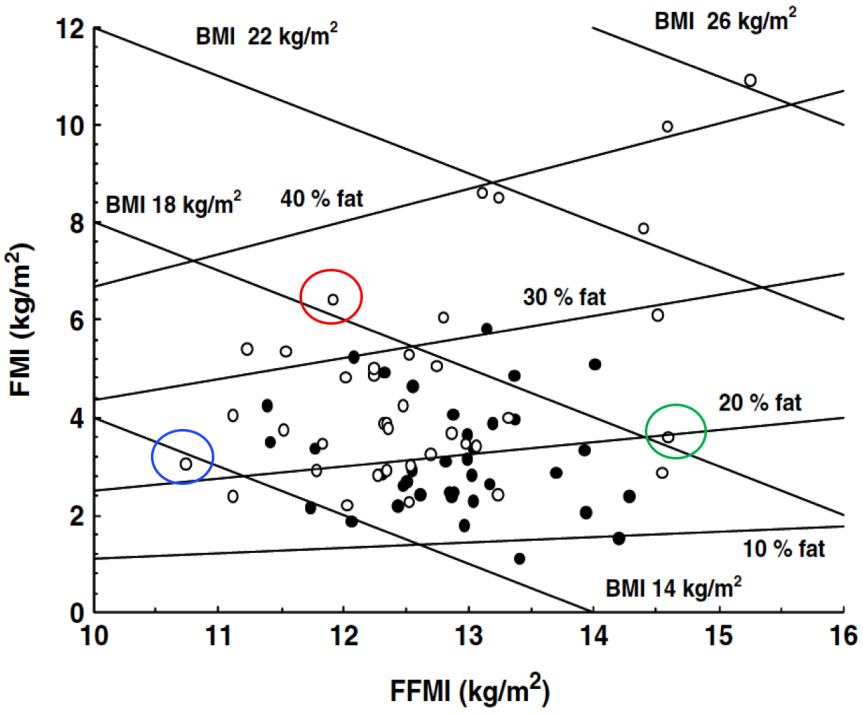


Figure 3-2 Hattori graph for a sample of 8-year-old children.
Image from Wells et al., 2007.

Comparisons of body composition in individuals of differing ethnicity offer another example of the limitations associated with BMI. I first described how body composition may differ among populations in Chapter 1, Section 1.4 on South Asian body composition. A 1994 study by Wang and colleagues obtained measures of body fat and BMI in men and women of European[5] and Asian (Chinese, Japanese, Korean and Filipino) descent. The authors reported that at a lower BMI, Asians had significantly more fat compared to Europeans (Wang et al., 1994). Deurenberg-Yap et al. (2002) found that Singaporean Chinese, Indians and Malays demonstrated the same %fat as European individuals, but had BMI values ~3kg/m^2 lower.

[5] When referring to the ethnic background of a study sample, I generally use the term(s) that were used in the publication I am citing. However, I use 'European' and 'white' interchangeably to denote individuals of European descent, and avoid the term 'Caucasian.'

Relationships between %fat and BMI also appear to differ between Polynesian and New Zealand European females (Rush et al., 1997). Similarly, correlations between BMI and body composition were less than straightforward for New Zealand European, Pacific Island and Asian Indian men. Namely, Pacific Islanders had higher SM mass, whilst Asian Indians had more fat and less muscle mass for a given BMI (Rush et al., 2004). These data indicate that FM/FFM ratios are not independent of ethnicity.

I aimed to recruit subjects whose ethnicity traces to a relatively specific geographical area in order to avoid the extra source of variation associated with a mixed-ethnicity sample. However, accurately measuring FM and FFM clearly requires a more sophisticated technique than BMI, irrespective of subject recruitment.

3.2.2 Body girth/circumference measurements

Compared to BMI, girth/circumference measurements including those of the head, upper arm, chest, waist, hip, thigh and calf offer more information about tissue distribution on the body. These measurements are relatively simple and easy and can be taken using a measuring tape. In some research centers and hospitals, three-dimensional (3D) photonic scanning is also available, which projects safe, white light onto the body inside a structure similar to a photo booth. Computer algorithms reconstruct skin surface topography from photonic data, and a 'virtual tape measure' is applied to the 3D image to quantify circumferences (Douros et al., 1999; Treleaven, 2004; Wells et al., 2011).

Girth measurements are an improvement over BMI because measures of body shape can offer additional information that may be helpful in making predictions about underlying tissues. For example, if waist circumference is found in an adult to be large relative to their hip circumference, or if waist circumference increases over time, this may indicate an increase in abdominally-deposited fat. This fat depot, also referred to as visceral AT (VAT) is of interest to researchers and

clinicians because it is associated with chronic disease risk (e.g. Ho et al., 2003; Araneta and Barrett-Connor, 2005; Zhu et al., 2005).

Indeed, indices incorporating waist measurements were, compared with BMI, better able to identify metabolic disease in two Arab cohorts (Al-Daghri et al., 2015). In a study of over 27,000 participants from 52 countries, waist-to-hip ratio was a stronger predictor of myocardial infarction risk than BMI (Yusuf et al., 2005). However, tape or 3D scan measurements of waist or other girths are ultimately limited because they cannot with suitable accuracy distinguish between underlying tissue types. A study by Araneta and Barrett-Connor (2005) demonstrates this. The authors both gathered anthropometric data and measured fat depots using CT imaging in Filipino, African American and white women. Adjusting for age, African American women had significantly higher outcomes for BMI and waist girth than Filipinas or white women, yet VAT was significantly higher in Filipinas compared to the other two groups (Araneta and Barrett-Connor, 2005).

The upper arm circumference has been used along with a measure of the triceps skinfold (see next section) to estimate arm muscle area (AMA) and arm fat area (AFA; Frisancho, 1974, 1981; Heymsfield et al., 1982). AMA and AFA have further been used as proxy measures for FFM and FM, respectively. This relies on a number of assumptions accompanying the calculations, including that the arm is cylindrical; that a circular core of muscle is surrounded by an evenly distributed band of subcutaneous fat; that the triceps skinfold measurement can accurately separate fat and non-fat tissue in the arm; and finally, that the skinfold is equal to double the arm subcutaneous fat thickness (Chomtho et al., 2006). In a study of children, Chomtho and coworkers (2006) found that arm circumference and AFA were reasonably useful in predicting FM as measured by criterion methods, and adequate for ranking children in terms of level of fatness. However, arm circumference and AMA performed poorly in predicting both regional and whole-body FFM.

Heymsfield and colleagues (1982) found that an old equation overestimated AMA by 20-25% in comparison to more accurate measures by CT; they revised the equation, which subsequently overestimated AMA by an average of 7-8%. The study of Baker and colleagues (2010) described in Chapter 1, Section 1.2.4 utilized the revised equation of Heymsfield et al. (1982) to calculate AMA in their sample of children from NHANES III. The authors note that previous studies found AMA to be strongly associated with levels of urinary creatinine, a byproduct of muscle metabolism. Indeed, proxy measures such as AFA and AMA are useful in large studies where more expensive and complex methods cannot feasibly be employed. However, these measurements are not adequate for assessing FM, FFM or SM in my sample.

Baker et al. (2010) further used a measure of head circumference as a proxy for brain mass in their study, an approach which has been used in many additional investigations in children (e.g. Dessens et al., 2000; Aylward et al., 2002; Hebestreit et al., 2003). With autopsy data, Epstein and Epstein (1978) demonstrated that brain weight scales closely with head circumference in individuals from birth to age 18 years. In an earlier study, Bray and colleagues (1969) showed that estimated intracranial volume correlated strongly with head circumference in 56 patients of varying age. However, more recent research by Bartholomeusz and coworkers (2002) suggested that head circumference may be a poor marker of brain volume, particularly in adults. They measured 76 healthy, mostly white male subjects using MRI, from which they obtained both head circumference and brain volume. Results indicated that a given adult head circumference measure could be associated with a range of brain sizes, likely due to the fact that brain volume decreases somewhat following adolescence, whilst achieved head size does not change (Bartholomeusz et al., 2002).

Based on these findings I concluded head circumference was not a sufficiently sensitive measurement of brain size to use in my study. Furthermore, an external

proxy of brain volume does not offer any information on specific brain components, which I require to test one of my hypotheses.

Additional studies I overviewed in Chapter 1 (Sections 1.2 and 1.4) utilized abdominal circumference as a proxy for internal organ size (e.g. Campbell and Thoms, 1977; Barker et al., 1993; Yajnik et al., 2003). An investigation by Vintzileos and coworkers (1985) found a strong correlation between liver length and abdominal circumference in fetuses, however I did not find reports of similar evidence in adults. In any case, to test my hypotheses that organs and tissues are differentially metabolically expensive and that the brain trades off against expensive organs, a more direct measure of organ size than abdominal circumference is required.

3.2.3 Regional skinfold thickness

Measuring regional skinfold thickness (SFT) is comparable to the measurement of body girths by tape measure with respect to ease of use and cost-effectiveness. The measurement involves isolating a fold of skin and underlying subcutaneous fat with the fingers. Handheld calipers are used to measure the size of the fold at specified sites on the body, often in biceps, triceps, subscapular and suprailiac regions. The latter two are on the upper back and above the hip, respectively.

Accuracy and precision may suffer when SFT measurements are attempted for overweight or obese individuals, or for those whose skin is particularly well-attached to the underlying tissue and more difficult to lift into a fold. The compressibility of skin may vary between individuals and introduce some degree of error (Durnin and Womersley, 1974).

Skinfold thicknesses have been used by researchers seeking to identify a simple technique which demonstrates good agreement with more accurate but at times impractical laboratory methods. For example, Durnin and Womersley (1974) developed regression equations using skinfold measurements from the biceps,

triceps, subscapular and suprailiac sites to predict body density and fatness in 481 men and women of varying age. These equations were widely used in subsequent research (Coward et al., 1988; Norgan, 2005).

However, some argued that the high degree of error associated with estimating FM and FFM from SFT measurements rendered the method unacceptable (e.g. Coward et al., 1988). Predicting FM from SFT requires two significant assumptions: first, that average whole-body SFT is adequately represented by chosen skinfold measurement sites, and second, that a constant relationship exists between subcutaneous fat and total FM (Lukaski, 1987; Coward et al., 1988; Wells and Fewtrell, 2006).

An additional limitation is that the validity of developed prediction equations may only be applicable in the populations from which they were derived (Lukaski, 1987; Rode and Shephard, 1994; Dioum et al., 2005; Norgan, 2005; Wells and Fewtrell, 2006). Many equations were derived in adults and children from European populations, therefore they would not be appropriate for use in my cohort.

As suggested by Wells and Fewtrell (2006), SFT measurements may be used most effectively as raw values, for example to quantify fat stored in specific subcutaneous depots and/or rank individuals in a sample by relative fatness. As the technique only directly measures skin and subcutaneous fat, attempts to assess the FFM component of body mass with SFT-derived equations is not appropriate (Wells and Fewtrell, 2006). SFT measurements would thus not allow me to collect adequate data for testing my study hypotheses.

3.2.4 Limb segment lengths

The human body can be divided and measured in several segments. Using a measuring tape, stadiometer or sliding calipers, one can obtain data on limb length (leg, arm), limb segment length (e.g. humerus, femur, tibia, foot) and trunk or head-trunk height. Head-trunk height is also referred to as sitting height. With these data

and also total stature, proportions can be calculated including relative leg length (RLL; leg length/stature) or sitting height ratio (sitting height/stature; Bogin and Varela-Silva, 2008, 2010). Limb/body segment measurements are not options for measuring soft tissues, however RLL or tibia length could both potentially be used as markers of postnatal experience to test my hypothesis concerning developmental mediation on trade-offs.

RLL has classically been used as a marker of growth in postnatal life, as the legs of humans are recognized during this period to grow quickly relative to other parts of the postcranial body (Cole, 2000; Bogin and Varela-Silva, 2008). Population secular increases in stature have been attributed largely to increased leg length (Tanner et al., 1982; Jantz and Jantz, 1999; Sanna and Soro; 2000; Cole, 2000). As RLL indexes growth postnatally and is apparently sensitive to environmental stimuli, it can act as a marker of environmental quality and nutritional experience in this period (Gunnell et al., 1998; Leitch, 2001; Bogin et al., 2002; Wadsworth et al., 2002; Wells et al., 2016c). Like birth weight, which is used in the current study as a marker of fetal experience, RLL is associated with adult chronic disease risk (Davey Smith et al., 2001; Lawlor et al., 2002, 2004; Ferrie et al., 2006). Importantly, several studies have suggested that RLL is independent of fetal weight gain, indexed by birth weight (e.g. Gunnell et al., 1999; Bogin and Baker, 2012; Pomeroy et al., 2014; Wells et al., 2016c), although some evidence suggests body and limb segments impacted *in utero* from maternal smoking or diabetes could affect birth length (e.g. Lindsay et al., 1997; Lampl et al., 2003; Lampl and Jeanty, 2004).

Although it has been used in studies less often than RLL, the tibia may in fact be the more sensitive component of the lower leg. Distal limb segments including the tibia and radius are more variable than proximal segments (Holliday and Ruff, 2001). I described the Peruvian study of Pomeroy and colleagues (2012) in Chapter 1, where the authors found that tibia length showed the greatest differences between differentially stressed highland and lowland children.

Additionally, following their investigation of secular changes in long bone lengths in US individuals, Jantz and Jantz (1999, pg. 57) reported that “lower limb bone secular change is more pronounced than upper limb bone change, and distal bones change more than proximal bones, particularly in the lower limb.”

Knee height is a similar measure which effectively indexes tibia length, and likewise serves as a marker of early-life nutrition (Bogin et al., 2014). Because taking knee height does not involve palpating the proximal and distal ends of the tibia through soft tissue, it may be an option for avoiding confounding in very overweight subjects, however the foot and ankle are included in the measurement. In a study of Maya children from Mexico, knee height as a measure of distal leg length and the knee height/stature ratio were more strongly associated with socioeconomic and environmental variables than was a variable reflecting ancestry (Vázquez-Vázquez et al., 2013).

I chose to measure tibia length in my sample as a marker of postnatal developmental experience. As noted above, the tibia appears to be the most plastic component of the lower limb in early life, and therefore may be a more sensitive marker than RLL. I considered that a more direct measurement of the tibia, accomplished by palpating the proximal and distal ends of the bone, was preferable to knee height, and feasible as my sample would not include very overweight or obese individuals.

3.3 Level IV (tissue-system) measurement outcomes and techniques

The review above has demonstrated that at Wang et al.’s (1992) ‘whole body’ level of assessment (V), only the anthropometric measurement of tibia length is appropriate for use in my study. I described how external circumference measurements would not give sufficiently detailed data on soft tissue components including brain and body organs; however, imaging methods such as CT and MRI

used at the tissue-system level (IV) are a further possibility. In contrast to the section above, here I organize the subsections by method, rather than outcome.

3.3.1 Computed tomography

Three-dimensional brain imaging by CT was first introduced by Hounsfield in the 1970s, and was thereafter utilized in many studies of normal and pathological brains (e.g. Hahn and Rim, 1976; Carlen et al., 1978; Brott et al., 1989). Its use was extended to measure visceral organs, muscle mass and AT[6] depots throughout the 70s and 80s, as reported in multiple studies (see Heymsfield et al., 1997).

CT data are contained in volume elements (voxels), which are two-dimensional picture elements (pixels; typically 1mm x 1mm) plus a third dimension reflecting slice thickness. In gray scale, voxels provide image contrast and show tissue composition, generating volumetric estimates of body and brain components (Heymsfield et al., 1997). The reconstruction of body composition data in each voxel is achieved via the differential attenuation of X-ray beams as they come in contact with fat, fat-free, brain and bone tissues (Heymsfield et al., 1997; Shen and Chen, 2008). Tissues are labeled based on attenuation values, which are measured in Hounsfield units (Després et al., 1996).

Using CT images, it is possible to delineate SM and organs among fat-free tissue, and also quantify AT components (Ellis, 2000). For example, studies have used CT to investigate associations between waist indices, abdominal fat and risk markers for chronic disease (Seidell et al., 1987; Schwartz et al., 1991). Kvist and colleagues (1988) further developed a method for quantifying whole-body AT

[6] 'Fat' and 'adipose tissue' are often used interchangeably, but following the five-level body composition model it is important to recognize they are separate components; fat typically refers to lipid (triglycerides), which is found in adipose tissue, but also in smaller amounts in other regions of the body (Shen et al., 2003).

volume using multiple CT image slices, and in an earlier study, Heymsfield et al. (1979) showed that CT could accurately quantify kidney, liver and spleen volumes.

Measuring total body mass and specific organ masses where CT image slices are taken at designated intervals across the body demonstrates excellent precision and accuracy (both <1% error; Ellis, 2000). Several validation studies have supported the use of the technique for estimating body tissues (Heymsfield et al., 1997). A number of more recent studies have also utilized CT to assess body composition (e.g. Goodpaster et al., 2006; Kuk et al., 2006; Irlbeck et al., 2010). However, undergoing a CT scan exposes subjects to multiple X-ray beams and a high degree of ionizing radiation (Després et al., 1996), which is a considerable disadvantage.

Yoon and colleagues (2008) reported the measurement of intra-abdominal AT by CT was valid and reproducible using a modified protocol which reduced the estimated radiation dose by ~75%. Indeed, if image resolution is reduced, the radiation dose can be decreased as well (Ellis, 2000). Nevertheless, the radiation exposure associated with CT may continue to limit its wider application in body composition research, particularly in healthy persons (Thomas et al., 2013). This extends to my study, for which CT did not represent a feasible imaging method.

3.3.2 Magnetic resonance imaging

Early work which led to the currently used method of MRI was carried out by researchers including Bloch et al. (1946), Purcell et al. (1946) and Damadian (1971). Central to MRI are the human body's billions of hydrogen atoms, which contain protons with a magnetic moment (Edelman and Warach, 1993; Heymsfield et al., 1996, 1997; Brown and Semelka, 2003). Subject to the Earth's relatively weak magnetic field, hydrogen protons are randomly configured. Within an MRI scanner, however, the protons' magnetic moments align with the much stronger magnetic field (B_0) generated by the MRI system (Heymsfield et al., 1997; Ellis, 2000). A rendering of the system is shown in Figure 3-3.

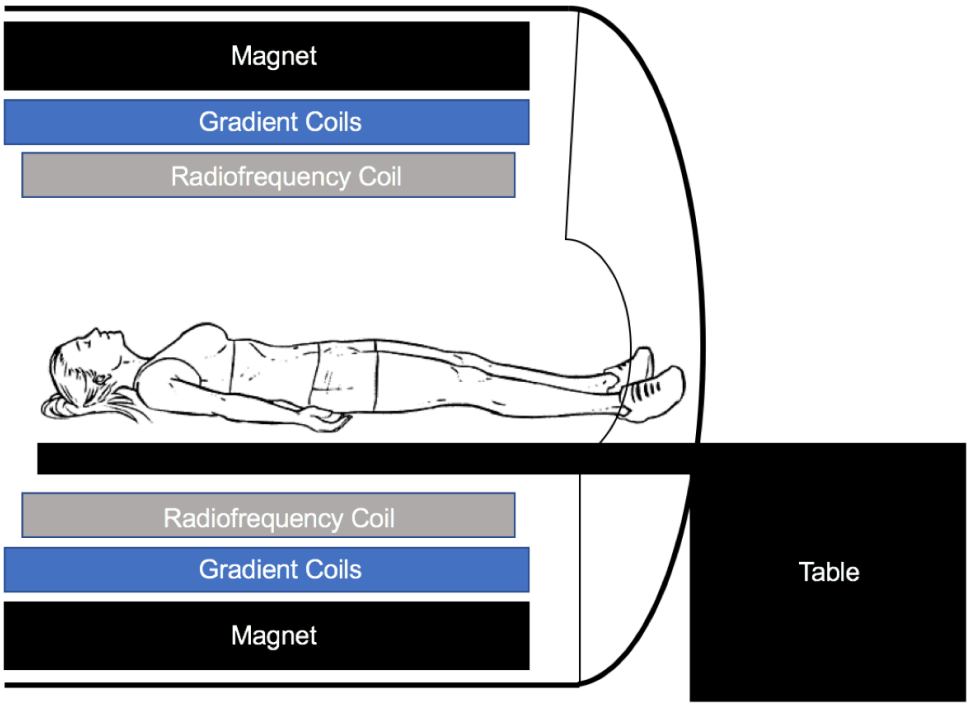


Figure 3-3 Simplified rendering of the MRI scanner.

During an MRI scan, applied pulses of radiofrequency (RF) energy at the resonant, or Larmor frequency are absorbed by a proportion of the body's hydrogen protons, disrupting their alignment with B_0 (Edelman and Warach, 1993). Following a RF pulse, protons undergo relaxation, or re-alignment with B_0, releasing energy that is used to generate MR images (Heymsfield et al., 1997; Ellis, 2000; Brown and Semelka, 2003; McRobbie et al., 2007). Additional magnetic field gradients interrupt B_0 to create spatial variations in field strength across the length of the subject's body, localizing MR signals to orient the image (McRobbie et al., 2007). Similar to CT, MR images are made up of pixels, the front face of many thousands of 3D voxels (with depth created by the slice thickness).

MRI's ability to provide image contrast and distinguish between different tissues is related to parameters known as T1 and T2. Both refer to the time (T) required for

hydrogen nuclei to return to alignment with B_0 (Brown and Semelka, 2003). For example, T1, the longitudinal relaxation time, is shorter for protons in fat compared to those found in water. Thus, differential relaxation allows fat and SM to be distinguished in the body, and white and gray matter separation in the brain. The generated contrast (i.e. variation in image signal intensity or brightness) can be optimized through the manipulation of the RF pulse parameters TE (time-to-echo) and TR (time-to-repeat; Heymsfield et al., 1997; McRobbie et al., 2007).

MRI is a state-of-the-art method and produces high quality data. With respect to the brain, it underpins the vast majority of the clinical and research literature, including structural, functional and diffusion imaging to assess various aspects of brain form and function in health and disease (e.g. DeYoe et al., 1994; den Heijer et al., 2003; Sowell et al., 2003; Debette et al., 2010; Anblagan et al., 2013; Kawadler et al., 2015; Lisofsky et al., 2015). It is possible using MRI to employ a volume-based approach (Fischl et al., 2002; Giorgio and De Stefano, 2013; Maclaren et al., 2014; Schmitter et al., 2015) and obtain highly accurate data on whole-brain volume and the volumes of specific regional components. MRI scanning is expensive, and scan times may be long relative to CT, however a principle advantage is that MRI does not subject individuals to ionizing radiation exposure. With access to a scanner, I was able to use MRI to quantify total and component brain volumes in my study cohort.

Once raw data are obtained (the scan protocol is described in Chapter 4), there are a number of options for extracting volumetric outcomes. In earlier studies, brain volumes were typically manually segmented (Morey et al., 2009; Maclaren et al., 2014), which can be extremely time-consuming; the volume or region of interest (ROI) must be traced in each slice of multi-slice datasets. Manual segmentation by an experienced clinician or researcher is considered the criterion against which many emerging semi- and fully-automated software methods are evaluated (e.g. Buckner et al., 2004; Morey et al., 2009; Lehmann et al., 2010), and the latter may be preferred for efficiency and practicality, particularly with large datasets.

A greater range of semi- and fully-automated methods are now available, as described by Giorgio and De Stefano (2013). Manual segmentation may be considered the reference method, however new methods which utilize computational algorithms could be argued to offer greater objectivity in brain volume assessment. Automated segmentation methods have been shown to be accurate and reproducible, and have been used in a number of studies to measure the whole brain and its gray and white matter components (Giorgio and De Stefano, 2013). Many involve the registration of 3D MR images to an electronic brain atlas, or template, allowing tissue classification within voxels according to prior tissue probabilities, which streamlines data acquisition and analysis (Klauschen et al., 2009).

One well-established, freely-available software package for the fully-automated segmentation of brain structures is called FreeSurfer, developed and made available by the Martinos Center for Biomedical Imaging at Harvard and MIT in Boston, USA, a leading imaging research group. FreeSurfer is a popular tool; it was described recently as “probably the most widely used software for [volume-based brain morphometry]” (Schmitter et al., 2015, pg. 9). It has been evaluated against manual brain volume measurements (e.g. Morey et al., 2009; Lehmann et al., 2010), and was recently used in a test-retest dataset which assessed the reproducibility of automated software within and across MRI scan sessions (Maclaren et al., 2014).

Morey et al. (2009) found reasonably good correlations between regions traced by hand and derived using FreeSurfer in 20 healthy adult controls (i.e. $r = 0.82$ for the hippocampus, and $r = 0.56$ for the amygdala). Using manual methods and FreeSurfer to measure bilateral temporal lobe structures in individuals with Alzheimer’s disease, semantic dementia, and controls, Lehmann and coworkers (2010) reported strong correlations between the methods for many of the structures, and observed comparable volume differences in a patient/control comparison. In their test-retest analysis, Maclaren et al. (2014, pg. 4) reported that

FreeSurfer demonstrated “generally good segmentation accuracy.” FreeSurfer has been utilized in previous studies carried out by my research group (e.g. Webb et al., 2012; Ranpura et al., 2013; Kawadler et al., 2013), and I chose to use it for brain volume segmentation in the current study.

MRI is also an option for obtaining detailed data on organs and tissues in the body, and I describe this further below.

3.3.2.1 MRI of the body

According to Heymsfield et al. (1997), Foster and colleagues’ (1984) demonstration that MRI could identify AT and SM in cadavers was the initial use of the technique in body composition research. This work was followed by a number of studies in the body composition literature (e.g. Hayes et al., 1988; Fowler et al., 1991; Ross et al., 1992). Compiled by Després et al. (1996), coefficients of variation for repeated measures of total body AT in earlier human MRI studies were 3.0 (Staten et al., 1989), 5.4 (Seidell et al., 1990), 2.5 (Ross et al., 1993) and 1.5 (Sohlström et al., 1993). The smaller values of 2.5 and 1.5 come from investigations where several image slices across the body were taken, rather than at one specific site (e.g. the umbilicus).

Ross and colleagues further quantified AT and lean tissues using MRI in a 1994 study. They described how the scan acquisition resulted in 41 images taken at 50mm intervals across the body for each subject, from which total-body adipose and lean tissue values were calculated. This involved setting a threshold to identify adipose and lean tissues in image pixels; calculating areas for adipose and lean components (pixels corresponding to each tissue were summed and multiplied by their surface area); multiplying areas by the slice thickness to get volumes; and then calculating total volume for both tissue types using a mathematical equation (Ross et al., 1992, 1994). The group used the same methods in a later study to assess the influence of diet and exercise on AT and SM in men (Ross et al., 1996).

The above whole-body multi-slice protocol is regarded as the reference method for obtaining total body volumes of AT and SM *in vivo* (Heymsfield et al., 1997; Shen et al., 2004), and it has subsequently been used in a number of other studies (e.g. Janssen et al., 2000; Park et al., 2001; Song et al., 2004; Kuk et al., 2008; Gallagher et al., 1998). Once a tissue volume is derived at the whole-body level, it is possible to convert it to mass using assumed constant tissue densities (e.g. 1.04 kg/l for SM and 0.92 kg/l for AT; Heymsfield et al., 1997).

Similar steps can be carried out to segment body organs from MR images. In a multi-slice dataset for a single subject, ROIs can be drawn along the edge of the organ so that it is entirely encircled in each image in the series. The organ areas as identified in each image slice in the series are then summed and multiplied by the slice thickness to obtain volume, and converted to mass using known densities. Software packages are available which have been used by a number of researchers for this purpose (e.g. Gallagher et al., 1998; 2006; Anblagan et al., 2013; Bosy-Westphal et al., 2013), however they may be expensive to purchase. Others have reported obtaining organ volumes from MR images with manual segmentation (e.g. Illner et al., 2000; Bosy-Westphal et al., 2004). This can be done using a free, downloadable image viewer, of which several are available.

One such image viewer is OsiriX, which has been used in a number of studies since it was released in 2004 (Fortin and Battié, 2012; Salaffi et al., 2013; Ahedi et al., 2014; Knight et al., 2015; Kartalis et al., 2016). In a number of these, authors used the OsiriX workstation to measure body tissues including muscle and bone (e.g. Fortin and Battié, 2012; Salaffi et al., 2013; Ahedi et al., 2014). A recent evaluation of image processing software rated OsiriX highly amongst several open-source packages (Valeri et al., 2015).

Considering the above, I chose to measure internal organs using MRI, and carry out post-processing to quantify volumes with OsiriX. I chose not to use MRI to quantify FM for three reasons. First, as described, a measure of AT, not fat, is

derived from imaging methods. Converting AT volume to FM requires assumptions regarding the density of AT and also its fat content (lipid fraction; Wells and Fewtrell, 2006), which are variable and difficult to estimate *in vivo* (Després et al., 1996). Second, measures of body fat by MRI cannot account for lipid that is not present in AT (Després et al., 1996; Wells and Fewtrell, 2006).

Third, measuring FM (and FFM) at the molecular level with a different technique than MRI would allow for independence among FM, FFM and brain/body organ outcomes, and therefore uncorrelated error. This similarly informed my decision for measuring SM, which, beyond CT and MRI, can also be obtained using DXA. DXA is technically an imaging method, however it is utilized extensively to construct 2C and multi-component models at the molecular level, so I describe it below rather than in the current section. I describe further in the section on DXA how I used it to derive SM mass in my sample, and the basis for this in the literature.

3.4 Level II (molecular level) measurement outcomes and techniques

I have described so far that I aim to obtain tibia length by anthropometry at Level V, and brain and body organ volumes by MRI at Level IV, the tissue-system level. As introduced in the first section of this chapter, 2C, 3C and 4C models of body composition assessment can be utilized at Level II, the molecular level, to model whole-body FM and FFM. Below, I initially overview how three different measurement techniques can be used to construct a 2C model. I then describe how these techniques can be used in conjunction and their data combined to construct 3C and 4C models.

3.4.1 Two-component (2C) models

Two-component models improve on Level V techniques such as BMI and anthropometry by measuring both FM and FFM, and avoiding the prediction of

these components from external measurements at one or a few sites on the body (Wells and Fewtrell, 2006). Molecular level 2C models are used extensively in body composition research (Heymsfield et al., 1996), however they do have limitations, which I discuss along with background to deuterium isotope dilution, DXA, and air-displacement plethysmography in the next three sections. Here I note both the outcome and measurement technique in the section headings, and provide a short summary of the information given in a fourth section at the end.

3.4.1.1 Total body water by deuterium isotope dilution

Water is the main component of body mass in humans and is clearly delineated at the molecular level (Wang et al., 1999; Ellis, 2000). Based on its presence in non-fat tissues, the measurement of total body water (TBW) allows for the quantification of FFM (Thomas et al., 1991), and FM can then be calculated if body weight is known (Wong, 2003). TBW techniques have been used to assess body composition in humans since the first half of the 20th century (Moore, 1946; Edelman et al., 1952).

One technique for measuring TBW is based on the dilution in the body water pool of a biological tracer. Deuterium (^{2}H), a stable isotope of hydrogen, can be used for this purpose (Schoeller, 1989; Moore, 1946). Discovered in 1931 by Urey, ^{2}H was first reported in the literature as an experimental tag for water by Hevesy and Hofer (Pinson, 1952; Schoeller et al., 1980). ^{2}H and the more common isotope of hydrogen (protium, ^{1}H) differ by one neutron. Due to the higher mass of ^{2}H, the isotopic ratio of deuterium:protium is quantifiable by mass spectrometry (Hachey et al., 1987).

Deuterium oxide ($^{2}H_{2}O$, or 'heavy water') is non-radioactive and non-toxic at tracer doses (Pinson, 1952; Thomas et al., 1991; Wong, 2003). Upon dose ingestion, ^{2}H is exchangeable with ^{1}H in the body, and, following the dilution principle, TBW is calculated as the amount of tracer added to the body water pool, divided by the tracer's concentration in the pool after equilibration (Edelman et al., 1952; Wong,

2003). Samples of a body fluid (e.g. saliva) are typically collected for analysis (Ellis, 2000).

TBW quantification in humans is subject to both biological variability and measurement error (Siri, 1961; Coward et al., 1988). Two main assumptions of this method are 1) that ^{2}H exchanges only with ^{1}H in the body water compartment (i.e. no non-aqueous exchange of hydrogen occurs; and 2) neither ^{2}H nor body water is metabolized during the equilibration period (Ellis, 2000).

Regarding the first assumption, at least 95% of the body's exchangeable hydrogen is in water; however, ingested $^{2}H_2O$ molecules may mix to some degree with hydrogen in protein, fat and carbohydrate (Moore, 1946; Culebras and Moore, 1977). The exchange of ^{2}H with non-aqueous organic solids has been investigated by several authors (e.g. Krogh and Ussing, 1936; Pinson, 1952; Culebras and Moore, 1977). The potential overestimation of TBW (~4%; Schoeller et al., 1980) necessitates a correction when calculating final body composition values (Racette et al., 1994), as described below.

With regard to the second assumption, Edelman and colleagues (1952) have suggested that water turnover during equilibration is minimal. For example, urine secretion in this period may account for less than 0.5% of TBW (Edelman et al., 1952). According to Wong (2003), the assumption that TBW remains at constant volume throughout equilibration is appropriate for healthy adults, with only minor tracer loss over a 4-hour equilibration period.

The following equation is used to calculate the dilution space (Halliday and Miller, 1977):

(3-1) $$N = \frac{TA}{a} * \frac{Ed-Et}{Es-Ep}$$

where

N= dilution space (ml)
a= portion of dose diluted (g)
T= tap water in which the dose portion was diluted (g)
A= dose given to subject (g)
E= isotopic enrichment in delta units relative to Vienna Standard Mean Ocean Water (VSMOW) standard

Ed= enrichment of diluted dose
Et= enrichment of tap water used as diluent
Es= enrichment of the post-dose sample
Ep= enrichment of the pre-dose sample

A correction is then applied to account for the non-aqueous exchange of hydrogen (Racette et al., 1994), giving TBW:

(3-2) $$TBW = N/1.044$$

Subjects' reported fluid intake during the equilibration period is subtracted from calculated TBW values to correct for the addition of fluid to the body water pool, which could lead to an overestimation of TBW. These values are then divided by 0.99337 to convert TBW in kg to liters. Assuming a hydration fraction of 73% (Pace and Rathbun, 1945; Fuller et al., 1992; Wang et al., 1999), FFM is calculated thus:

(3-3) $$FFM = TBW/0.73$$

and FFM is subtracted from total weight to get FM, giving a 2C model. This model assumes a constant density of FFM (Wells et al., 1999; Ellis, 2000).

The dilution method has been reported to measure TBW with good precision and accuracy (1-2%; Wang et al., 1999). In a study by Fuller et al. (1992) the method's

propagation of error in estimating FM was only ±0.62kg. However, error in TBW-derived estimates of FFM (the larger body compartment, relative to FM) may translate to a larger error for calculated FM (Withers et al., 1999). In addition, the hydration and density of FFM varies between individuals (Wells et al., 1999) and may vary with increasing age. Relatively little is known about how these properties vary by ethnicity, as most research on FFM hydration and density has been done in non-human animals and populations of European ethnicity (Wang et al., 1999).

Deurenberg-Yap and coworkers (2001) assessed 2C model outcomes, with a specific focus on %fat, against a criterion 4C model in three ethnic groups from Singapore. At a group level, comparisons of isotope dilution with the 4C model revealed relatively small differences in %fat (0.0-1.4 %), in comparison with the other 2C models. These data suggest the potential suitability of a 2C model by ^2H_2O dilution at the group level. However, the authors concluded significant error and the violation of assumptions of FFM properties rendered 2C models unadvisable for assessing body composition among these ethnic groups at the individual level (Deurenberg-Yap et al., 2001).

3.4.1.2 Bone mineral content by dual-energy X-ray absorptiometry

DXA scanning estimates bone mineral content (BMC) and soft tissue via the differential attenuation of two distinct energy beams by a subject's body (Laskey, 1996; Heymsfield et al., 1997; Wells and Fewtrell, 2006). The method was originally developed to measure bone mineral, for which its precision has been reported at ~1% (Mazess et al., 1990; Roubenoff et al., 1993; Laskey, 1996).

As it accounts for soft tissue overlying bone, DXA can also quantify FM and FFM to obtain a 2C model (Roubenoff et al., 1993; Laskey, 1996; Heymsfield et al., 1997). The two energy beams measure only two tissue types in each image pixel: either bone and soft tissue, or FM and FFM (minus mineral) in pixels without bone (Pietrobelli et al., 1996; Heymsfield et al., 1997). For the roughly 1/3 of pixels

containing bone, FM and FFM are estimated from non-bone pixels in the same region (Laskey, 1996; Ellis, 2000). Bone and soft tissue components are identified across image pixels by DXA system algorithms (Pietrobelli et al., 1996).

The ease with which a 2C body composition model can be derived, along with data on tissue distribution, is advantageous and a strong motivation for using DXA in research and clinical practice (if one has access to a scanner). Other advantages include a short scan time (5-10 minutes) and very low radiation exposure compared to imaging methods like CT (Laskey, 1996).

A number of studies have utilized DXA to assess body composition (Lohman et al., 2000), however several point to the potential error resulting from incorrect assumptions of FFM density and hydration in DXA estimations of soft tissue (Roubenoff et al., 1993; Laskey, 1996; Clasey et al., 1999). Others have argued that such error occurs, but is very small (Pietrobelli et al.,1998; Testolin et al., 2000; Lohman et al., 2000).

In Deurenberg-Yap et al.'s (2001) test of 2C models against the 4C model, the authors argued that variation in model outcomes resulted from the use of assumed values of FFM properties that were not valid in their study populations. Specifically, they found that DXA underestimated %fat across groups of Singaporean Chinese, Malays and Indians; error was in the range of 2.1-4.2 %fat, compared to the 4C model (Deurenberg-Yap et al., 2001).

Williams and colleagues (2006) evaluated DXA against the 4C model in patients and healthy controls, finding a variable degree of bias in body composition associated with age, sex, disease state and body size. Error might be introduced in DXA estimations of FM and FFM due to variability in tissue thickness across the body, or between individuals (Roubenoff et al., 1993; Williams et al., 2006). For example, bias has been positively associated with tissue depth (Laskey, 1996; Mitchell et al., 2000). In a study by Van der Ploeg et al. (2003), variation in

anteroposterior tissue thickness may have accounted for DXA's underestimation of fat, particularly for lean subjects, relative to the 4C model.

Overall, research suggests that DXA whole-body soft tissue outcomes should be interpreted with caution (Roubenoff et al., 1993; Deurenberg-Yap et al., 2001; Van der Ploeg et al., 2003; Williams et al., 2006; Wells and Fewtrell, 2006). Beyond its utility for accurately estimating BMC, DXA may be best utilized to asses regional FM and FFM, particularly in the limbs; determining trunk composition requires a higher degree of prediction due to the amount and distribution of bone in the truncal region (Wells and Fewtrell, 2006).

DXA therefore offers an option for measuring lean tissue in the limbs, and using this as a measure of whole-body SM mass. Following Kim et al. (2002), the sum of DXA's estimates of lean tissue in both the arms and legs can be referred to as appendicular lean soft tissue (ALST), which comprises skeletal muscle, skin, and connective tissues, but not fat and bone mineral (Kim et al., 2002). SM represents the largest FFM component in the adult body, and approximately 73-75% of its mass is found in the appendages (Heymsfield et al., 1990; Wang et al., 1996; Fuller et al., 1999; Kim et al., 2002). In turn, the largest component of ALST is SM (Figure 3-4), so that measuring ALST by DXA is a useful and more practical option for quantifying SM than either CT or MRI. From here, I use ALST and SM synonymously.

In an early assessment of the use of DXA for quantifying SM, Heymsfield and coworkers (1990) found DXA's estimates were highly correlated with those achieved using both simpler and more complex techniques. DXA only slightly overestimated SM compared to CT estimates in a later study, so that Wang et al. (1996) recommended its use as a practical alternative method for SM quantification *in vivo*. Indeed, DXA has subsequently been utilized by many researchers for this purpose (e.g. Gallagher et al., 1997; Starling et al., 1999; Kyle, et al., 2001; Iannuzzi-Sucich et al., 2002; Szulc et al., 2004; Moon et al., 2014).

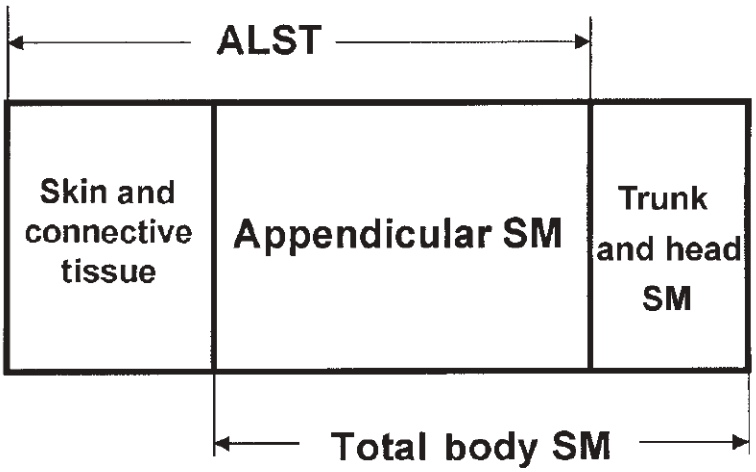


Figure 3-4 Appendicular skeletal muscle as a component of appendicular lean soft tissue and total body SM.
From Kim et al., 2002.

In their 2002 paper, Kim and colleagues assessed DXA-derived ALST against whole-body SM derived from MRI. For the latter, they utilized the reference whole-body multi-slice method I described above in Section 3.3.2.1. As shown in Figure 3-5 below, they found a close correlation between the outcomes.

Based on the recognized proportion of appendicular SM to total SM (as noted above, ~73-75%), some authors have obtained ALST from DXA and divided the value by 0.75 to achieve total SM (e.g. Wang et al., 1996; Iannuzzi-Sucich et al., 2002). Others have utilized ALST as SM without the additional calculation (e.g. Gallagher et al., 1997; Illner et al., 2000; Kyle et al., 2001; Kim et al., 2002; Szulc et al., 2004). I took the latter approach in my study to avoid making the assumption that appendicular SM represents 75% of whole-body SM in my sample. Deriving my SM measure from DXA's ALST estimate is advantageous as it means that SM will be independent of FM, FFM, brain and body organ outcomes.

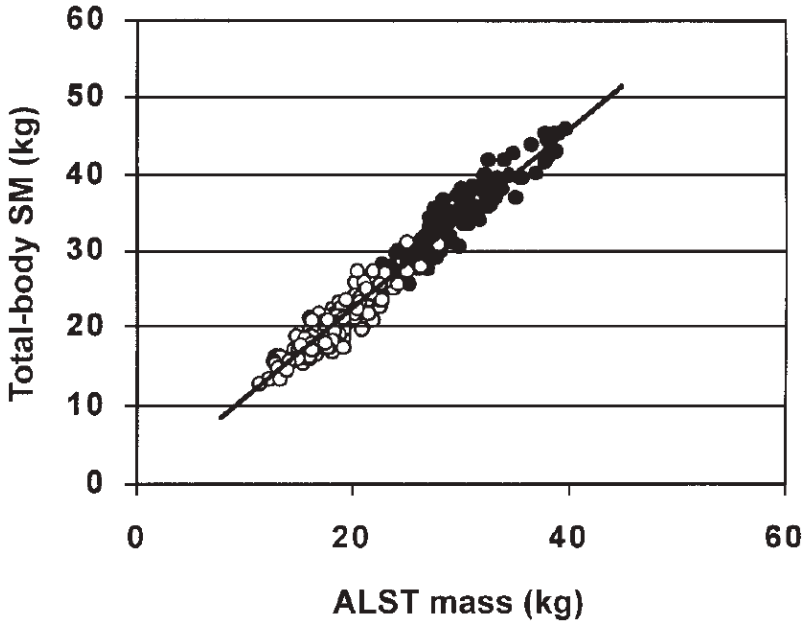


Figure 3-5 Total-body skeletal muscle estimated by MRI against appendicular lean soft tissue measured by DXA.
Women are open circles, men are shaded circles, $r^2 = 0.96$; from Kim et al., 2002.

Below, I return to the topic of the 2C model and explore how a third measurement technique, air-displacement plethysmography, can be used for this purpose.

3.4.1.3 Body volume by air-displacement plethysmography

Air-displacement plethysmography (ADP) is a densitometric technique, one of the oldest and most frequently used methods for generating 2C body composition models (Behnke et al., 1942; Keys and Brozek, 1953; Siri, 1961; Brozek et al., 1963; Heymsfield et al., 1997). ADP has largely superseded the classic technique of underwater weighing, pioneered by Behnke and colleagues (1942), which measures body volume via submersion in and displacement of water by the body (Ellis, 2000). ADP instead derives body density by quantifying the volume of air displaced by an individual within a closed chamber (Figure 3-6). This is often

undertaken using instrumentation known as the BodPod (Dempster and Aitkins, 1995; McCrory et al., 1995; Wells and Fuller, 2001; Fields et al., 2002).

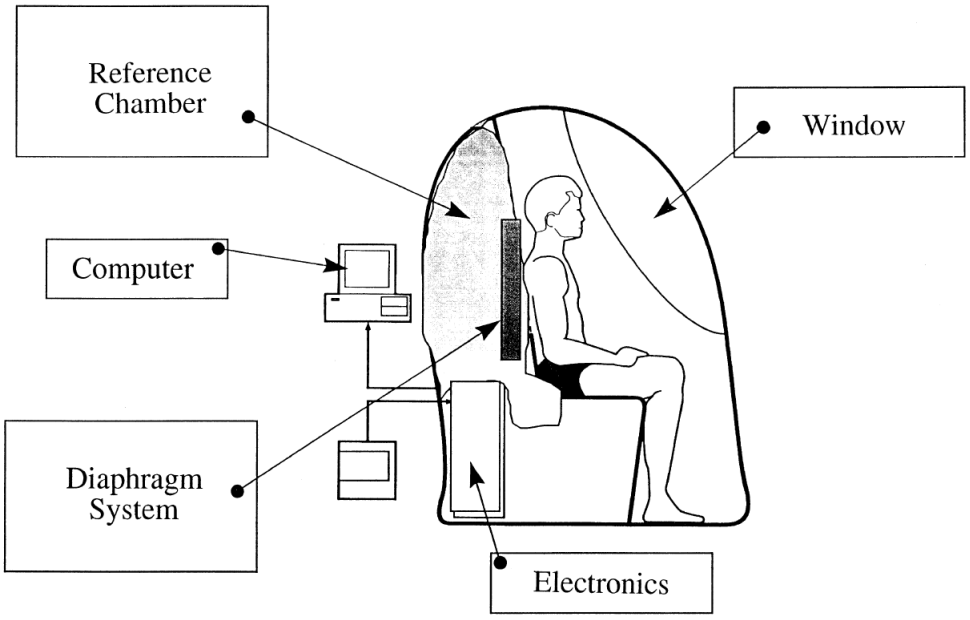


Figure 3-6 General components of an ADP system.
Image from Ellis, 2000, originally adapted from Dempster and Aitkens, 1995.

The BodPod system's front measurement chamber, where the subject sits, is separated from a rear reference chamber by an oscillating diaphragm that induces subtle changes in pressure (Ellis, 2000). The principle of an inverse relationship between pressure and volume at a constant temperature – Boyle's Law – is used to determine the subject's body volume (Ellis, 2000; Fields et al., 2002). Raw body volume (RBV) is corrected for thoracic gas volume (TGV) and surface area artefact (SAA; warmer, more compressible air close to the surface of the skin) to derive actual body volume (ABV). As outlined in Dewit et al. (2000), TGV is predicted from tidal volume (TV) and functional residual capacity (FRC):

(3-4) $$TGV = FRC + 0.5TV$$

The sex-specific equations of Crapo et al. (1982) predict FRC, whilst TV is an assumed constant which also differs for males and females. A study by Demerath and colleagues (2002) indicated that using predicted TGV, rather than directly measuring this outcome during ADP assessment, did not have a significant effect on estimations of FM in adults.

Surface area (SA) is calculated using the equation of Du Bois and Du Bois (1916). SA is multiplied by a constant provided by the manufacturer to obtain SAA (Dewit et al., 2000). The following equation yields ABV:

(3-5) $$ABV = RBV + 0.4TGV - SAA$$

To construct a 2C model, whole-body density is calculated as the mass of the subject divided by ABV, and assumed fixed densities of FM and FFM are used to estimate the FM:FFM ratio (Wells and Fewtrell, 2006).

Specifically, following Siri (1961), %fat is calculated as shown below:

(3-6) $$\%fat = \frac{x}{d-y}$$

where d is body density, and x and y are empirical constants derived using density values of 0.9 kg/l for FM, and 1.1 kg/l for FFM (Siri, 1961). Percentage fat and body weight outcomes can be used to obtain values for FM and FFM (Dewit et al., 2000; Wells and Fuller, 2001).

Assuming a density of 0.9 kg/l for FM has been deemed appropriate based on several studies (Fidanza et al., 1953; Keys and Brozek, 1953; Siri, 1961). Research has validated ADP using the BodPod in adults and children (McCrory et al., 1995; Demerath et al., 2002; Fields et al., 2002) over a range of body size (Wells and Fuller, 2001) with generally good precision, although some studies

have reported underestimations (Wagner et al., 2000) and overestimations (Collins et al., 1999) of body density.

However, as described in the preceding sections regarding TBW and DXA 2C models, the assumption of constant FFM properties remains a major limitation of the densitometric 2C model (Siri, 1961; Ellis, 2000; Fields et al., 2002; Wells and Fewtrell, 2006). An individual's FM or FFM may be estimated incorrectly if variable proportions of water, mineral or protein result in a value for FFM density that differs from 1.1 kg/l. For example, evidence has suggested black individuals have a higher FFM density than whites, which could result in spurious estimates of FFM if an equation based on density measured in the latter group is used for the former group (Schutte et al., 1984; Wagner and Heyward, 2000). As yet unpublished data from my research group suggests that the density of FFM differs between Europeans and South Asians, as well.

In a study comparing ADP's 2C model estimate of FM with the 4C model in women, Fields and colleagues (2001) found that the BodPod under-predicted %fat. With additional measurements, they concluded this was related to incorrect estimation of the FFM hydration fraction. The authors suggested the use of ADP data in the 4C rather than 2C model, when possible (Fields et al., 2001).

3.4.1.4 2C model summary

As noted above, human body composition differs in association with various characteristics including age, body size, sex, ethnic background and disease state. In particular, these characteristics have been associated with variability in the FFM component of body composition, whilst FM is relatively homogeneous at the molecular level. Assuming relative homogeneity of FFM – namely, the constancy of water, protein and bone mineral across populations and subject characteristics – can lead to error in estimating body composition using a 2C model (Wells et al., 1999; Withers et al., 1999). In my study recruitment, I aimed to control for many of

the characteristics listed above – e.g. age, sex, ethnicity and health – however variability in FFM properties would still be expected among individuals.

Indeed, it has been suggested that 2C model error is largely due to the violation of biological assumptions, rather than a result of technical inaccuracy associated with DXA, ADP and isotope dilution measurements (Withers et al., 1999). Data should be combined from two or more of these techniques in a multi-component model, if feasible, to reduce reliance on assumptions and estimate FM and FFM more accurately (Jebb et al., 1993; Jebb and Elia, 1993; Withers et al., 1999; Norgan, 2005; Wells and Fewtrell, 2006). Considering the limitations of the 2C model, and following these authors, I did not utilize a 2C approach in my study.

Below, I describe the 3C and 4C multi-component models. Figure 3-7 visualizes the additional properties that these models assess, in relation to the 2C model.

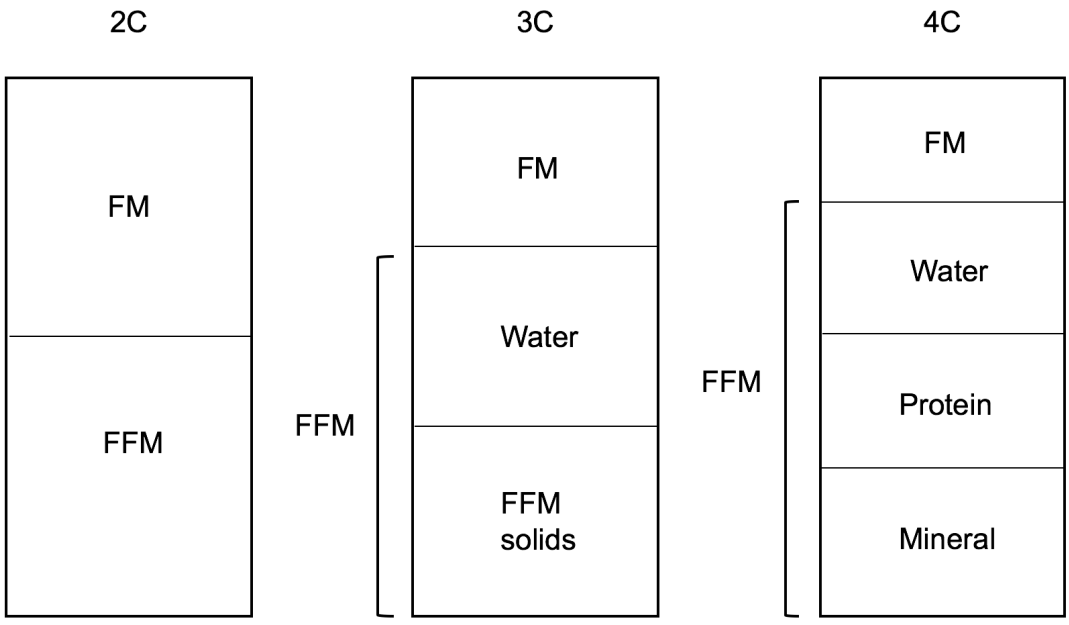


Figure 3-7 2C, 3C and 4C body composition models.

3.4.2 Multi-component models

The 3C model is a multi-component model that incorporates data on body volume and body water, often obtained via ADP and isotope dilution, respectively. With the further addition of body weight, the 3C model divides the body into FM, water, and fat-free dry matter, where the latter includes protein and mineral (Figure 3-7; Siri, 1961; Fuller, et al., 1992). The water content of FFM is not assumed in this model, and the derivation of FFM hydration and density values is possible. However, a constant, whole-body ratio of protein to mineral in fat-free dry matter is assumed (Fuller et al., 1992; Wells et al., 1999; Ellis, 2000), so that protein and mineral are considered together (protein+mineral).

Following Fuller et al. (1992), the density of protein+mineral and fat (D_{pm+f}) in kg/l is calculated thus:

(3-7) $$D_{pm} + f = \frac{Wt - TBW_m}{BV - TBW_v}$$

Wt is body weight in kg, TBW_m is total body water mass in kg, BV is body volume in liters, and TBW_v is total body water volume in liters. FM is obtained from the following calculation (Fuller et al., 1992):

(3-8) $$FM = \left(\left(\frac{2.2199}{D_{pm+f}}\right) - 1.4646\right) * (Wt - TBW_m)$$

where 2.2199 and 1.4646 are constants derived from assumed density values of fat, water (at 36°C) and protein+mineral (Fuller et al., 1992).

With the 4C model, the assumed constant ratio of protein:mineral in fat-free dry matter is avoided with the addition of BMC data from DXA. This allows for the division of the body into four components: fat, water, protein and mineral (Figure 3-7; Heymsfield et al., 1996, 1997; Fuller et al., 1992; Wells et al., 1999; Ellis,

2000), and may improve accuracy over the 3C model. Like the 3C model, the hydration and density of FFM can be estimated. A remaining assumption of the 4C model is that the ratio of bone mineral:total body mineral demonstrates a constant relationship (Fuller et al., 1992; Wells et al., 1999).

The calculations are similar to those above (Fuller et al., 1992). First, one calculates the density of protein and fat (D_{p+f}) in kg/l:

(3-9) $$D_{p+f} = \frac{Wt - TBW_m - TBMM}{BV - TBW_v - TBMV}$$

Here again, Wt is body weight in kg, TBW_m is total body water mass in kg, BV is body volume in liters, and TBW_v is total body water volume in liters. The added outcomes are total body mineral mass (TBMM) in kg, and total body mineral volume (TBMV) in liters. TBMM is equal to BMC measured by DXA, multiplied by the value 1.2741 (Brozek et al., 1963). TBMV is obtained from the calculation BMC x 0.4195 (Fuller et al., 1992). FM is then calculated with the following equation:

(3-10) $$FM = \left(\left(\frac{2.7474}{D_{p+f}}\right) - 2.0503\right) * (Wt - TBW_m - TBMM)$$

where, similar to the 3C model, 2.7474 and 2.0503 are values derived from the assumed densities of the four components (Fuller et al., 1992; Wells et al., 1999). FFM can be calculated with the following:

(3-11) $$FFM = Wt - FM$$

Although it is not entirely free of assumptions, the 4C model is considered the gold-standard *in vivo* body composition technique: by combining several measurements, it minimizes assumptions to the extent currently possible. With the 4C model, researchers can accurately measure body composition and also

evaluate the validity of simpler methods, which obviates the need to rely on relatively impractical chemical cadaver analyses (Heymsfield et al., 1997; Wells and Fewtrell, 2006). An additional question, though, is relevant to the multi-component model's use of several measurements; namely, whether errors associated with the individual measures are propagated and ultimately result in larger error for final outcomes (Fuller et al., 1992; Withers et al., 1999).

According to a review of models by Withers and coworkers (1999), 3C and 4C models offer increased accuracy that is not attenuated by propagated error. Likewise, Fuller and colleagues (1992) found no evidence that individual measurement errors were additive. Estimation with the 4C model of both FM and FFM produced in their sample an overall error of ~1% body weight, which is lower than the error associated with body composition outcomes derived using a single technique. Error propagation for the estimation of FM was ±0.54 kg and ±0.49 kg with 4C and 3C models, respectively (Fuller et al., 1992). In a study comparing 2C and multi-component models, Wells et al. (1999) reported similar propagation of error to the study of Fuller et al. (1992), with a precision of ±0.5 kg for FM and FFM from 3C and 4C models.

An evaluation of various body composition models in African American and white children found that 4C models demonstrated the greatest degree of reliability, relative to 3C models and simpler methods (Bray et al., 2002). More recently, the 4C model was used to validate field and 2C techniques, including ADP and DXA, in a cohort of Indian adults (Kuriyan et al., 2014). Numerous others have utilized the 4C model to assess body composition or serve as a criterion method (e.g. Gallagher et al., 1996; Williams et al., 2006; Chomtho et al., 2008; Deurenberg-Yap et al., 2001; Pourhassan et al., 2013; Wells et al., 2015).

As I had access to isotope dilution, DXA and ADP techniques, I was able to utilize the gold-standard 4C model to predict FM and FFM in my sample. In the next

section I describe the method I used to measure REE, after which I give a summary table of my study outcomes and methods.

3.5 Resting energy expenditure by indirect calorimetry

The quantification of REE by indirect calorimetry (IC) will allow me to test my first hypothesis, that organs and tissues demonstrate differential metabolic cost, as measured in kcal/kg/day (see Chapter 2). I first described REE in Chapter 1. This outcome constitutes the number of kilocalories used daily by the body for functions including respiration, circulation and cellular homeostasis. It is the main component of TEE, and it is measured at rest in a post-absorptive state (Gannon et al., 2000; Wang et al., 2001; Mittelsteadt et al., 2013).

TEE reflects the total heat energy used by the body to carry out all functions over a 24-hour period (Haugen et al., 2007). There is some variation in the literature regarding what proportion of TEE is accounted for by REE. Estimates range from 60-90% (Bogardus et al., 1986; Owen, 1988; McClave and Snider, 1992; Matarese, 1997; Case et al., 1997; Gannon et al., 2000; Ruggiero et al., 2008; Gurven et al., 2016), which may be due to differences in the way authors define REE. The remaining proportion of TEE is attributed to thermogenesis, physical activity, anabolism (growth) and the thermic effect of food (Wang et al., 2001).

The assessment of heat loss in animals through direct measurement dates to Lavoisier and Laplace at the end of the 18th century, and IC has been used to measure energy expenditure in humans for about a century (Passmore and Durnin, 1955; Jéquier et al., 1987; Henry, 2005). Where direct calorimetry measures heat lost from the body, IC estimates heat loss as a function of the oxidative process (Jéquier et al., 1987). Specifically, an indirect calorimeter measures oxygen consumption and the production of carbon dioxide; with these data, established equations are used to calculate REE (see Weir, 1949; McClave and Snider, 1992; Matarese, 1997; Case et al., 1997). Although the full equation technically takes into account urinary nitrogen production, Weir (1949) argued that

error introduced by failing to correct for this was negligible (1% for each ~12% calories from protein), and would likely cancel out as, broadly, humans appear to consume similar amounts of dietary protein.

The relative availability and ease of use of IC equipment renders this method, compared with direct calorimetry, more feasible for research studies and clinical assessment (Lam and Ravussin, 2017). Before its production ceased, the Deltatrac II metabolic monitor was validated and widely used for measuring REE in adults and children, and utilized as the standard for validation studies of new equipment (Phang et al., 1990; Weissman et al., 1990; Bauer et al., 2001; Littlewood et al., 2002; Alam et al., 2005). I had access to a Deltatrac II system, and therefore used it to measure REE in my study participants.

REE has demonstrated intra-individual variation in healthy volunteers undergoing repeated measurements, both over several months and with hourly-repeated tests on consecutive days (McClave and Snider, 1992). This indicates that a one-time measurement may not offer the most accurate estimate of an individual's REE. Nevertheless, a single measurement is preferable to the estimation of REE using predictive equations. These have demonstrated variable efficacy in different ethnic groups, and may only be appropriate when used in the population from which they were derived (Case et al., 1997; Wang et al., 2001).

Following IC measurements, REE was calculated with the equation of Weir (1949):

(3-12) $$(3.941 * V0_2) + (1.106 * VC0_2)$$

The table given below (Table 3.1) summarizes the techniques I selected to obtain data of the highest possible quality in my sample. I list the instruments used, which will be described along with more practical aspects of data collection in Chapter 4.

As described in the current chapter, I largely discarded techniques at Wang et al.'s (1992) whole-body level, which offer limited detail and accuracy, in favor of more difficult to acquire, more accurate methods such as MRI and the 4C model.

Table 3.1 Summary of measurement outcomes and techniques

Method	***Instrument***	***Outcome(s)***
Anthropometry	Stadiometer, calipers	Height, tibia length
MRI	MRI scanner	Brain and organ volumes
4C model		
Air-displacement plethysmography	BodPod, scale	Body volume*, weight*
Dual-energy X-ray absorptiometry	DXA scanner	Bone mineral content*, appendicular skeletal muscle mass
Deuterium isotope dilution	$^{2}H_2O$ dose and saliva samples	Total body water*
Indirect calorimetry	Deltatrac II indirect calorimeter	Resting energy expenditure

*combined in 4C model to derive FM and FFM

4 Recruitment, ethics and methodology

In the previous chapter I described how I selected methods for data collection out of several available. This chapter provides details on the practical aspects of data collection using the chosen methods. I also describe recruitment, ethical considerations, methods of data post-processing and statistical analysis, and consideration of potential confounders.

4.1 Criteria of inclusion/exclusion

For the current study I sought participants who demonstrated the following characteristics:

- Aged 20-28 years
- Of South Asian ancestry
- Female
- Nulliparous
- BMI in the range 17-28 kg/m^2
- Generally healthy, non-smoking
- Born at term (≥37 weeks gestation)

I excluded potential participants if they reported:

- Having any condition likely to affect growth and/or metabolism
- Taking medications with the potential to affect metabolism
- Weight change greater than 3kg in the past three months
- Contraindications for MRI scanning, including pacemakers or other metallic implants, or severe claustrophobia

The relatively narrow age range of 20-28 was chosen to limit variability in body composition brought about by pubertal growth and later changes associated with

aging. The decision was made to recruit a single sex, rather than split the sample size between males and females. I chose females, as they engender a particular anthropological interest as the physical and metabolic niche of subsequent generations. However, I sought to include in the present cohort only women who were currently nulliparous. This was to avoid potential confounding by body composition variability associated with differential parity.

Only women who had been born at term were recruited to control for the possibility that any observed tissue trade-offs would have developed specifically in association with pre-term birth. The BMI range was set so as not to include very underweight or obese women, as the aim of the study was to assess relationships among body components across a normative range of body size and composition. In general, Asian populations demonstrate lower mean or median BMI compared to non-Asian populations (Lancet, 2004). Because the BMI distribution is shifted to the left for Asians, obesity-related health risks may occur below a BMI of 30 kg/m^2 (Lancet, 2004; Unni et al., 2009), thus our upper BMI cutoff was set at 28 kg/m^2. The exclusion criterion related to recent weight change aimed to avoid potential confounding by fluctuating body composition, particularly FM.

The decision to recruit South Asian women was discussed in Chapter 1. Additional motivations were: 1) research studies in non-European/North American populations remain relatively rare, and 2) of the UK population identifying as non-white, South Asians make up a relatively large proportion, with many residing in London (Office for National Statistics, 2012). The latter point was considered to increase the feasibility of successful recruitment and the likelihood of achieving the target sample size, as the study was carried out in London, UK (see below).

I specifically aimed to recruit women with Indian, Pakistani, Bangladeshi and Sri Lankan ancestry. Nepal was excluded in an attempt to avoid recruiting subjects who may have lived at and adapted to a high-altitude environment. The

geographical location of the target countries on the Indian subcontinent is shown in Figure 4-1.

Figure 4-1 Map of the Indian subcontinent, highlighting India, Pakistan, Bangladesh and Sri Lanka.

Ancestry was ascertained by subject self-identification, confirmed by the subject's four grandparents also being Indian, Pakistani, Bangladeshi or Sri Lankan. It was not a requirement that subjects themselves were born in one of the four target countries. Further information on the ethnic background and birthplace of participants in the final sample is given in Chapter 5, Section 5.1.

4.2 Sample size calculation

Considering limitations of time and funding to cover costly measurement techniques such as MRI scanning, a sample size of 70 was originally conceived for its feasibility. A power analysis showed that this sample size would yield 80% power to detect a correlation of 0.33 and explain 10% of the variance in the outcome at a significance level of 0.05. This r value is suggested to represent a medium effect size (Cohen, 1992).

4.3 Study site and recruitment procedures

The study was carried out in London, UK, with all data collection procedures performed at the UCL Great Ormond Street Institute of Child Health (GOSICH) and Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH). Recruitment was carried out in London, mainly within and around UCL, although study details were also circulated at the University of Cambridge.

Methods of recruitment included hanging posters in buildings at UCL and surrounding universities (e.g. the London School of Hygiene and Tropical Medicine, SOAS), and circulating study details via email, for example to staff and students of GOSICH and UCL societies (e.g. the Indian Society, the Hindu Society). The study was also advertised in an online student newsletter at the London School of Economics, and on more than one occasion in the weekly student newsletter of the UCL Union. Word-of-mouth proved to be an important tool through the assistance of colleagues, friends and already-recruited participants. Two subjects were recruited from Cambridge after a University of Cambridge student shared details of the study within her college, and a GOSICH colleague invited me to attend a Sikh Gurdwara (temple) in London to connect with potential participants. The poster mentioned above, and additional recruitment materials described below are included in the Appendix.

My contact details were included on posters and in online advertisements about the study, and most often, potential participants would send an email or text message expressing their interest in joining. In response, I would send the individual a copy of the study information sheet, and ask them to contact me again to set up a phone interview if they remained interested after reading a more detailed description of the study. In the phone interview, I asked for the individual's age, information about the ancestry and birthplace of their parents and grandparents, whether or not they were or ever had been pregnant, and whether they had any medical conditions or took any medications. I asked if they knew their birth weight and gestational age, or if they believed they could obtain this information from a parent or relative.

I asked about smoking; as detailed above, we sought to recruit only non-smokers. I also asked about alcohol use, as we wanted to avoid recruiting subjects who appeared to drink excessively, however all individuals reported moderate or no drinking. I explained the MRI process for those who had not been scanned previously, and discussed the potential for claustrophobia and other contraindications for scanning. Individuals were asked whether they had gained or lost more than 3kg in the previous 3 months, and if they knew or could estimate with confidence their height and current weight. Height and weight were used to calculate BMI and screen out individuals above or below the specified BMI range.

4.4 Ethical considerations

4.4.1 Informing subjects and getting consent

Potential participants were informed of why the study was being done, why they were being asked to participate, and what each aspect of the data collection would entail for them personally. All were assessed regarding their suitability and safety for MRI scanning, both before an appointment for data collection was booked, and again immediately prior to scanning. We requested participant GP details prior to scanning per standard protocol. It was communicated to all subjects that incidental

MRI findings are rare, but in the case of such findings their GP would be notified in the first instance.

Each participant was given as much time as they wished to ask any questions before deciding whether they wanted to participate in the study, and advised that they were free to ask questions or voice any concerns at any point after joining. Consent was taken in writing before any measurements were done. Subjects were informed that they were free to withdraw from the study at any point without needing to give a reason. They were given at the end of the study information sheet details to contact the UCL office for Research Incidents and Complaints, or GOSH's Patient Advice and Liaison Service, in the event they wished to make a comment or complaint.

4.4.2 Potential risks and burdens and how I sought to minimize them

All measurements were harmless and non-invasive, and used in many prior studies of both adults and children.

I made it clear to all participants that taking part in the study was not a clinical assessment, and that it was important they consult their GP if they had any specific health concerns.

The REE measurement by IC was done following an overnight fast, as per protocol. I conveyed this information to potential participants before booking them in for an appointment, in case they would not wish to fast. I scheduled visits to GOSICH/GOSH so that the REE measure could be performed in the morning, and afterwards I provided subjects with a light breakfast before further measurements were carried out.

The DXA scan exposes subjects to a very small amount of ionizing radiation. This exposure is at maximum 2 microSv, which is well below daily background radiation

in the UK (approximately 7 microSv, depending on location), or that experienced during a trans-Atlantic flight (Williams et al., 2006). However, pregnancy is a contraindication for DXA. Potential subjects were asked in the recruitment interview if they were pregnant, and an item on the consent form asked whether the subject was, or potentially could be, pregnant. They signed the consent form just prior to scanning.

Individuals who suffer from claustrophobia may experience distress during MRI scanning, or during the BodPod measurement, as both techniques require participants to enter a narrow, confined space. Potential subjects were asked about their susceptibility to claustrophobia during recruitment, as noted above, with the conditions of the MRI and BodPod measurements described to them in full.

Incidental findings of pathology on the MRI scan had the potential to cause distress, although, as subjects were informed prior to the measurement, such findings are rare. An MRI scan may also present risk of heating of the skin or discomfort due to persistent loud noise during image acquisition. The risk is always present of accidental injury if metallic objects, either internal or external to the subject, are brought in contact with the scanner's strong magnetic field.

Injury due to the movement of metallic objects in or around the body was very unlikely to occur: individuals were carefully checked before entering the scanner room, and MRI-safe equipment was used. Heating of the skin was avoided with appropriate precautions taken by the radiographers, including proper placement of the participant on the scanner table. Ear protection was provided to avoid uncomfortable effects of noise. Throughout each scan, radiographers remained in direct contact with the subject, and were able to stop scanning immediately if the subject signaled distress or discomfort.

4.4.3 Data anonymization and security

Each participant was given a study identification number following recruitment, and this number was used on all forms throughout the data collection process to maintain confidentiality. Paper data forms and MRI scans, which were collected from radiology on DVDs, were stored in a locked filing cabinet in a locked office on a floor with keycard access.

The document that linked ID codes to participants' full names was the consent form. These were stored on the same keycard-protected floor in a separate, locked cabinet. All electronic data (containing ID code only) were stored and analyzed on a password-protected computer. MR image data were transferred to a password-protected, encrypted hard drive for analysis.

Saliva samples were stored in a GOSICH laboratory, on a floor with keycard access. Samples were marked by date of collection and subject ID code. All samples will be destroyed upon completion of the project in accordance with the Human Tissue Act Code of Practice on Disposal.

4.4.4 Ethical approval

Ethical approval for the study was granted by a NHS Research Ethics Committee of the Health Research Authority on 15 October 2014 (REC reference: 14/LO/1684; IRAS project ID: 151208). I was issued an amended letter on 7 November 2014 to include the version number and date of the project protocol, which was missing from the original favorable opinion letter.

The study was granted approval by the Joint Research and Development Office of GOSICH and GOSH on 21 November 2014 (R&D reference: 14NT03). Completed risk assessments for the study were approved by the Head of Laboratory Management and Safety at GOSICH on 24 December 2014.

4.5 Methods of measurement

Following the recruitment interview, eligible individuals who still wished to join the study were booked in for a data collection appointment at GOSICH/GOSH. I arranged this, and booked scans in advance for DXA and MRI with GOSH radiology. Subjects visited on one occasion, or on two separate days which, in the majority of cases, occurred within a fortnight of one another. All participants initially reported to GOSICH, where I met them and took their written, informed consent to join the study (a copy of the consent form is included in the Appendix). Measurements were then carried out at GOSH.

Overall, subjects underwent 12 separate measurements, as described in the study information sheet (see Appendix). The data from a select number of these measurements are used in the analyses in this thesis; remaining data will be analyzed in future studies. This section describes the measures used for this study only, with the subsection headings reflecting the outcome variable/s collected. There was some variation in the order of measurements for each subject, largely related to the time of the subject's arrival on the day and the availability of slots for DXA and MRI. However, the order in which measurements are detailed below reflects the order in which most subjects experienced them. I give information on how data were obtained from each measurement, and describe further calculations and sample/data processing where relevant.

4.5.1 Birth weight

I asked participants to fill out a questionnaire, which they sent in advance or brought with them to the data collection appointment. The questionnaire included space for the participant to report their birth weight, obtained by their own knowledge, the recollection of a parent/relative, or documentation to which they had access. I also asked for information on gestational age in order to derive birth weight standard deviation scores (SDS). This is described further in Chapter 9, where birth weight data are used in analyses.

4.5.2 Height

Height was taken in duplicate to the nearest 0.1cm using a wall-mounted stadiometer (Holtain), with subjects standing and unshod, and the head assessed for alignment with the standard Frankfurt plane. The average of the two measurements was used for analysis.

4.5.3 Resting energy expenditure

Subjects were asked to fast overnight prior to the REE measurement (e.g. if their data collection appointment was 9am, they were requested to refrain from eating or drinking anything apart from water from 9pm the previous evening). Following standard protocol the measurement was carried out in the morning, in a thermoneutral environment. The subject lay supine on a padded hospital table under a ventilated plastic canopy connected by cylindrical pump to a Deltatrac II indirect calorimetry system (Datex-Engstrom Corp, Helsinki, Finland). They were asked to remain awake, relax and breathe normally during the measurement, which lasted approximately 25 minutes.

A gas calibration of the metabolic cart was carried out prior to each measurement session. During the measurement, ambient air is drawn through the pump and directed to a mixing chamber within the machine. Measured concentration differences between inspired oxygen and expired carbon dioxide are used to calculate REE (Littlewood et al., 2002). These data were recorded minute by minute from the display on the Deltatrac monitor, and used in the Weir equation (given in Chapter 3, Section 3.5) to calculate a final REE value for each subject in kcal/24hr units.

4.5.4 Body volume and weight

Body volume was measured by ADP using the BodPod system (BodPod, Cosmed, Rome, Italy). Weight was also obtained during this measurement. Subjects wore clothing that were minimal and tight-fitting (e.g. a swimming costume, bra and

pants), and they covered their hair with a swim cap. Shoes, jewelry and glasses were removed. For calibration, the volume of gas in the BodPod's front chamber was measured first with the chamber empty, and then containing a 50-liter cylinder.

After being weighed to the nearest 0.01kg, subjects entered the BodPod. Gas volume in the chamber was measured again, now with the subject inside and the door closed; they were asked to limit bodily movement, breathe normally, and refrain from speaking. One complete test was comprised of two or three volume measurements (~50 seconds in duration each), depending on consistency. If the first two measurements differed by >150ml, a third was performed.

Mean volume was calculated from the two measurements (or the two of three which agreed within 150ml), after which the entire process was repeated for a second test. A second weight measurement was taken (as with height, the average of the two weight measures was used for analysis). If calculated mean density was consistent between the two tests (within 0.007 kg/l; Wells and Fuller, 2001), a third test was not performed.

RBV was corrected for TGV and SAA to derive ABV, as described in Chapter 3, Section 3.4.1.3. ABV was used in the 4C model (Chapter 3, Section 3.4.2).

4.5.5 Tibia length

Large sliding calipers (Harpenden) were used to measure, to the nearest mm, tibia length as the distance from the medial tibial plateau to the inferior edge of the medial malleolus (Cameron, 2004) on the left leg only. This measure was taken in duplicate and the values averaged.

4.5.6 Bone mineral content and SM

All subjects underwent a DXA scan of approximately 5 minutes' duration, whilst wearing light clothing. The scan, which I performed, involved the subject lying

supine on the bed of a Lunar Prodigy fan beam scanner (GE Medical Systems, Madison, WI), as the scanner arm moved rectilinearly from head to toe. Information on subject weight and height was recorded in the integrated computer system, allowing scan depth to be automatically adjusted (Williams et al., 2006).

Measures of BMC were obtained directly from the scanner software (Encore, Version 14.10.022). Precision of BMC quantification by this method was reported to be 1.1% (Kiebzak et al., 2000). BMC data were incorporated in the 4C model, as described in Chapter 3, Section 3.4.2. Estimated ALST in the arms and legs was also obtained directly from the software to be used as a measure of SM mass, following previous authors (Heymsfield et al., 1990; Sparti et al., 1997; Illner et al., 2000; Kim et al., 2002).

4.5.7 Total body water

TBW was measured using ^{2}H-labeled water dilution with a dose equivalent to 0.05g $^{2}H_2O$/kg body weight (99.9 atom % ^{2}H, Sigma Chemical Co., Poole, UK). The dose was filtered and mixed with approximately 100ml of tap water. From the mixture, an aliquot was drawn to be analyzed along with physiological samples in lieu of relying on enrichment data provided by the manufacturer (Wong, 2003).

Subjects drank the $^{2}H_2O$ dose following the collection of a baseline saliva sample using an absorbent salivette (Sarstedt, Rommelsdorf, Germany). The human body naturally contains deuterium ions, thus the pre-dose sample is required to document subjects' baseline isotopic enrichment. In nature, ^{2}H abundance is 0.0156%, relative to the abundance of ^{1}H (99.9844%), although the precise amount varies geographically and seasonally. The average amount of ^{2}H is reported to be 1.5g in an adult (Wong, 2003).

The equilibration period from ingestion of the deuterium dose to the post-dose saliva sample was 4 hours (Williams et al., 2006). Subjects reported any liquids consumed during this period. They did not consume food or drink for 30 minutes

prior to the post-dose sample, which was collected in the same manner as the pre-dose. Saliva was collected from salivettes via centrifugation as soon as possible and stored at -20˚C. I prepared and processed all ^2H_2O doses and saliva samples at GOSICH.

4.5.7.1 Deuterium isotope sample analysis

The aliquotted ^2H_2O dose was gravimetrically diluted with double-distilled water (Milli-Q water, Millipore) to an enrichment approximately equivalent to the post-dose saliva sample. This is necessary to measure the isotopic concentration of the dose, as highly enriched samples can contaminate the mass spectrometer, and traceable analytical standards were not available at higher levels of enrichment. Specifically, 200µl of the dose was diluted in a 50ml flask, with the weight of both dose and diluent recorded.

For analysis, 250µl samples (in duplicate) of dilute dose, diluent, pre-dose sample, and post-dose sample were added to 12ml septum-sealed vials (Exetainer, Labco Ltd.) along with platinum catalyst rods (Thermo). Five standard waters were used to normalize deuterium isotope data against the international standard VSMOW; like the samples, they were prepared in duplicate.

Vials were flush-filled with 2% hydrogen in helium at 75ml/min for 5 minutes and then left to equilibrate for 24 hours at 25ºC. During this time, the hydrogen and deuterium (^1H and ^2H) of water equilibrates with hydrogen gas in the headspace, catalyzed by the platinum.

The enrichment of ^1H to ^2H in the headspace was measured by continuous-flow isotope ratio mass spectrometry (Thermo Delta XP with Gasbench sample introduction). With 10 gas injections into the mass spectrometer of 100µl, ^1H to ^2H enrichment was compared with a pure reference gas (99.999% hydrogen). This yields a chromatogram (Figure 4-2 below), which shows the results as 10 peaks.

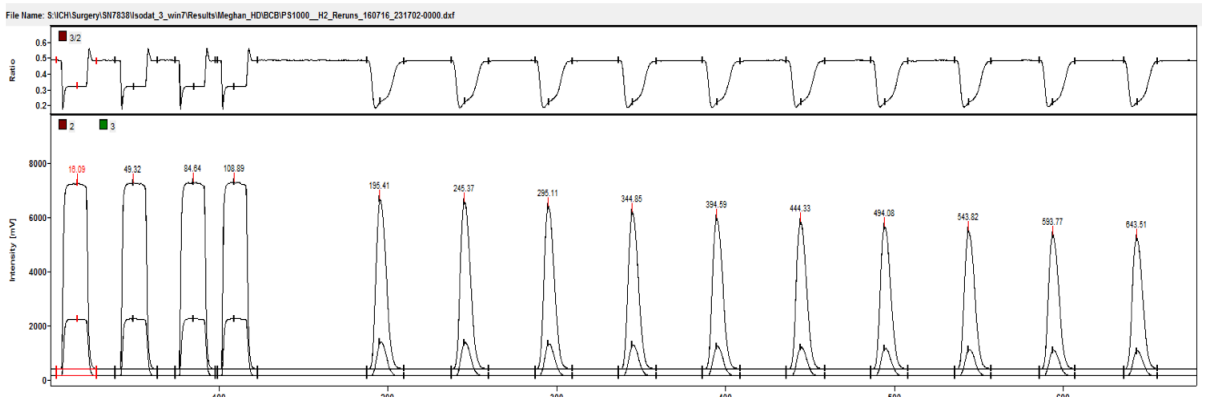


Figure 4-2 Sample chromatogram.

The standard deviation of the 10 peaks was routinely found to be <1.0‰. $^{1}H/^{2}H$ enrichment in the headspace was converted to actual enrichments in the sample by reference to the 5 water standards referred to above. A sample standard curve used to make the calculation is shown below in Figure 4-3.

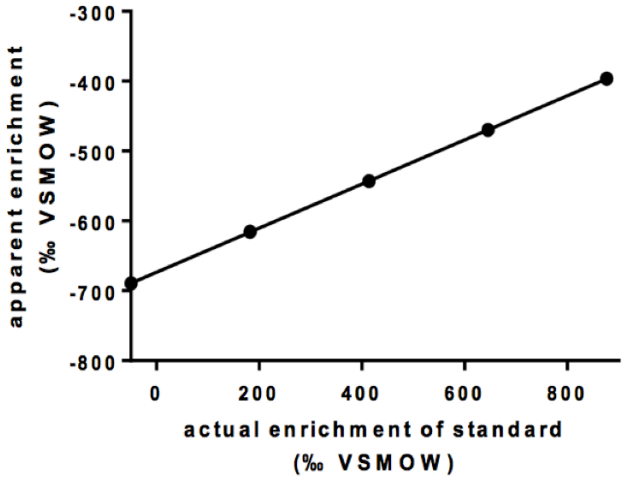


Figure 4-3 Sample standard curve for calculating actual enrichment.

For these example data, the linear regression yields r^2=1.000, slope 0.3158 and intercept -673.5, so that measured sample enrichments were converted to actual enrichments by the equation:

(4-1) $$Actual\ enrichment = \frac{measured\ enrichment + 673.5}{0.3158}$$

Following calculation of the actual enrichments, agreement between the duplicates was assessed. Samples were repeated where the difference in actual enrichment between the two duplicates was more than 5‰. Final data were used to calculate TBW as described in Chapter 3, Section 3.4.1.1, Equations 3-1 and 3-2, and incorporated in the 4C model.

4.5.8 Brain and body organ volumes

4.5.8.1 MRI protocol

Subjects were scanned at GOSH on a 3-Tesla (3T) Siemens Magnetom Prisma scanner (Siemens, Erlangen, Germany). The protocol included the following acquisitions:

- For 3D brain volume, a T1-weighted MPRAGE (Magnetization Prepared Rapid Gradient Echo; TR = 2300ms, TE = 2.74ms, flip angle = 8°, voxel size = 1mm^3, duration = 5 minutes).
- For the abdomen, a 3D isotropic T2-weighted turbo spin echo SPACE sequence (TR = 2000ms, TE = 220ms, flip angle = variable, voxel size = 1.5mm^3, duration = 7 minutes).
- For the chest, a T2-weighted TrueFISP (True fast imaging with steady state precession) with breath-hold (TR = 475.4ms, TE = 1.53ms, flip angle = 47°, voxel size = 1.5 x 1.5 x 4.0mm, duration = 20 seconds).

All images were visually assessed by trained radiographers during scanning for artefacts or anatomical abnormalities.

4.5.8.2 Brain volumetric analysis using FreeSurfer

High-resolution T1-weighted MR images were automatically segmented using FreeSurfer, the open source software suite for MR image analysis described in Chapter 3, Section 3.3.2 (version 5.3.0 for Mac OS X, https://surfer.nmr.mgh.harvard.edu). Figure 4-4 shows raw T1-weighted brain data in, from left to right, axial, coronal and sagittal orientations.

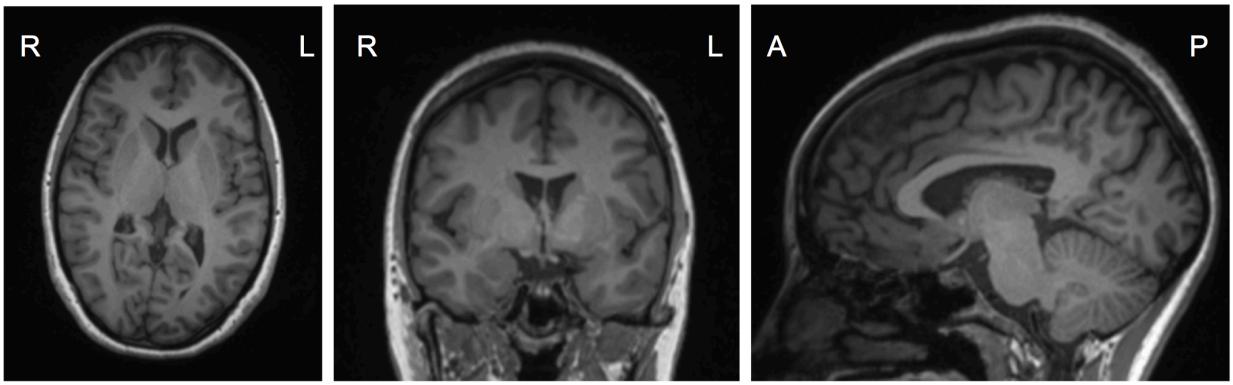


Figure 4-4 T1-weighted brain images.

The technical aspects of FreeSurfer's processing pipeline have been discussed extensively in the literature (e.g. Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 2002; Fischl et al., 2004a,b; Segonne et al., 2004; Jovicich et al., 2006).

Core elements of the pipeline include affine registration to the MNI305 atlas (Collins et al., 1994), bias field and motion correction (Reuter et al., 2010), skull stripping and removal of non-brain tissue (Segonne et al., 2004), automated Talairach transformation (spatial normalization), intensity normalization, and segmentation of white matter, cortical gray matter, and subcortical gray matter structures (Fischl et al., 2002; Fischl et al., 2004a). FreeSurfer's procedure of

probabilistic labeling of tissues at each voxel was found to be comparable to manual rating (Fischl et al., 2002).

Figure 4-5 shows subcortical structures (caudate, pallidum, putamen, amygdala, hippocampus), white matter and gray matter labeled by color. The image, in coronal orientation, was loaded in FreeSurfer's volume viewer. The nucleus accumbens, cerebellum cortex and cerebellum white matter were quantified, but are not shown in the figure.

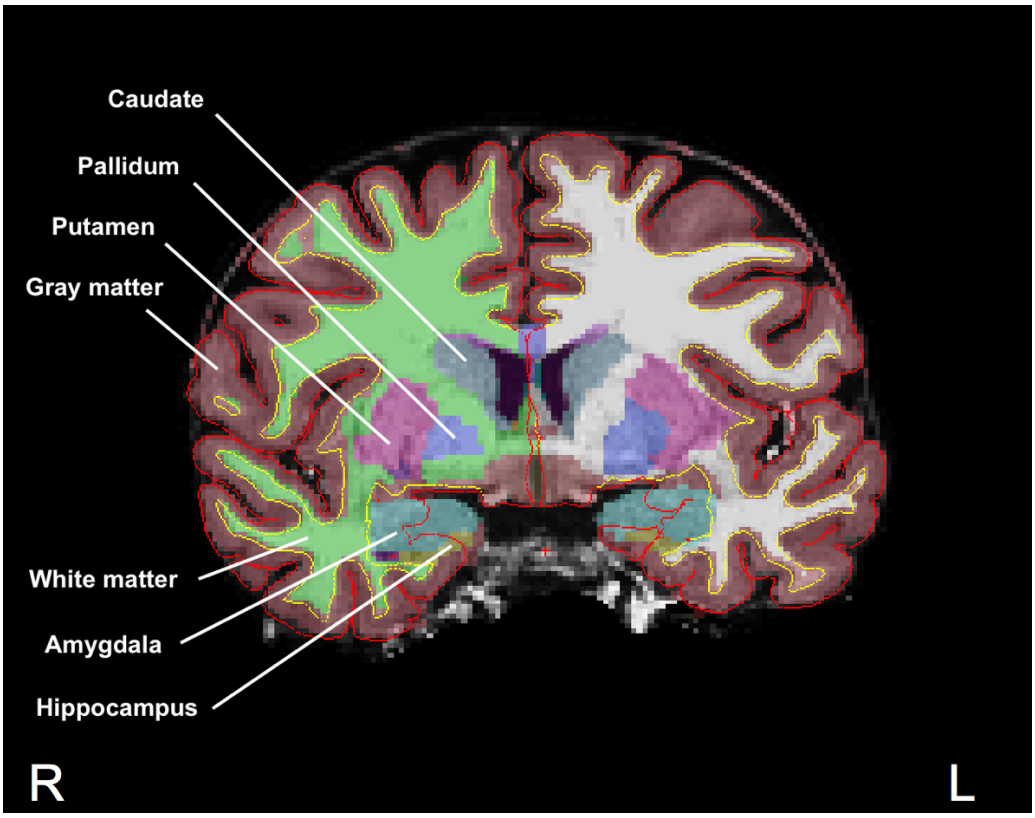


Figure 4-5 FreeSurfer volume-based labeling.

Following FreeSurfer processing, all segmentations were visually inspected. Certain aspects of the output can be manually edited with FreeSurfer tools if the segmentation appears incorrect (https://surfer.nmr.mgh.harvard.edu/fswiki/Edits).

I used the tools to make edits for several subjects where: incompletely stripped skull or dura interfered with brain surface delineation; small groups of voxels well within the white matter boundary were not labeled as white matter; or where white matter voxels were mislabeled near the white-gray boundary. In the latter case, 'control points' were added to 'push out' the white matter to meet the gray matter boundary. All edited datasets were re-processed with corrections incorporated.

4.5.8.3 Re-segmenting brain subcortical structures using FIRST

My original intention was to use the volume segmentation from FreeSurfer to quantify all brain tissue structures. However, upon visual inspection, FreeSurfer's subcortical segmentation output appeared incorrect in several subject datasets, particularly for the putamen and pallidum. These issues could not be addressed with FreeSurfer editing tools.

The segmentation tool FIRST (FMRIB's Integrated Registration and Segmentation Tool), part of the FSL library for brain imaging analysis, was used in an attempt to improve on FreeSurfer's results for subcortical structures. Like FreeSurfer, FSL is a fully-automated, widely-used toolkit (e.g. Zivadinov et al., 2012; Tondelli et al., 2012; Witte et al., 2014; Pardini et al., 2014), made available by the Oxford Centre for Functional MRI of the Brain (FMRIB) at Oxford University in the UK (https://fsl.fmrib.ox.ac.uk; Woolrich et al., 2009; Jenkinson et al., 2012). FIRST, described by Patenaude et al. (2011), employs a Bayesian approach to the labelling and segmentation of brain volumes. The default settings of the segmentation script have been empirically optimized for each subcortical structure (Patenaude et al., 2011).

Figure 4-6 shows FreeSurfer-derived subcortical structures loaded in FslView, FSL's brain volume viewer. Figure 4-7 shows the same structures, in the same subject, derived using FIRST. Upon visual inspection, the FIRST segmentation generally appeared more accurate across subjects.

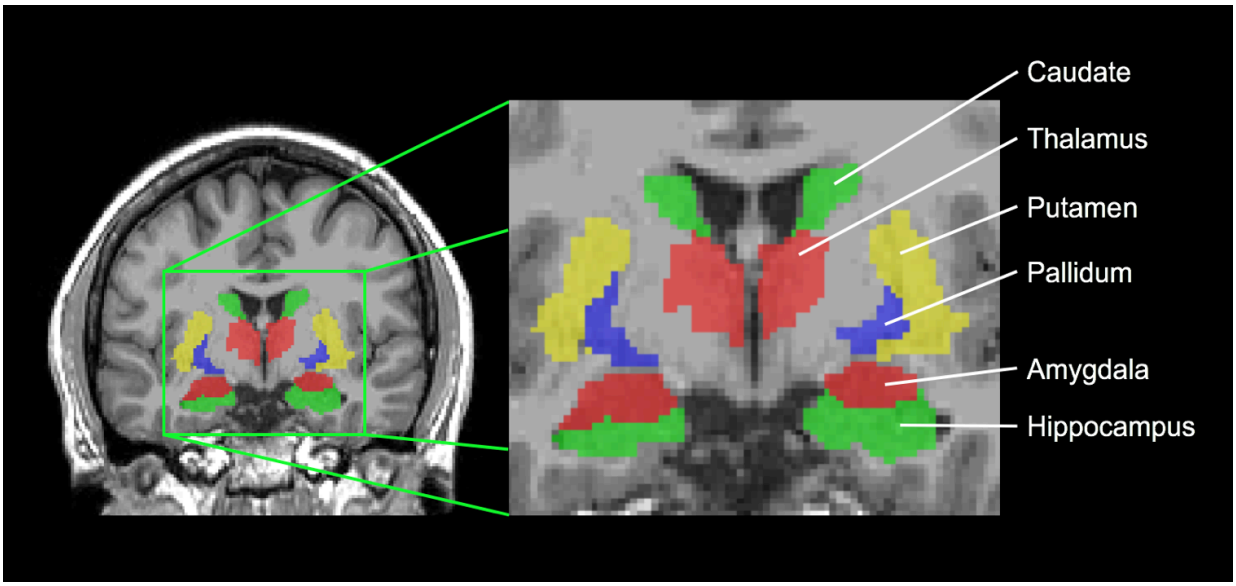


Figure 4-6 FreeSurfer-derived subcortical structures in FslView.

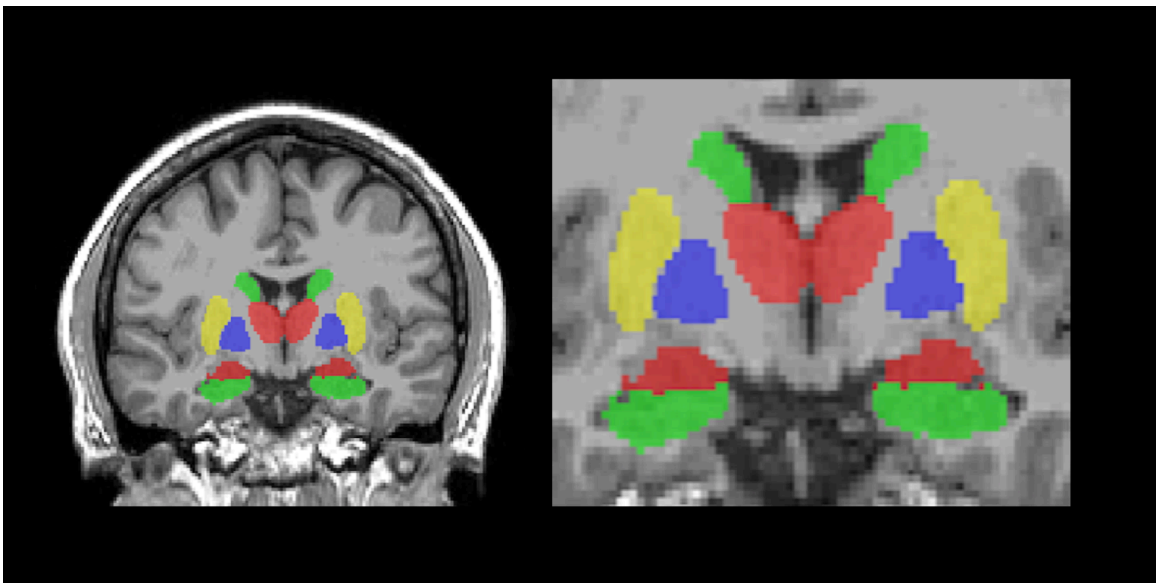


Figure 4-7 FIRST-derived subcortical structures in FslView.

FreeSurfer-derived white matter volume (the white matter 'mask') was corrected so that white matter boundaries were consistent with FIRST-segmented

subcortical structures. This was done by subtracting FreeSurfer's subcortical segmentation from the white matter mask; re-labeling these areas as white matter; and then subtracting FIRST subcortical structures from the modified white matter mask. Finally, manual edits were carried out to correct for any areas mislabeled in this process, for example spuriously labeled white matter at the caudate-cerebrospinal fluid (CSF) boundary. Figures 4-8 and 4-9 demonstrate these steps. FreeSurfer-derived gray matter, and cerebellum gray and white matter volumes were not modified.

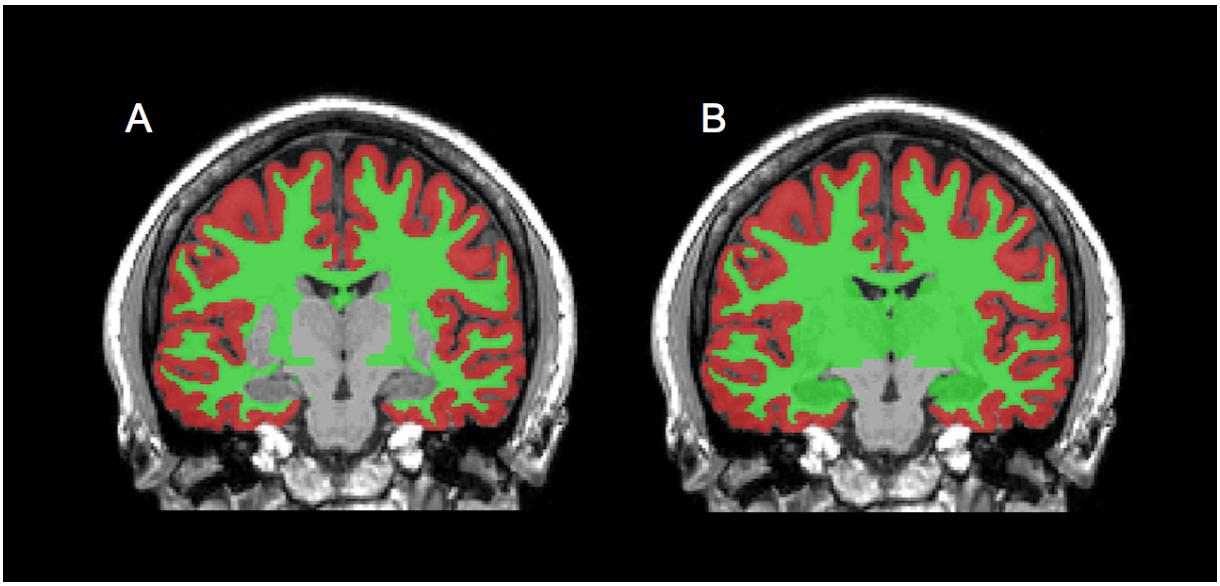


Figure 4-8 FreeSurfer-derived subcortical structures subtracted from the white matter mask (A), and re-labeled as white matter (B).

Included in Figure 4-9 below is an example of the manual correction necessary at the caudate-CSF boundary: the arrows indicate where mislabeled white matter was corrected in the final dataset.

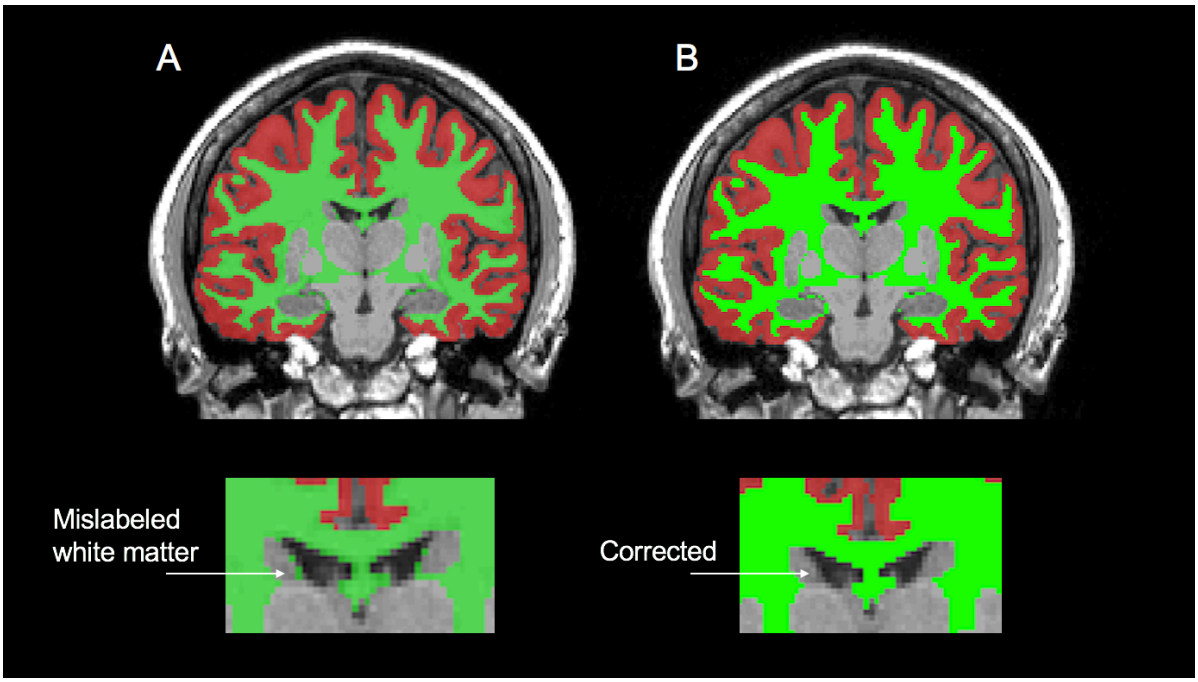


Figure 4-9 FIRST-derived subcortical structures subtracted from the modified white matter mask (A), and the final mask following edits (B).

Following FreeSurfer and FIRST processing, output included 12 brain volume variables: gray matter, white matter, cerebellum cortex, cerebellum white matter, amygdala, hippocampus, caudate, nucleus accumbens, putamen, pallidum, thalamus, and total intracranial volume (TIV). TIV is utilized as a covariate in statistical analyses. Buckner et al. (2004) describe FreeSurfer's approach to estimating TIV; Figure 4-10 below shows the area included in the TIV measure.

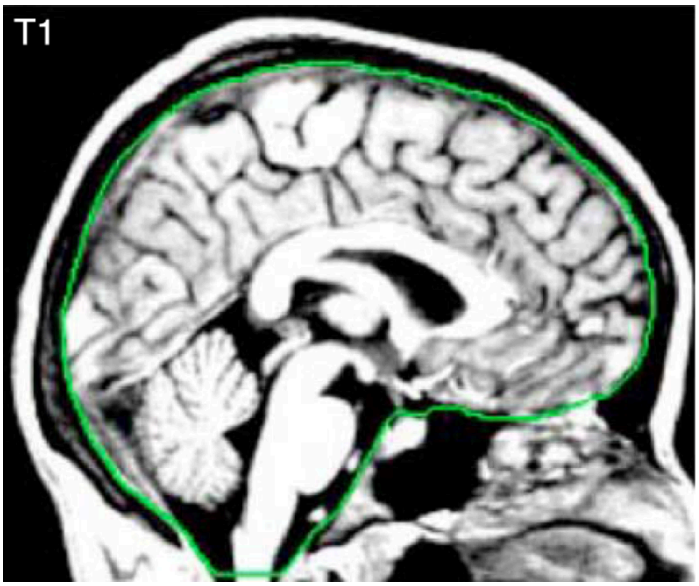


Figure 4-10 Area included in TIV.
Image from Buckner et al., 2004.

I combined caudate, putamen and nucleus accumbens structures to make a striatum volume variable. I combined all brain volumes given by FreeSurfer and FIRST, except TIV, to make a 'composite' brain variable. Composite brain includes the pallidum and thalamus, however these variables are not tested further as discrete variables. Composite brain captures the majority of tissue in the brain, but excludes the CSF. I use the term 'composite' rather than 'total' to reflect the fact that the CSF and additional small components (e.g. substantia nigra, hypothalamus, pituitary gland) are not incorporated. The brain stem is likewise not included.

For use in analyses in Chapters 5 through 9, I thus had 7 brain component volume variables (gray matter, white matter, cerebellum cortex, cerebellum white matter, amygdala, hippocampus, striatum), 1 global brain measure (composite brain) and 1 covariate (TIV).

I describe as ‘gray matter’ what could also be referred to as cortical or cerebral gray matter, the large mass of brain tissue visible as a series of folds when viewing the whole brain. In a slice of brain, ‘white matter’ is that which is seen to underlie the gray matter. For gray and white matter of the cerebellum I refer to ‘cerebellum cortex’ and ‘cerebellum white matter.’ The amygdala, hippocampus and striatum are smaller gray matter volumes located subcortically, thus I refer to these throughout as subcortical structures.

In addition to gray and white matter, which are the two largest tissue components in the brain, I chose to include the amygdala, hippocampus, striatum and cerebellum in analyses following evidence in the literature of their potential relevance in human evolution (e.g. Balsters et al., 2010; Barger et al., 2014; Barton and Venditti, 2014; Raghanti et al., 2016).

Figure 4-11 below is given to clarify from which software package each brain outcome was derived, the components of the striatum variable, and the components which comprise the composite brain variable.

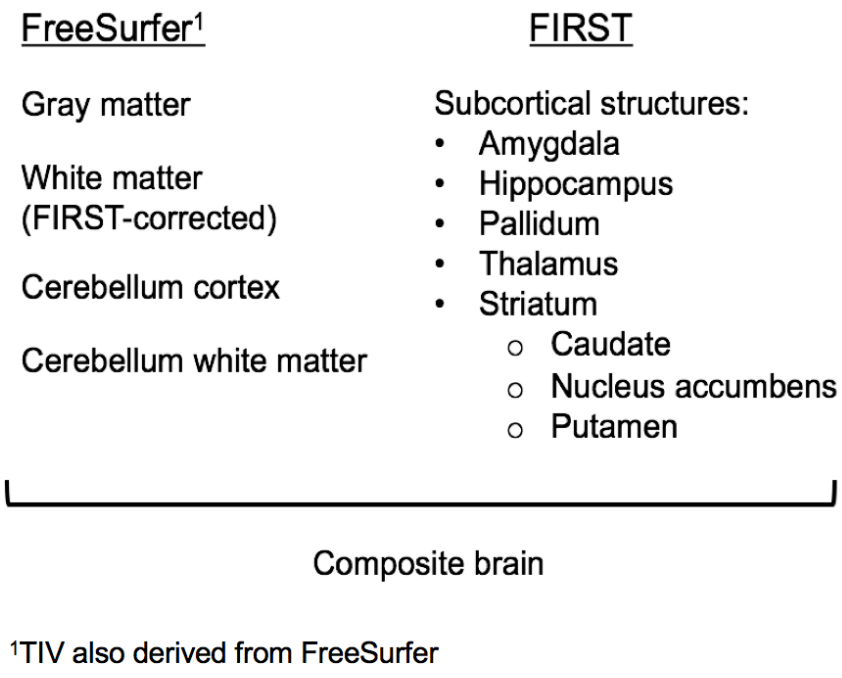


Figure 4-11 Brain volume variables derived from FreeSurfer and FIRST.

4.5.8.4 Body organs – OsiriX

I manually segmented the heart, liver, kidneys and spleen from raw MRI data using the OsiriX viewer for DICOM images (Version 8.5, http://www.osirix-viewer.com; Rosset et al., 2004). I chose to segment the heart, liver and kidneys because they are known to be high-metabolic rate tissues (Elia, 1991; 1992). The spleen is also likely to have a high SMR (Heymsfield et al., 2012a), and these four organs were feasible to extract from the MR images. I created a 'composite organ' variable by summing the four organs. The specific organs predicted by the ETH and TPH to trade off with the brain – the gut and the pancreas – were unfortunately not obtainable due to excessive image artefact in the relevant areas.

As briefly discussed in Chapter 3, Section 3.3.2.1, the manual estimation of organ volumes involves drawing an ROI along the border where the organ can be seen to meet surrounding tissue. Figure 4-12 shows an ROI around the heart.

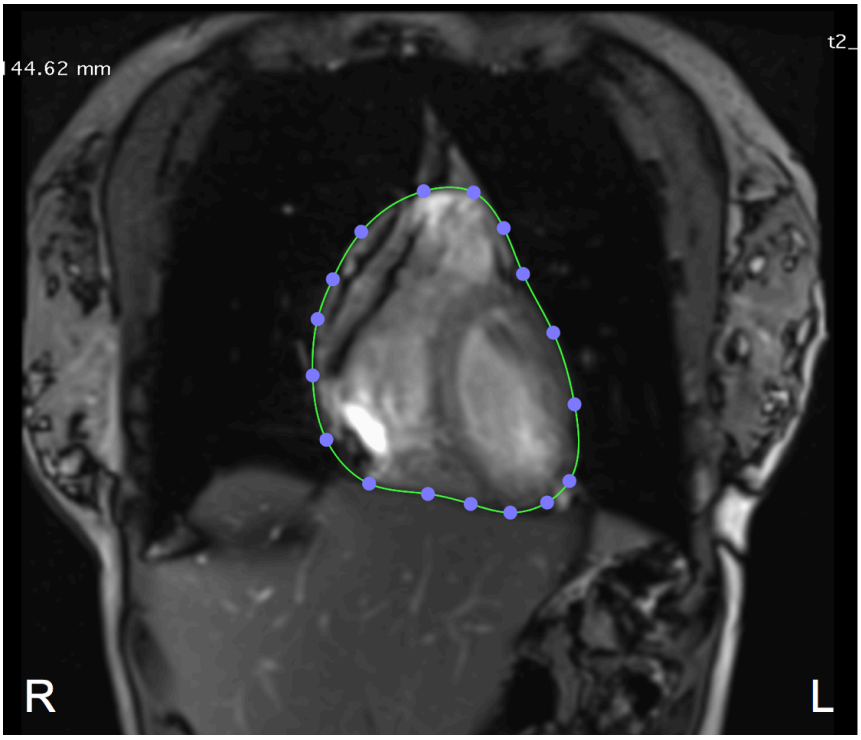


Figure 4-12 ROI around the heart in coronal orientation.

Once again, as first described in Chapter 3, an ROI is drawn on every image in a series of images; each MRI scan produces a variable number of image 'slices'. Area is automatically calculated for each ROI in OsiriX. When ROIs are drawn in all images, the software automatically calculates organ volume by summing the ROIs and multiplying by the slice thickness.

The series containing the image in Figure 4-12 contained 22 images where the heart was visible, thus I drew 21 additional ROIs in order to estimate heart volume for this subject. The liver series for this same subject contained 39 images. There is some variation in the number of image slices in a series owing to body size and

positioning of the subject during scanning. As chest scans were done during breath-hold, they were shorter and had relatively large slice gaps compared to the abdominal scans, which were more comprehensive. Thus, abdominal scan series, from which I measured the kidneys, contained a greater number of images. For example, there were 73 image slices for this subject's right kidney, measured in the axial plane (Figure 4-13).

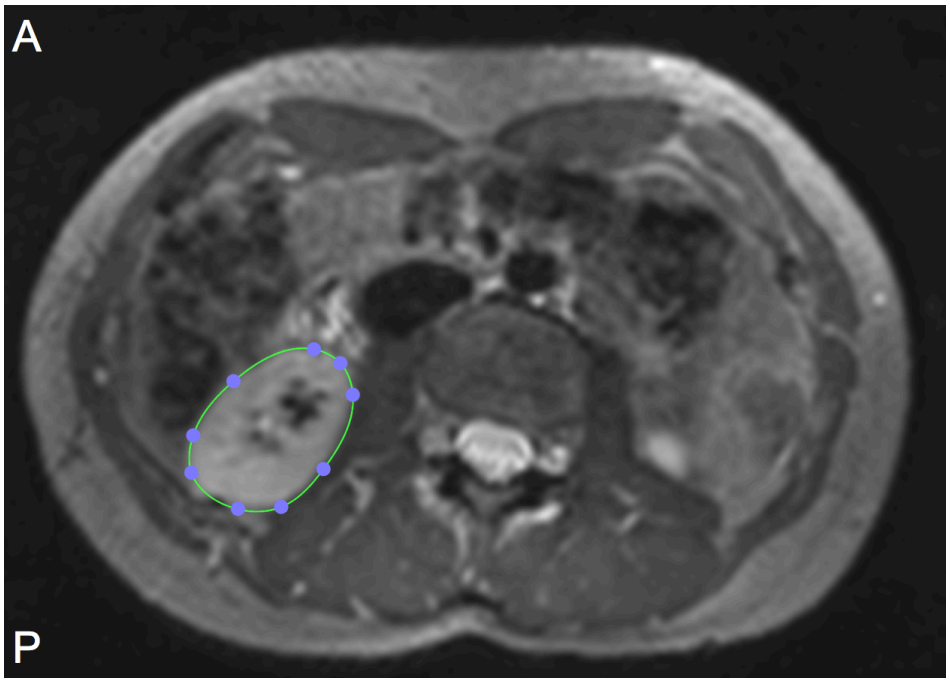


Figure 4-13 ROI around the right kidney in axial orientation.

I measured each organ for each subject twice and took the average as the final data, as the mean of two or more measurements is likely to give a better estimate of the true size of an object than a single measurement (Harris and Smith, 2009). I concluded that the number of images in each series made it less likely for repeat measurements in a single day to introduce bias. For this reason, and with time constraints in mind, I did not consider it a requirement for repeat measures to be performed after a specific length of time (i.e. on separate days, although repeat measures for some subjects ultimately did occur on different days).

The calculated technical error of measurement (TEM), as a percentage (following Perini et al., 2005), was as follows for duplicate measures of each organ: 1.9 for the heart (n = 67), 1.1 for the left kidney (n = 68), 0.7 for the right kidney (n = 68), 0.7 for liver (n = 67), and 1.4 for the spleen (n = 61). I explain below why the sample sizes given here do not match my study sample size of 70.

The kidneys were generally clearest in images, and most easily delineated from surrounding tissue. The greater number of image slices decreased the contribution of measurement error on any given slice to overall measurement error for the particular organ. Additionally, the kidneys were segmented, as shown above, in axial orientation: this decreased the area for each slice, and likewise minimized error if an ROI was poorly-drawn.

The heart and liver were segmented in coronal orientation (e.g. Figure 4-12). Although the border between the heart and surrounding tissue was clear in most images, in others it was difficult to distinguish from surrounding blood vessels, and this may have contributed to poorer repeatability, relative to the other organs. Like the kidneys, spleen ROIs were drawn in axial orientation, however image contrast was generally poorer than that seen for the kidneys. Figure 4-14 below demonstrates an ROI drawn around the spleen (this image series had 48 slices total).

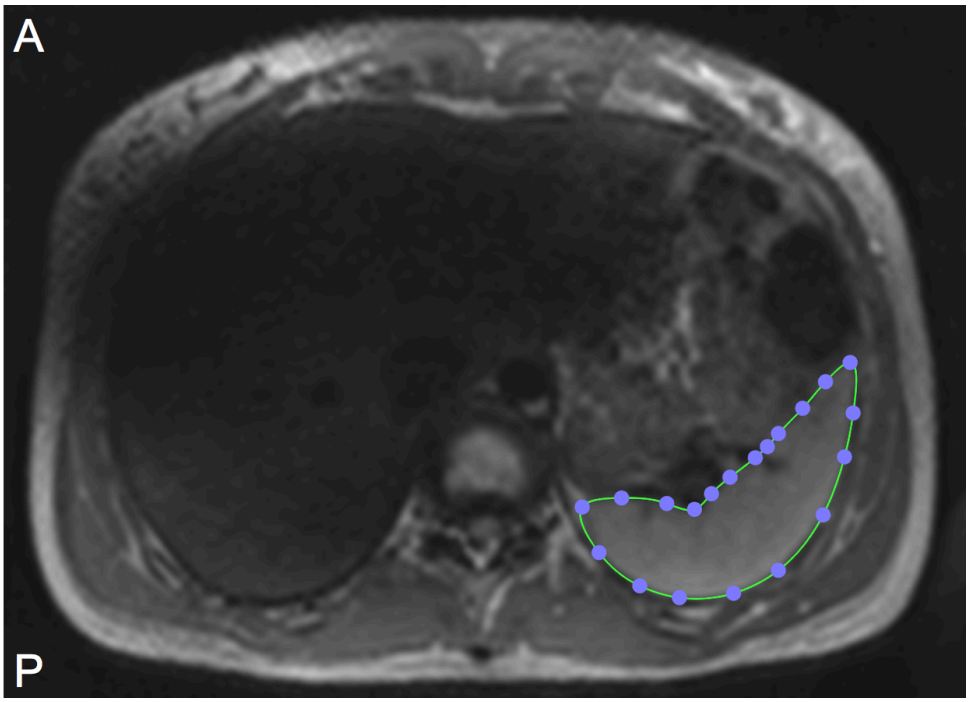


Figure 4-14 ROI around the spleen in axial orientation.

The scan sequences used to image the abdomen (SPACE) and chest (TrueFISP) were described in Section 4.5.8.1. Overall, the heart and liver were segmented from the TrueFISP images, and the kidneys and spleen derived from the SPACE. However, for a small number of subjects, it was necessary to take an alternative approach.

The first three subjects recruited and scanned did not have the TrueFISP, as it was included in the scan protocol following their visits for data collection. Thus, the heart and liver could not be segmented from the TrueFISP for these participants.

The SPACE sequence was included in the scan protocol from the start of data collection for the study. However, for one subject, a scanner fault caused the sequence to fail and the data were not collected. A second subject was unable to limit her body movement sufficiently during the SPACE (a relatively long scan of approximately 20 minutes), and movement artefact precluded segmentation of the

kidneys and spleen. For 7 additional subjects, the spleen was not clearly visible on SPACE images and could not be segmented, although kidney segmentation was unaffected.

My approach was to segment the missing organs from an available alternative. For the liver, I used TIRM (turbo inversion recovery magnitude) sequences; these were shorter sequences done prior to the main protocol sequences to confirm normal anatomy. A sub-sample of 30 subjects was chosen where the liver could be segmented from both TIRM and TrueFISP images (and which attempted to represent the range of liver size in the full sample). Single measures were taken for this exercise. I regressed the TrueFISP values measured in the 30 subjects on TIRM values measured in the same 30 subjects, and used the derived equation to correct the liver values – also measured on TIRM – for the 3 subjects where segmentation from TrueFISP was not possible. Similar prediction equations have been used previously, for example in the recent publication by Devakumar and colleagues (2015).

A similar process was undertaken for the kidneys and spleen, although here TrueFISP, rather than TIRM, images were used as the best available alternative. Data for two out of nine missing spleen volumes could not be derived by the prediction method, as it was not possible to segment the spleen from any of the images obtained for these two subjects.

With respect to the heart, it was visible in TIRM images for two of three subjects missing the TrueFISP sequence. However, as the heart was not visible in TIRM images for the majority of the sample, the process of correction by sub-sample analysis and regression described above could not be carried out. Figure 4-15 is a flow chart demonstrating the approach for obtaining final data for each organ.

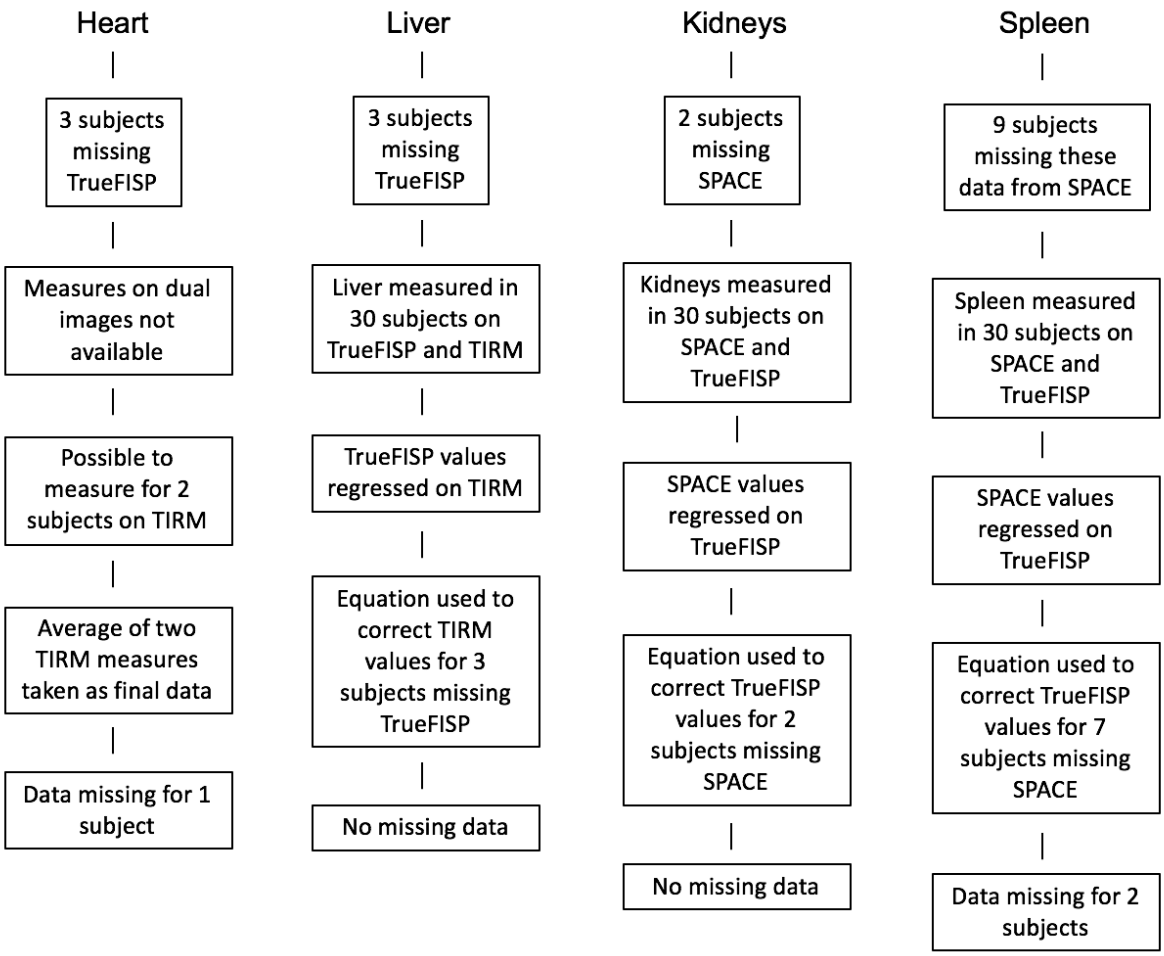


Figure 4-15 Dealing with missing body organ data.

4.6 Potential confounders

This section introduces variables that will be explored as potential confounders. In Chapter 5 I assess their association with study variables to determine whether it is indeed appropriate to include them as confounders in my analyses. If it is determined they should be included, findings related to their impact on results will be discussed where appropriate in subsequent chapters.

4.6.1 Place of birth

As discussed in Chapter 1, regional environmental characteristics are recognized to contribute to geographical variability in body composition. This informed the decision to recruit women of South Asian ancestry only, and avoid extra sources of variation that would be expected in a sample with a wider range of ethnicity. However, my participants were not exclusively born in South Asia (see Chapter 5, Section 5.1), and variation in place of birth may also represent a source of added variation due to differing environmental experience. Place of birth (South Asia, or elsewhere) will thus be investigated as a potentially confounding variable.

4.6.2 Physical activity

Physical activity (PA) may impact on body composition, REE, or relationships therein. To collect information on PA, subjects were asked to fill out a version of the International Physical Activity Questionnaire (IPAQ). "[D]eveloped as an instrument for cross-national monitoring of physical activity and inactivity" (Craig et al., 2003, pg. 1381), the IPAQ has demonstrated reliability and validity across populations (Craig et al., 2003; Rosenberg et al., 2008), although some studies have reported discrepancies between IPAQ and accelerometer data (Boon et al., 2010; Cerin et al., 2016).

There are short and long versions of the IPAQ, and I opted to use the latter for this study (see Appendix). It offers more information than the short version, but in fact is not appreciably longer. Questions cover four domains of activity: work; active transportation; domestic and yard work; and leisure-time. The questionnaire asks individuals to recount PA relevant to these domains in the last 7 days. I followed the guidelines for data processing and analysis of the IPAQ (downloadable from the IPAQ web page, https://sites.google.com/site/theipaq/) to obtain PA in MET (metabolic equivalent) minutes/week. The MET value reflects energy expended during an activity relative to energy expended by the body at rest. PA in kcal/week was derived following the equations outlined in Bushman (2012).

4.6.3 Menstrual cycle

I controlled for known effects of reproduction on body composition by recruiting nulliparous women only. However, it was not feasible in recruitment to control for all aspects of reproductive function which have the potential to impact on body/brain composition or REE. For example, previous research found that REE "cannot be assumed to be 'stable' in all women" across the menstrual cycle (Henry et al., 2003, pg. 817). Additionally, cyclical hormonal changes throughout the menstrual cycle are increasingly recognized to associate with changes in the structure of brain components, including the hippocampus, in rodents and humans (Galea et al., 2008; Lisofsky et al., 2015; Barth et al., 2016). Thus, variation in REE or brain volume measurements could be attributable to variation in the phase of the menstrual cycle at which participants were seen for data collection.

To allow menstrual phase to be treated as a potential confounder, I asked participants to report in the questionnaire they returned the date on which they had most recently begun menses, and whether they generally experienced a regular menstrual cycle. I used this information to estimate on which day of a participant's cycle their data collection visit fell, similar to the method of Henry et al. (2003).

4.7 Statistics

4.7.1 Assessing normality

Variables were assessed for normality using Shapiro-Wilk tests and histograms. All brain variables were normally distributed, as was FFM; FM demonstrated a slight positive (right) skew, but Shapiro-Wilk was not significant. Shapiro-Wilk was significant for REE, however this was due to the influence of a single outlier. Otherwise, the data demonstrated a normal distribution.

Of the organs, liver volume demonstrated normality; the heart, kidneys and spleen were right-skewed. This caused the composite organ variable to skew slightly positive as well, although Shapiro-Wilk was only borderline significant. As with

REE, one or two outliers were found to cause the skew, and with their removal the distributions were normal. The spleen, however, remained non-normal following the removal of two outliers. Any outliers removed to explore normality were subsequently returned to the dataset.

Following natural log-transformation all variables demonstrated normality. Log-transformed variables are utilized in Chapter 5, where I initially explore relationships amongst organs and tissues, and in Chapters 7, 8 and 9, where I test for trade-offs, and for evidence of developmental mediation on observed trade-offs using markers of early-life growth.

4.7.2 Testing study hypotheses

I utilize Pearson correlation, regression and graphical analyses to describe the data and test my study hypotheses. There is some variation in the use of these statistical methods in Chapters 5-9, thus I include in each of these chapters a section which describes the statistical methods for the current chapter analysis.

All statistical analyses were carried out using the R language for statistical computing in RStudio (Version 1.0.136) with two-tailed tests for significance at an alpha level of 0.05.

4.7.3 Potential confounding variables

4.7.3.1 Place of birth

A binary birthplace variable was generated to reflect those subjects who were born in South Asia (n = 33), and those born elsewhere (n = 37).

4.7.3.2 Physical activity

PA in kcal/week was calculated as described in Section 4.6.2 for 69 subjects; one subject did not return the IPAQ. The plotted data revealed an extreme outlier,

shown in Figure 4-16. I removed this individual's data, and derived a variable I called PA1 for the remaining 68 individuals.

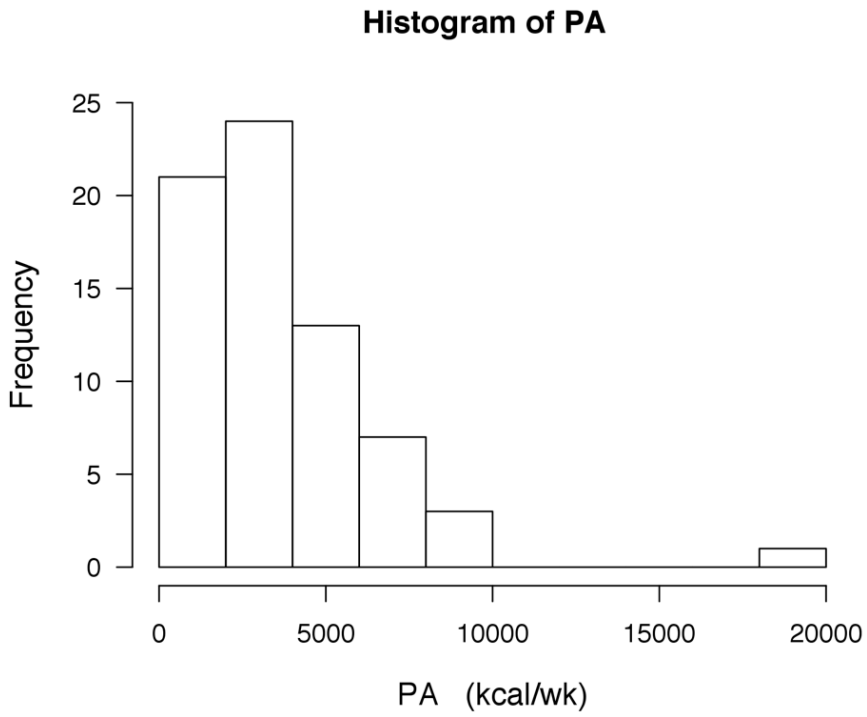


Figure 4-16 Physical activity data outlier.

Although they did not appear as obvious outliers, a further 10 subjects reported levels of physical activity which seemed unrealistic, given additional information I collected. For example, some participants who reported being undergraduate or graduate students also reported a considerable amount of moderate or vigorous physical activity associated with the IPAQ's work domain, which seemed inconsistent with their student status.

According to the instructions given to participants on the questionnaire, "moderate activities refer to activities that take moderate physical effort and make you breathe

somewhat harder than normal," whilst "vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal."

I made a second variable called PA2 which does not include data from these 10 individuals; I tested both PA1 and PA2 for potential confounding.

4.7.3.3 Menstrual cycle

I calculated three different variables to assess the potential effects of variation in menstrual cycle stage at the time of data collection. Each variable is comprised of a single number for each participant: estimated day-of-cycle at data collection visit. I obtained the number by counting days between the participant's visit and their reported first day of last menstruation. The first variable, MC1, included data from subjects who, based on this reporting, appeared to experience a normative cycle range of 21-35 days (n = 48).

In their 2003 paper on metabolism and the menstrual cycle, Henry and colleagues reported for their subjects a wider range of cycle length, from 24-41 days. The variable MC2 includes those participants in my own sample whose reported data puts them within the range 21-41 days, which increased the sample size to 54. I also calculated a variable, MC3, which extended the upper range value to 45 days, and the sample size increased by 4 to 58. Data were missing for 12 subjects.

4.8 Summary of study methods, techniques and outcomes

Similar to Table 3.1 at the end of Chapter 3, I offer a table below summarizing measurement methods, instruments and outcomes. I have added more detail regarding the specific organ and brain components I described in this chapter. I have also added birth weight and gestational age.

Table 4.1 Final summary of measurement methods and outcomes

Method	***Instrument***	***Outcome(s)***
Anthropometry	Stadiometer, calipers	Height, tibia length
MRI (head)	3T scanner	Individual gray matter, white matter, cerebellum cortex, cerebellum white matter, amygdala, hippocampus, striatum volumes Composite brain volume
MRI (body)	3T scanner	Individual heart, liver, kidney and spleen volumes Composite organ volume
4C model	BodPod, scale, Lunar Prodigy scanner, $^{2}H_{2}O$ dose and saliva samples	FM, FFM
DXA	Lunar Prodigy scanner	SM
Indirect calorimetry	Deltatrac II indirect calorimeter	Resting energy expenditure
Recruitment screening and collection of subject background information	Questionnaire, and UK-WHO reference birth centiles to derive SDS (see Chapter 9)	Birth weight and gestational age

5 Description of the sample

In this chapter I describe my study sample, which I recruited and measured as detailed in Chapter 4. I begin by providing frequency statistics in Section 5.1 for ethnicity, place of birth and profession, as reported by participants. Section 5.2 details statistical methodology. My aim in Sections 5.3 and 5.4 is to describe the average body and brain composition of participants, and the range of variability in outcomes. Before testing tissue trade-offs in Chapters 7 and 8, I show how components of the body and brain relate to one another and with weight and height. This allows me to test part (iv) of my second hypothesis, set out in Chapter 2, which predicts positive associations amongst body components, and also amongst brain components, but predicts that potentially fewer positive relationships will be found between body and brain components. Section 5.5 examines whether place of birth, physical activity and menstrual cycle variables should be included in further analyses as potential confounders. Finally, I discuss the chapter's findings in Section 5.6.

5.1 Ethnic background, place of birth and profession

As described in the previous chapter, a key criterion of inclusion for the present study was South Asian ancestry. Table 5.1 shows that the majority of the sample (n = 70) reported Indian ethnicity. Mixed South Asian ancestry identifies those subjects who reported an ethnic background characterized by a mix of two or more of the four target ethnicities. For example, a subject might have reported one set of grandparents from India and the other set from Pakistan, or a mix of Pakistani, Indian and Bangladeshi ancestry among her grandparents.

As seen in the final row of Table 5.1 below, one subject reported that her parents and grandparents were born on Mauritius, an island nation located in the Indian Ocean. This subject reported that her ancestors emigrated to Mauritius from the Indian subcontinent, and was considered eligible for the study.

Table 5.1 Reported ethnic ancestry of the sample

Ethnic ancestry	***n***
Indian	36
Pakistani	8
Bangladeshi	8
Sri Lankan	8
Mixed South Asian ancestry	9
Mauritian	1

Table 5.2 shows that roughly half of the sample was born in one of the countries listed in Table 5.1. Table 5.3 gives information on place of birth for the rest of the sample.

Table 5.2 Frequency statistics for place of birth

	n	**South Asia**	**Elsewhere**
Place of birth	70	33	37

Table 5.3 Place of birth for subjects born outside South Asia

Place of birth	***n***
United Kingdom	25
United States	5
Canada	3
Australia	1
Mexico	1
Saudi Arabia	1
Kenya	1

For those born outside the UK, age of emigration to the UK varied, with a mean age of 18.7 years (range = 2-28 years). Out of this group, the majority moved to London in their early to mid-twenties to attend undergraduate or graduate-level university programs. In the full sample, the vast majority were students, as shown in Table 5.4.

Table 5.4 Frequency of reported professions

Profession	***n***
Student	59
Researcher	2
Teacher	1
Communications professional	2
Management accountant	1
Doctor	2
Physiotherapist	1
Banker	1
Pharmacist	1

5.2 Statistical methods

For statistical analyses in this chapter, all brain and body composition variables were log-transformed to capture their allometric relationships. Pearson correlation coefficients were calculated for body composition and brain variables with height or weight. Correlations were also calculated among body and brain components, and the data were plotted to visualize relationships. In Section 5.5, independent sample t-tests and Pearson correlations were utilized to assess potential confounders.

5.3 Composite brain and body composition variables

This section begins to explore correlations among my main study variables, which were summarized in Table 4.1 at the end of Chapter 4. I focus on tissues and organs of the body and the composite brain variable, aiming to elucidate their relationships with one another, and with weight and height. Brain component volumes are examined in more detail in Section 5.4. First, Table 5.5 contains descriptive statistics for age, weight, height and BMI in the sample. As noted in Chapter 4, BMI in the range 17-28 kg/m^2 was a criterion of inclusion, however two subjects inaccurately estimated their height and weight in the recruitment interview; therefore, my final sample range extends from 17-30 kg/m^2.

Table 5.5 Characteristics of 70 female subjects

	Mean±SD	Range
Age, yr	24±2.4	20-28
Weight, kg	57.8±9.3	40.7-81.1
Height, cm	161.2±6.6	147.8-177.3
BMI, kg/m^2	22.3±3.5	17.2-30.3

Figure 5-1 below shows subjects' weight plotted against their height on a log-log axis. Weight and height are positively correlated (r = 0.34, p = 0.005). The plot demonstrates the range of variability in body size in the sample.

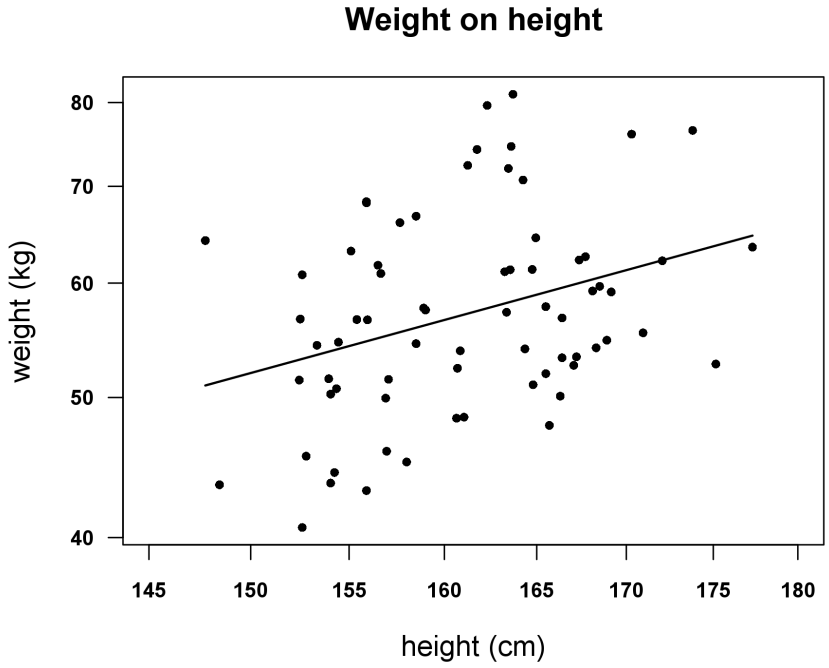


Figure 5-1 Weight plotted against height for 70 subjects.
$r^2 = 0.12$, $p = 0.005$

Table 5.6 contains descriptive statistics for FM, FFM, SM, body organs, composite organ, and composite brain. With regard to the body variables, as described in Chapter 3, skeletal muscle and organs are considered components of FFM, as they are largely fat-free tissues. However, as described in Chapters 3 and 4, I obtained these outcomes using independent methods: FFM by the 4C model, SM by DXA, and body organs by MRI.

Coefficients of variation (CV) are included in Table 5.6 to show the relative variability of the outcomes. The CV is highest for FM and spleen volume, and lower for FFM. CVs for the heart, liver and kidney are virtually identical. ('Kidney' is data for the right and left kidney, combined in a single variable.) Compared to the body outcomes, the CV for the brain is the lowest at 7.5%.

Table 5.6 Raw values and CV percentages for FM, FFM, SM, body organs and composite brain

Body/brain outcomes	*n*	Mean±SD	Range	CV %
FM, kg	70	20.3±6.7	8.3-40.1	32.9
FFM, kg	70	37.6±4.3	28.4-48.8	11.4
SM, kg	70	15.3±2.2	10.8-20.2	14.2
Organ volumes, cm^3				
Heart	69	499±89	330-780	17.8
Liver	70	1140±202	722-1599	17.7
Kidney	70	277±48	197-453	17.5
Spleen	68	132±46	73-310	34.9
Composite organ	67	2055±327	1410-2811	15.9
Composite brain, cm^3	70	1041±78	851-1210	7.5

In order to shed light on whether bigger individuals in the sample tend to have bigger tissues, Table 5.7 shows correlations of the variables in Table 5.6 with weight and height.

Table 5.7 Pearson correlation coefficients for weight and height with FM, FFM, SM, body organs and composite brain

Body/brain outcomes	Weight r, *p*	Height r, *p*
FM, kg	0.88, <0.001	0.12, 0.33
FFM, kg	0.76, <0.001	0.54, <0.001
SM, kg	0.73, <0.001	0.49, <0.001
Organ volumes, cm^3		
Heart	0.63, <0.001	0.50, <0.001
Liver	0.63, <0.001	0.46, <0.001
Kidney	0.58, <0.001	0.42, <0.001
Spleen	0.38, 0.001	0.31, 0.009
Composite organ	0.70, <0.001	0.53, <0.001
Composite brain, cm^3	0.04, 0.72	0.41, <0.001

All body components are more strongly related to weight than they are to height, demonstrated by the relative size of the correlation coefficients. In contrast, composite brain is related to height, but not weight. Of the body variables, the only non-significant correlation is that between FM and height, although FM is strongly correlated with weight. With respect to the organs specifically, heart and liver demonstrate the largest coefficients with both weight and height, while coefficients for the spleen are smallest.

Overall, the data suggest that individuals who weigh more have bigger organs and larger body tissues, but brain size does not track weight in the same way. Individuals who are taller on average have larger organs and body tissues, and larger brain size, although being taller is not associated with having more fat mass.

The following figures demonstrate these relationships visually, with FM, FFM, SM, organs, and composite brain plotted against weight and height.

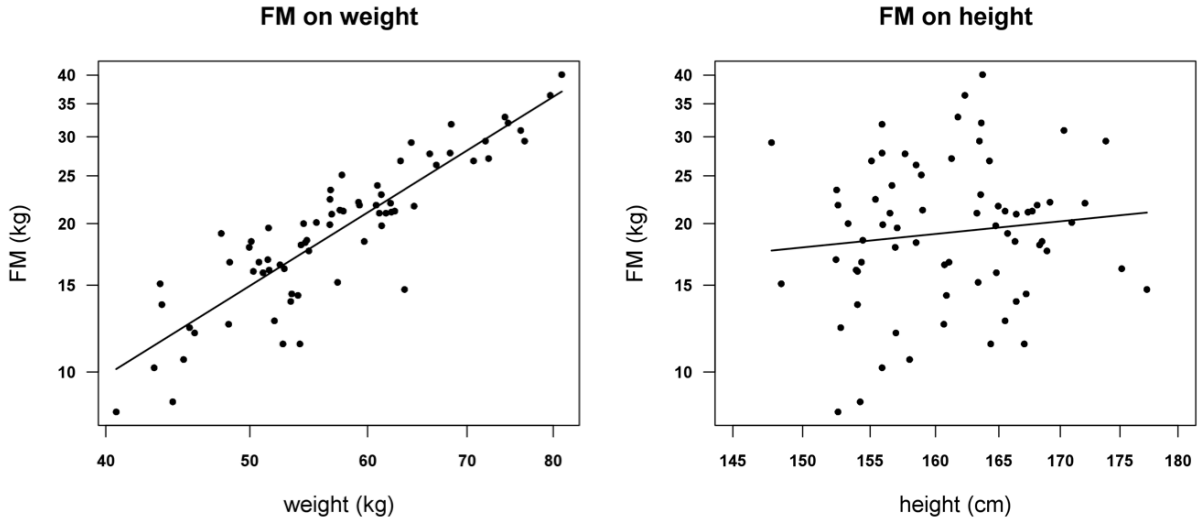


Figure 5-2 FM against weight and height (n = 70).
With weight ($r^2 = 0.77$, $p < 0.001$), with height ($r^2 = 0.01$, $p = 0.33$).

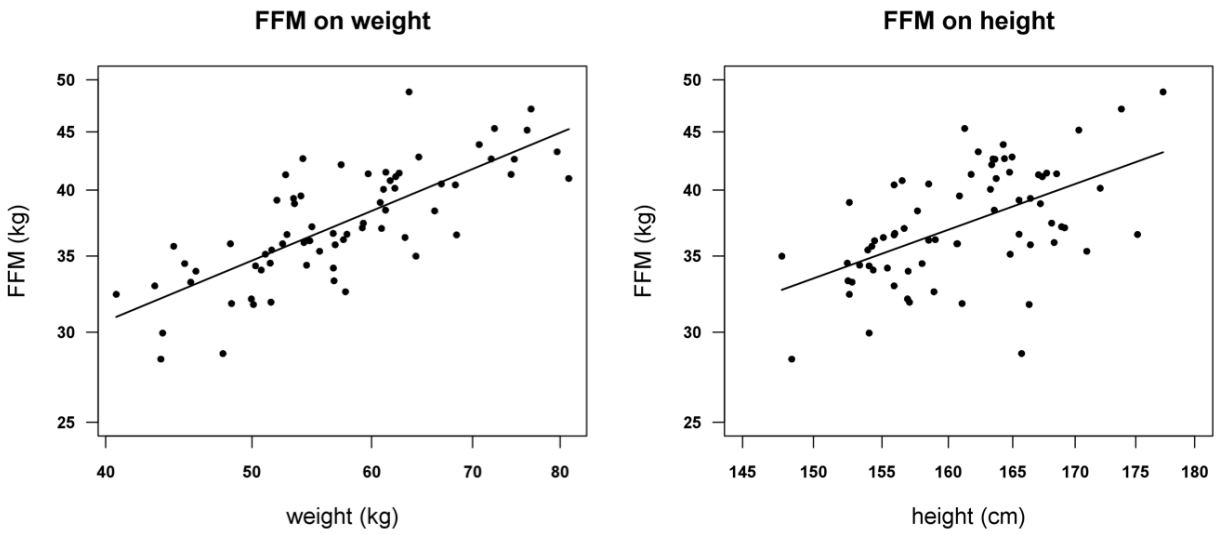


Figure 5-3 FFM against weight and height (n = 70).
With weight ($r^2 = 0.58$, $p < 0.001$), with height ($r^2 = 0.29$, $p < 0.001$).

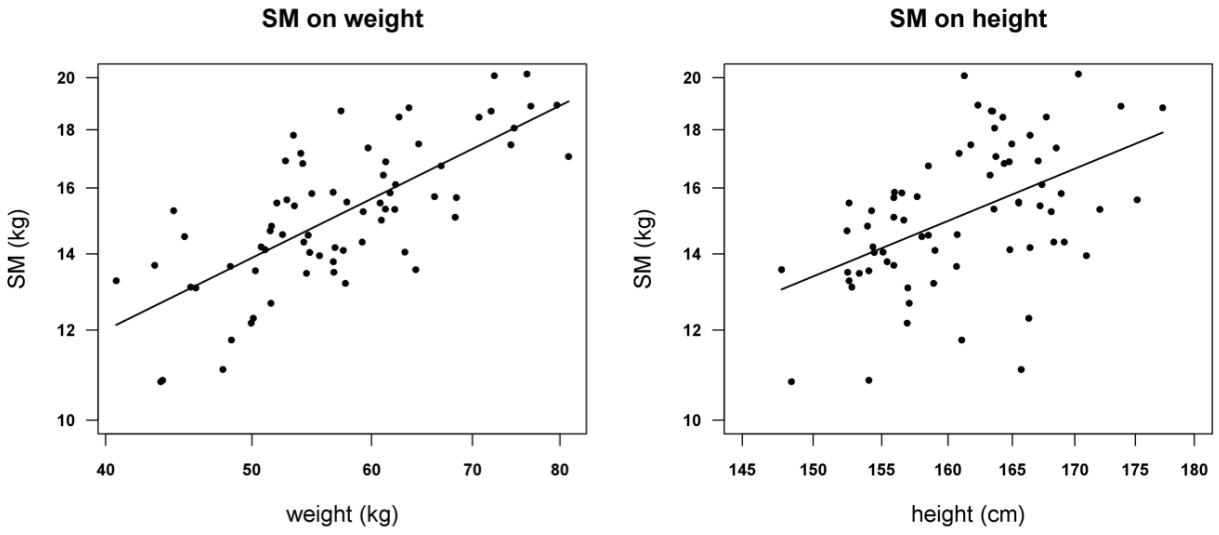


Figure 5-4 SM against weight and height (n = 70).
With weight ($r^2 = 0.53$, $p < 0.001$), with height ($r^2 = 0.24$, $p < 0.001$).

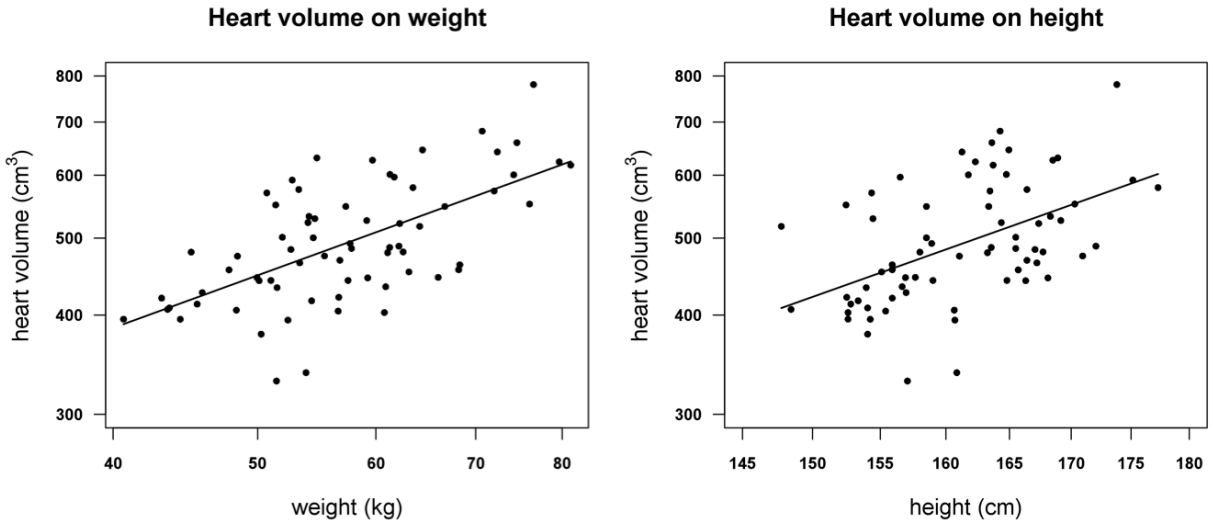


Figure 5-5 Heart volume against weight and height (n = 69).
With weight ($r^2 = 0.40$, $p < 0.001$), with height ($r^2 = 0.25$, $p < 0.001$).

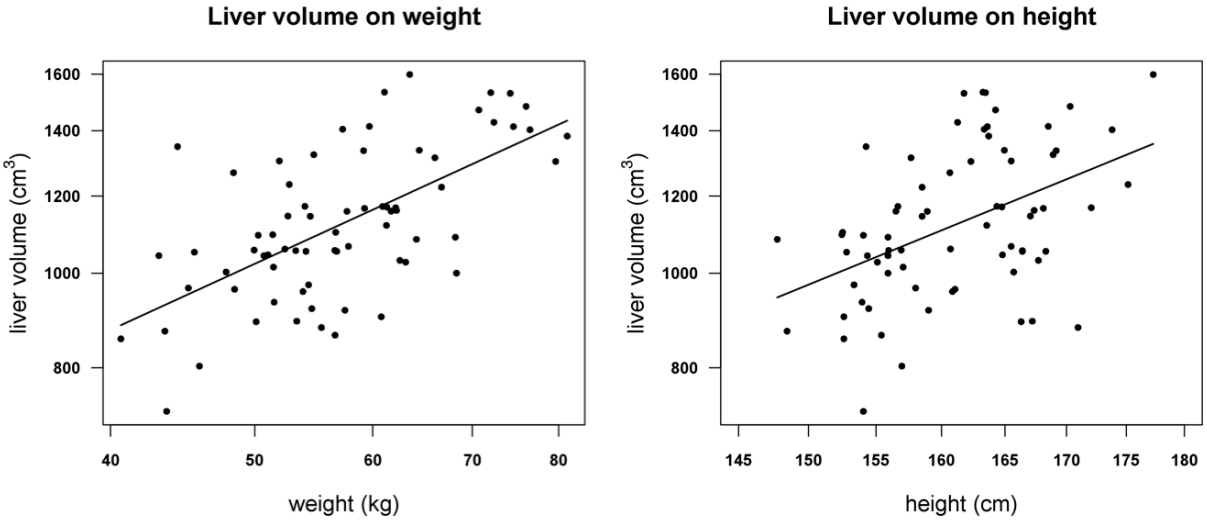


Figure 5-6 Liver volume against weight and height (n = 70).
With weight ($r^2 = 0.40$, $p < 0.001$), with height ($r^2 = 0.21$, $p < 0.001$).

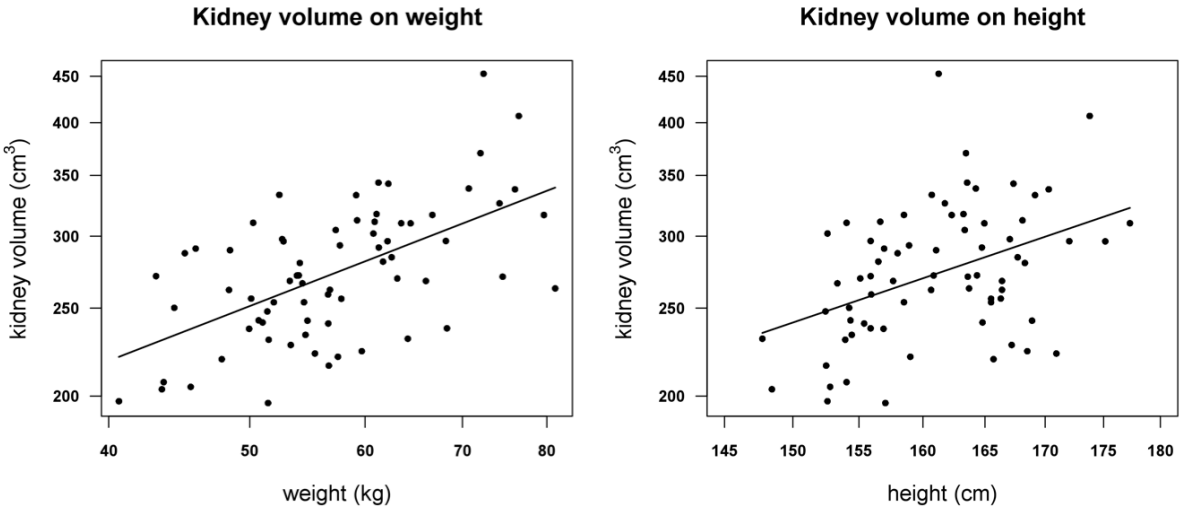


Figure 5-7 Kidney volume against weight and height (n = 70).
With weight ($r^2 = 0.34$, $p < 0.001$), with height ($r^2 = 0.18$, $p < 0.001$).

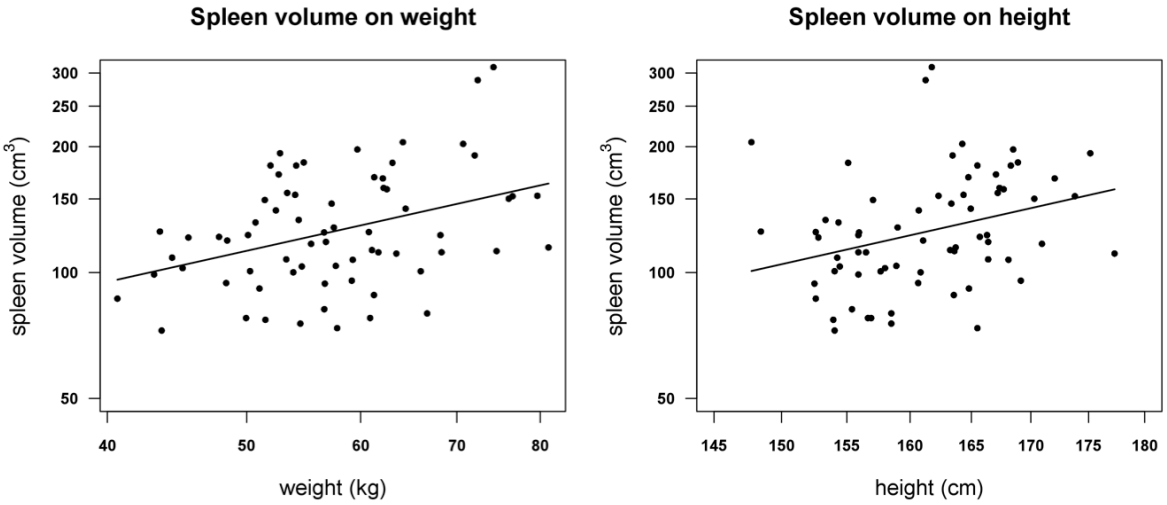


Figure 5-8 Spleen volume against weight and height (n = 68).
With weight ($r^2 = 0.14$, $p = 0.001$), with height ($r^2 = 0.10$, $p = 0.009$).

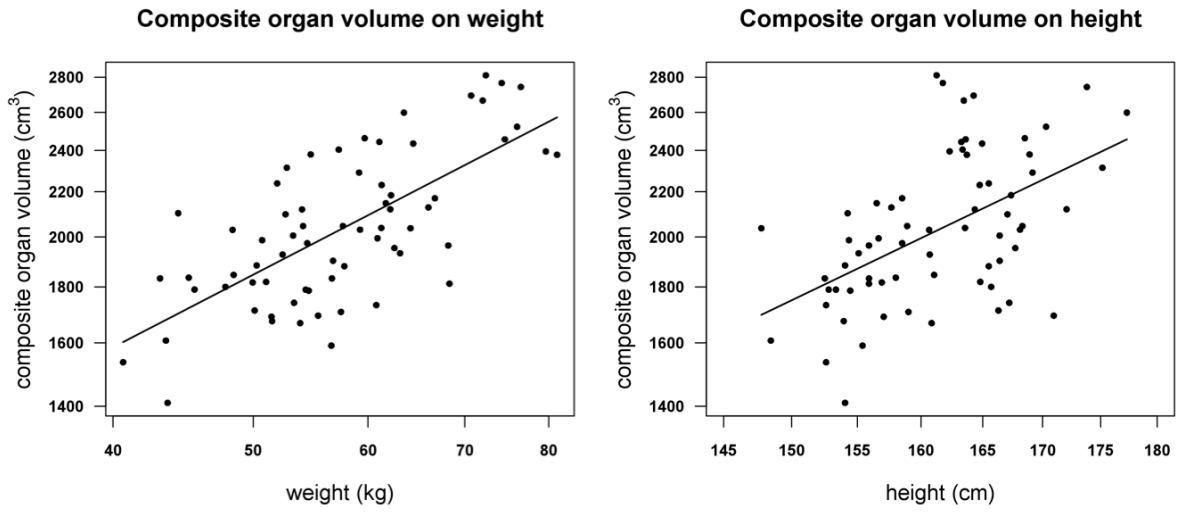


Figure 5-9 Composite organ against weight and height (n = 67).
With weight ($r^2 = 0.49$, $p < 0.001$), with height ($r^2 = 0.28$, $p < 0.001$).

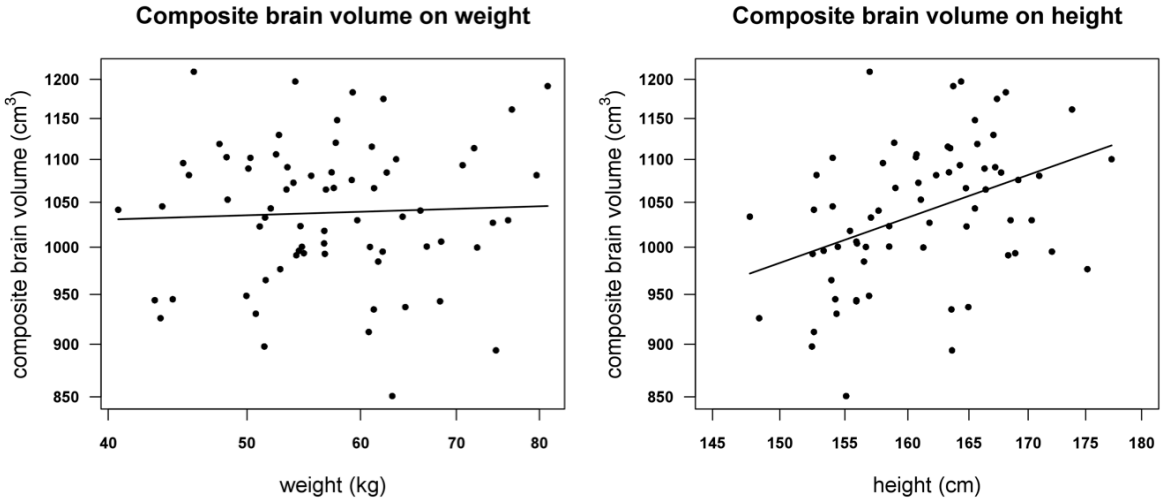


Figure 5-10 Composite brain against weight and height (n = 70).
With weight ($r^2 = 0.002$, $p = 0.72$), with height ($r^2 = 0.17$, $p < 0.001$).

To examine how the components plotted against weight and height above relate to one another, Table 5.8 gives a matrix of correlations.

Table 5.8 Matrix of Pearson correlation coefficients among FM, FFM, SM, body organs and composite brain

	FM	FFM	SM	Heart	Liver	Kidney	Spleen
FFM	0.38[2]						
SM	0.36[2]	0.95[1]					
Heart	0.44[1]	0.66[1]	0.65[1]				
Liver	0.38[2]	0.71[1]	0.72[1]	0.64[1]			
Kidney	0.37[2]	0.67[1]	0.63[1]	0.48[1]	0.58[1]		
Spleen	0.24	0.43[1]	0.42[1]	0.48[1]	0.41[1]	0.34[2]	
Brain	-0.07	0.16	0.13	0.03	0.09	0.19	0.04

[1]$p < 0.001$, [2]$p < 0.01$; FM, FFM and SM are in kg, organs and brain are in cm^3

As SM accounts for a large proportion of FFM, these variables are predictably strongly correlated, although SM also correlates well with body organs, which in turn scale to a variable degree with FFM. The relationship between FFM or SM with liver volume is strongest among the organs, although coefficients for the heart and kidney with FFM or SM are not markedly smaller. The composite brain variable, in contrast, is not related to FM, FFM, SM, body organs, or composite organ volume (r = 0.15, $p = 0.22$ for the latter).

All organs are associated with one another, with the strongest relationships seen between the heart and liver, and the liver and kidneys. Among the organs, the correlation between the kidneys and spleen is weakest. The composite organ variable is strongly correlated with FFM (r = 0.79, $p < 0.001$) and SM (r = 0.78, $p < 0.001$), and significantly but more weakly correlated with FM (r = 0.43, $p < 0.001$) (results not shown in the table).

FM is significantly correlated with most fat-free tissue variables. The relationships are generally weaker than those found among the fat-free components, however there is some overlap in the size of the coefficients. FM's strongest relationship is with the heart, whilst the FM-spleen correlation is borderline significant (p = 0.05).

These findings demonstrate that a majority of the main study variables correlate positively with one another. The data cannot indicate whether FFM components like the organs and SM grow in concert with one another per se, however if one component is relatively large in adulthood, it appears the other has some tendency, varying between outcomes, to be relatively large as well. Made up largely of fat-free tissues, the brain may also be considered an FFM component: it is comprised mainly of water (~78%), whilst proteins are 8% of its weight (McIlwain and Bachelard, 1985). The brain does not, however, fit the pattern of close relationships seen for FFM tissues of the body.

Plots are given below of composite brain against FFM, SM, composite organ, and liver volume. As shown, these outcomes are not significantly correlated, however the relationships are relevant to the trade-offs discussed in Chapter 7.

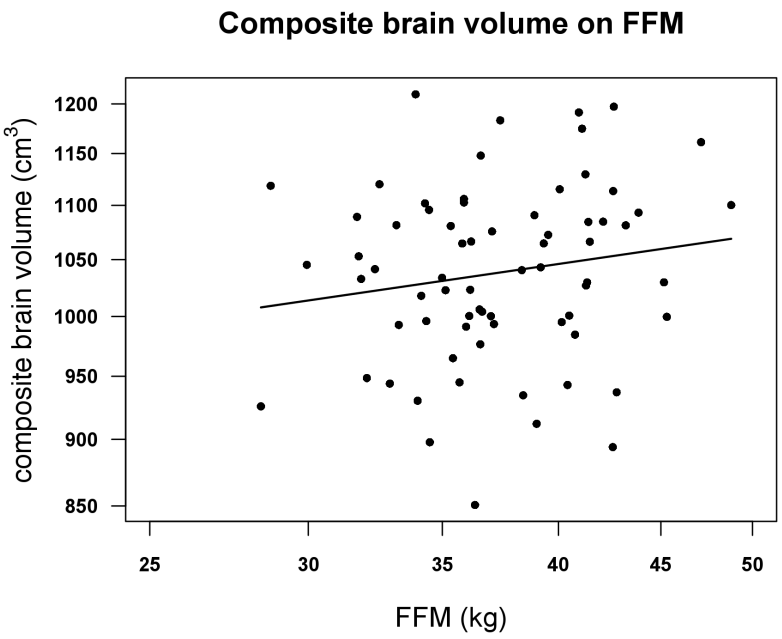


Figure 5-11 Composite brain volume against FFM.
$r^2 = 0.03$, $p = 0.18$; n = 70

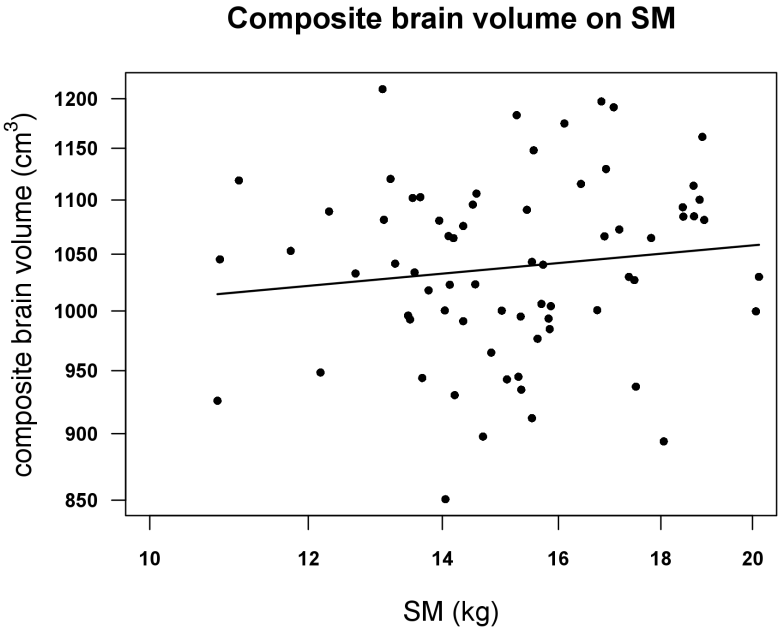


Figure 5-12 Composite brain volume against SM.
$r^2 = 0.02$, $p = 0.29$; n = 70

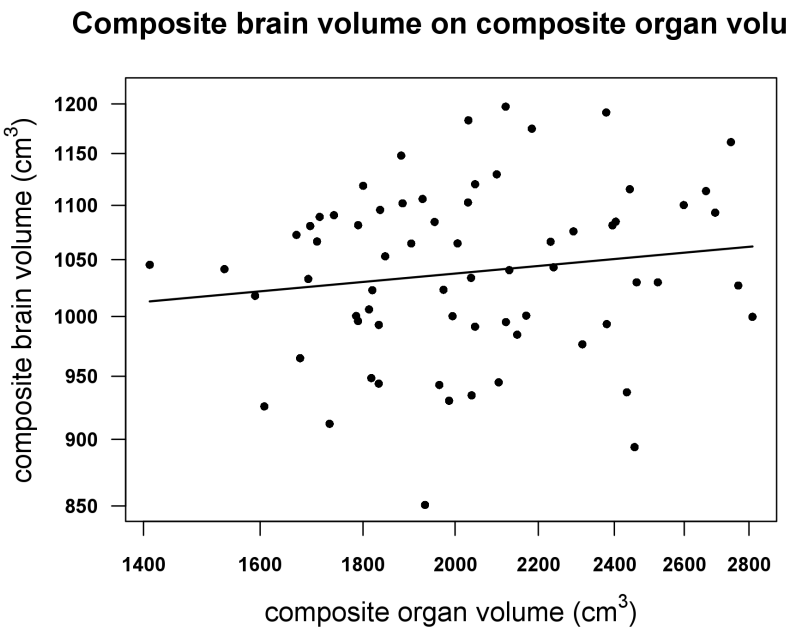


Figure 5-13 Composite brain against composite organ.
$r^2 = 0.02$, $p = 0.22$; n = 67

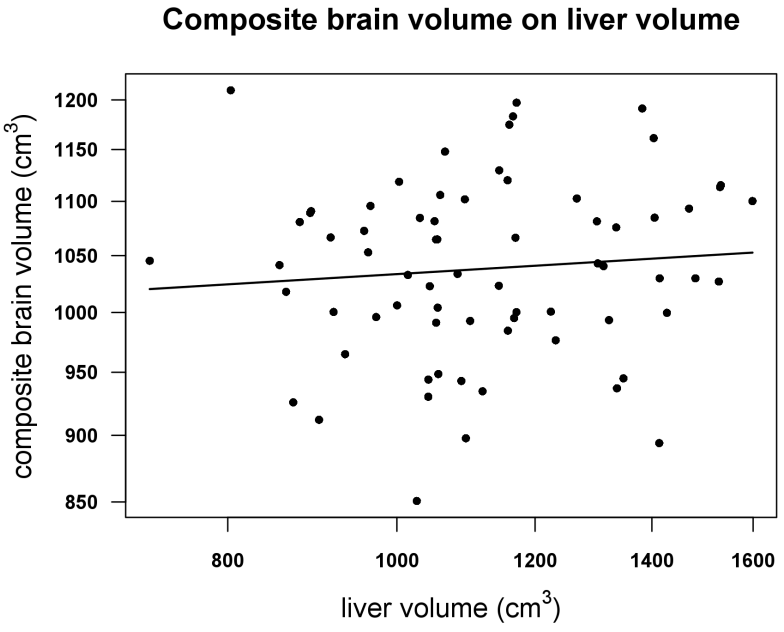


Figure 5-14 Composite brain volume against liver volume.
$r^2 = 0.008$, $p = 0.45$; n = 70

5.4 Brain component volume outcomes

This section looks in more detail at specific components of the brain, to ascertain how they relate to weight, height, body tissues, and one another. In Chapter 7 I test whether, beyond composite brain, these specific brain volumes demonstrate trade-offs with body tissues.

Table 5.9 first gives descriptive statistics for each component, and a measure of their variability, indicated by the CVs. White matter brain volumes appear to be more variable than their gray matter counterparts. The amygdala, the smallest of the subcortical structures, demonstrates a relatively large CV in comparison to the hippocampus and striatum. For all variables, n = 70.

Table 5.9 Raw values and coefficients of variation for brain volume measures

Brain components, cm^3	Mean±SD	Range	CV %
Gray matter	458±35	384-535	7.7
White matter	410±43	299-509	10.4
Cerebellum	127±11	102-154	8.7
Cerebellum cortex	96.4±8.4	78.5-117.7	8.7
Cerebellum white matter	31.0±4.1	21.9-42.1	13.1
Subcortical structures			
Amygdala	2.5±0.3	1.8-3.2	11.7
Hippocampus	7.3±0.5	5.8-9.1	7.3
Striatum	18.0±1.5	15.4-21.6	8.2

Table 5.10 below shows correlations of brain component volumes with weight and height. Consistent with the relationship seen for composite brain above, three brain components correlate with height. The larger coefficients are found for gray and white matter, although the coefficient for cerebellum white matter is similar in size. Cerebellum white matter was the only component also related to weight.

Table 5.10 Pearson correlation coefficients for brain component volume measures with weight and height

Brain components, cm^3	Weight r, *p*	Height r, *p*
Gray matter	-0.05, 0.70	0.34, 0.004
White matter	0.08, 0.52	0.39, <0.001
Cerebellum cortex	0.06, 0.64	0.21, 0.08
Cerebellum white matter	0.26, 0.03	0.30, 0.01
Amygdala	0.14, 0.24	0.09, 0.47
Hippocampus	-0.05, 0.66	0.09, 0.44
Striatum	0.02, 0.88	0.20, 0.10

Plots are given below of cerebellum white matter on weight, and of gray matter, white matter, cerebellum cortex, and cerebellum white matter on height. I do not show plots of the remaining relationships, which are highly non-significant.

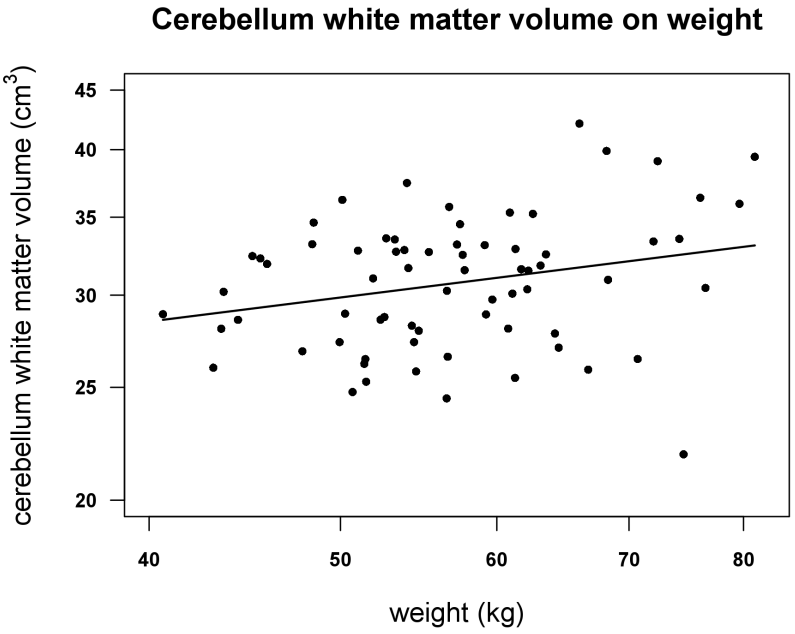


Figure 5-15 Cerebellum white matter against weight.
$r^2 = 0.07$, $p = 0.03$; n = 70

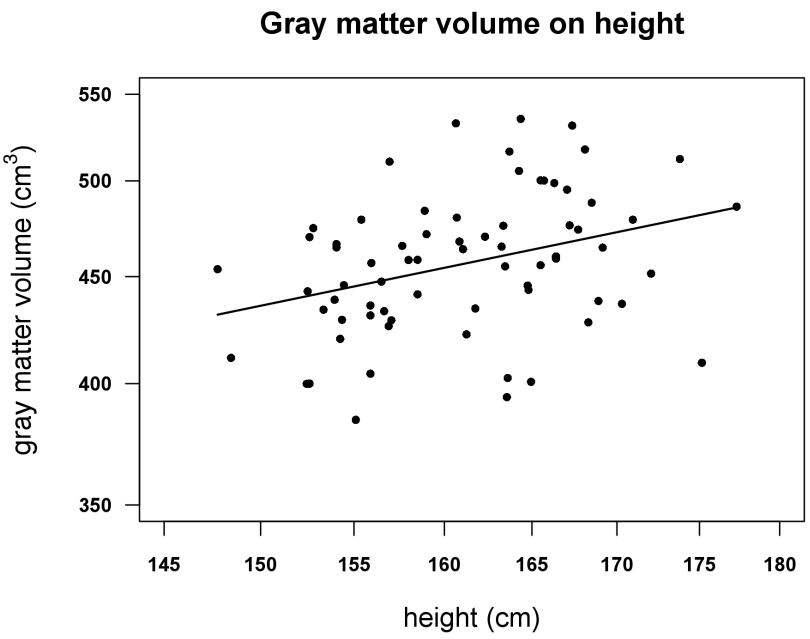


Figure 5-16 Gray matter volume against height.
$r^2 = 0.12$, $p = 0.004$; n = 70

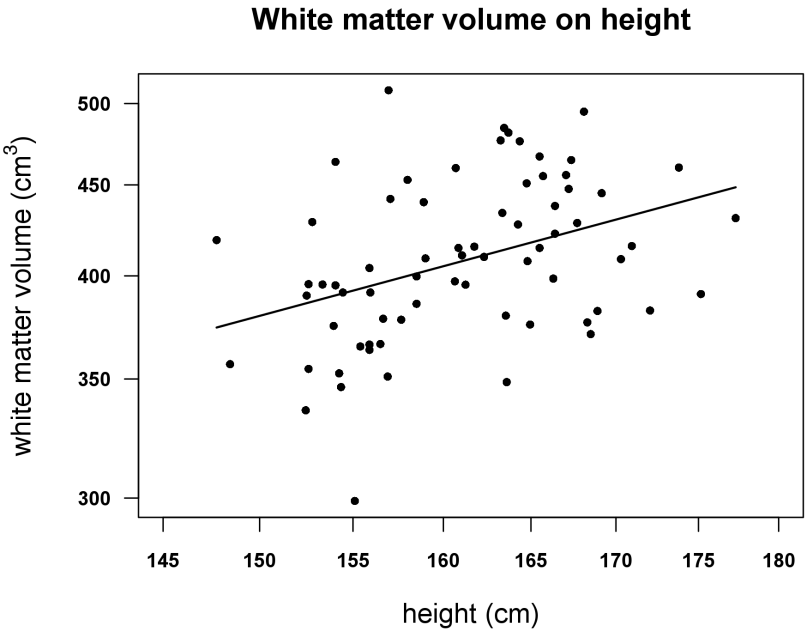


Figure 5-17 White matter volume against height.
$r^2 = 0.15$, $p < 0.001$; n = 70

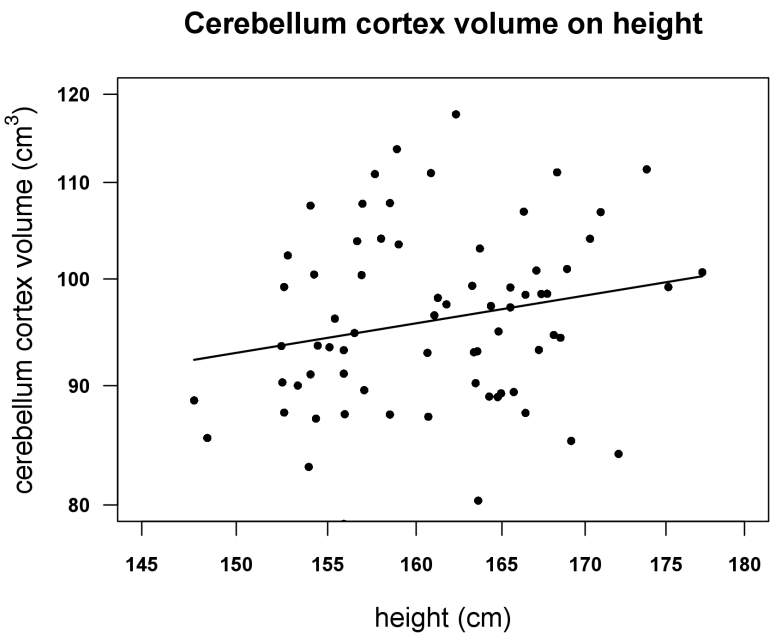


Figure 5-18 Cerebellum cortex volume against height.
$r^2 = 0.04$, $p = 0.08$; n = 70

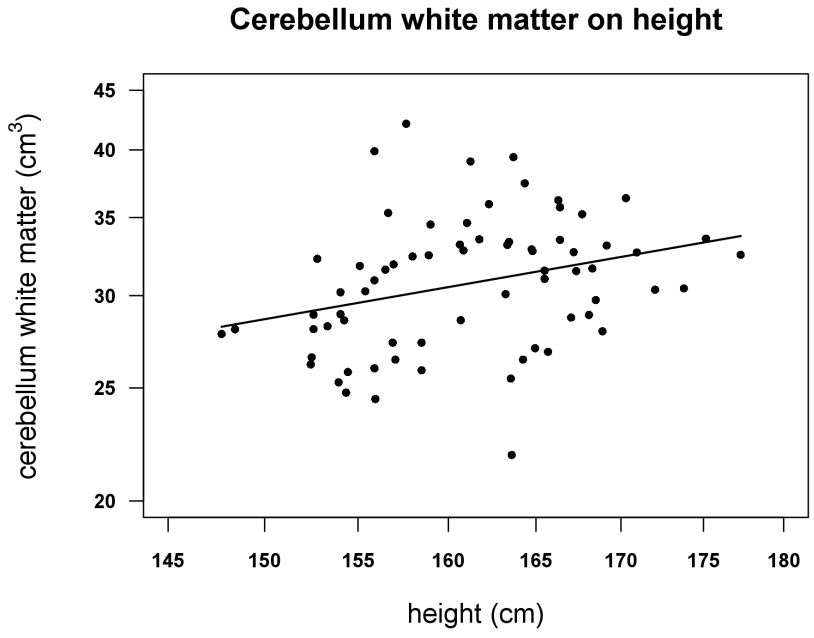


Figure 5-19 Cerebellum white matter against height.
$r^2 = 0.09$, $p = 0.01$; n = 70

In Section 5.3, I found that composite brain volume was not significantly related to any body composition variables. Table 5.11 below shows correlations of brain volume components with FM, FFM, SM and body organs. Consistent with the results for composite brain, there is a lack of association in most cases. The majority of brain components, including gray matter, cerebellum cortex, the amygdala, hippocampus and striatum, are not correlated with any body composition variables.

In contrast, cerebellum white matter is related to FFM (p = 0.02) and kidney volume (p = 0.03), and its relationship with SM approaches significance (p = 0.06). A significant, positive association is also found between kidney volume and white matter volume (p = 0.03). In each case, the correlation is positive.

Table 5.11 Pearson correlation coefficients for brain component volume measures with FM, FFM, SM and body organs

	FM	FFM	SM	Heart	Liver	Kidney	Spleen
Gray matter	-0.15	0.09	0.04	0.01	0.03	0.04	-0.06
White matter	-0.03	0.18	0.17	0.05	0.13	0.27[1]	0.10
Cerebellum cortex	0.02	0.06	0.05	0.03	-0.01	0.06	-0.03
Cerebellum white matter	0.16	0.27[1]	0.22	0.06	0.14	0.26[1]	0.20
Amygdala	0.12	0.09	0.07	0.01	0.06	0.18	-0.15
Hippocampus	-0.04	-0.06	-0.02	-0.13	-0.01	0.11	-0.08
Striatum	-0.02	0.05	-0.004	-0.01	0.02	0.21	-0.01

[1] $p < 0.05$, all other correlations non-significant; FM, FFM and SM are in kg units; brain volumes and body organs are in cm^3

The following figures (5-20 to 5-22) are plots of the significant relationships shown in Table 5.11.

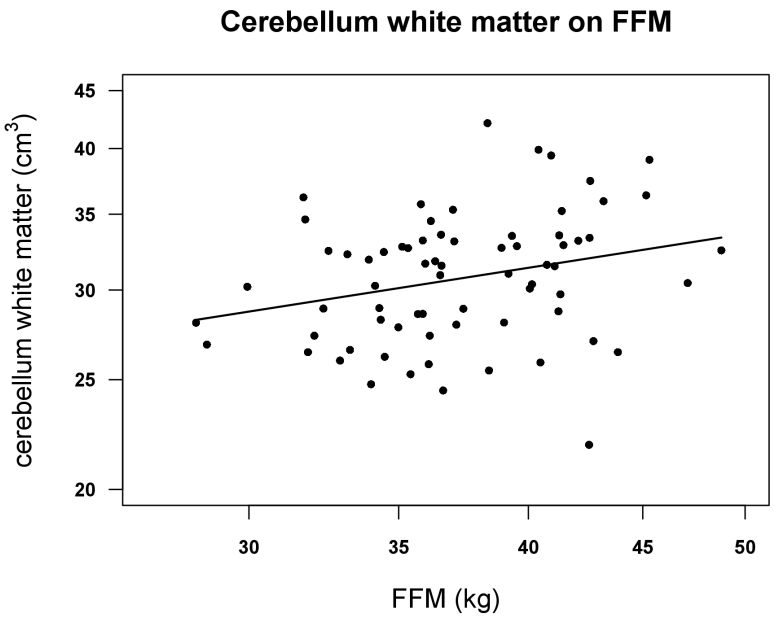


Figure 5-20 Cerebellum white matter against FFM.
r^2 = 0.07, *p* = 0.02; n = 70

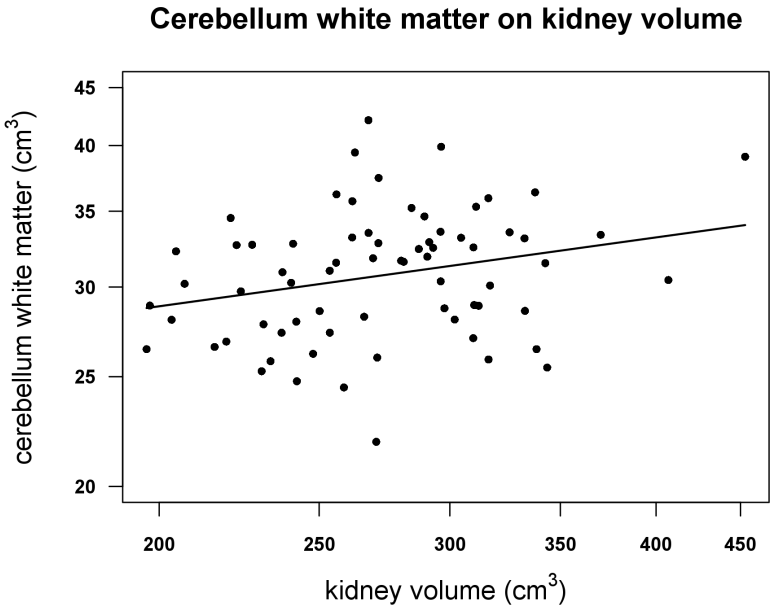


Figure 5-21 Cerebellum white matter against kidney volume.
r^2 = 0.07, *p* = 0.03; n = 70

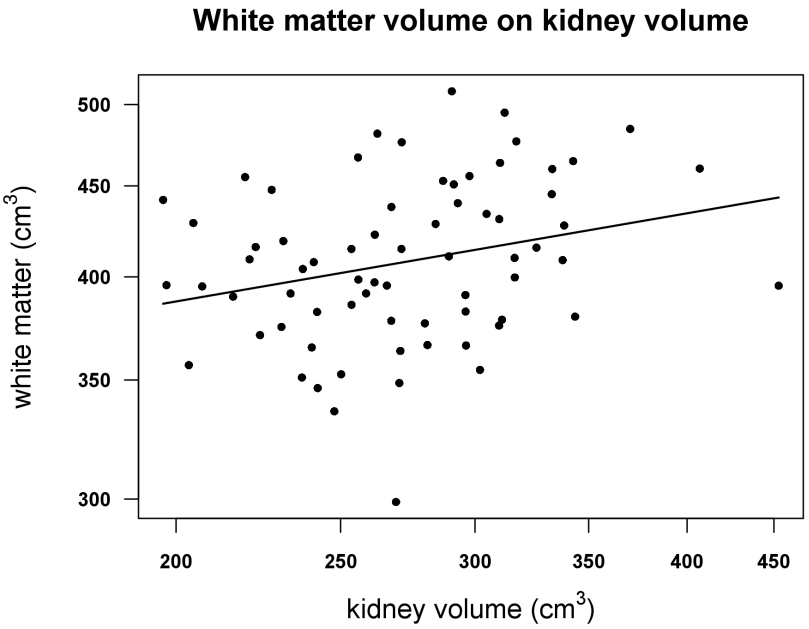


Figure 5-22 White matter volume against kidney volume.
$r^2 = 0.07$, $p = 0.03$; n = 70

The correlation matrix given in Table 5.12 shows relationships among brain components. I include composite brain to show how specific brain volumes are related to the larger whole. Perhaps unsurprisingly, the two largest brain tissue volumes – gray and white matter – correlate strongly with composite brain, and fairly strongly with each other. Both gray and white matter are also significantly correlated with each of the subcortical structures, but with smaller coefficients.

Table 5.12 Matrix of Pearson correlation coefficients among brain components

	GM	WM	CCortex	CWM	Amygdala	Hippo	Striatum
WM	0.72[1]						
CCortex	0.33[2]	0.21					
CWM	0.29[3]	0.29[3]	0.53[1]				
Amygdala	0.32[2]	0.31[2]	0.20	0.17			
Hippo	0.51[1]	0.56[1]	0.19	0.05	0.55[1]		
Striatum	0.53[1]	0.54[1]	0.23	0.30[3]	0.46[1]	0.49[1]	
Composite brain	0.91[1]	0.92[1]	0.40[1]	0.41[1]	0.36[2]	0.58[1]	0.60[1]

[1]$p < 0.001$, [2]$p < 0.01$, [3]$p < 0.05$; all others non-significant; all variables are in cm^3; GM = gray matter; WM = white matter; CCortex = cerebellum cortex; CWM = cerebellum white matter

Gray matter, white matter and subcortical volumes, which comprise the cerebrum, generally correlate less strongly with cerebellum outcomes. The cerebellum, with its white matter underlying a gray matter cortex, similar to the cerebrum, is the 'hindbrain' situated posteriorly, underneath the cerebrum (Figure 5-23 below).

The cerebrum and cerebellum can be partitioned into separate structures (i.e. with their own values for gray and white matter quantified by FreeSurfer), however the gray matter of the cerebral cortex and the cerebellum are in fact 'extensively interconnected' (Rilling, 2006), which renders their relatively weak association somewhat surprising. This is discussed further in the final section of the chapter.

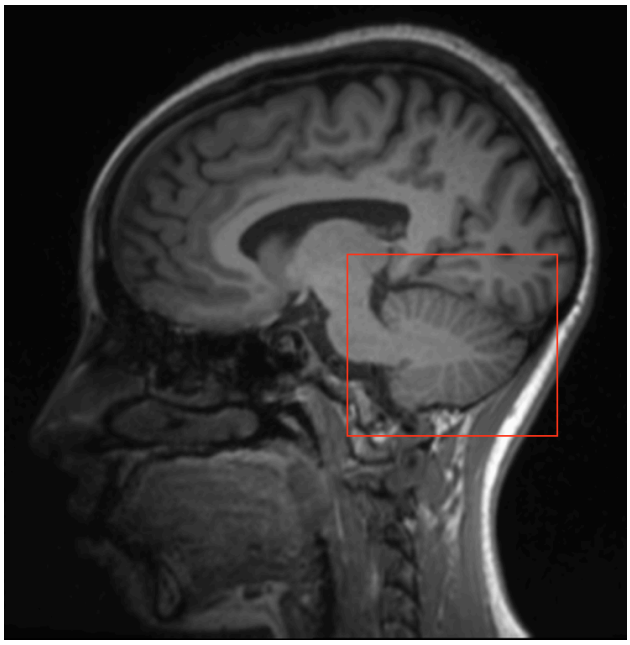


Figure 5-23 A sagittal view of the brain with the cerebellum highlighted.

As seen in Table 5.12, associations among the subcortical structures are all highly significant, with that between the amygdala and hippocampus demonstrating the largest coefficient. In comparison to the hippocampus and striatum, the amygdala correlates more weakly with the composite brain variable.

These findings show that a majority of the brain volume variables correlate positively with one another, and thus, as seen within the body, a larger brain component in adulthood tends to be associated with other relatively large brain components. However, there is variation within this broad pattern, and the relationships seen among specific brain volumes are overall fewer and somewhat weaker than those observed among body outcomes.

5.5 Potential confounders

In this section, I examine whether place of birth, physical activity and menstrual cycle variables are appropriate to include in main analyses as potential confounders.

5.5.1 Place of birth

A potentially confounding variable is associated with both the outcome and the predictor of interest. Birthplace is a binary variable, thus I cannot meaningfully test for associations between it and body/brain components. Instead, I use independent sample t-tests to determine whether my main variables of interest significantly differ between those subjects born in South Asia, and those born elsewhere. Table 5.13 below shows means and *p* values for each test. Birthplace codes subjects born in South Asia as 0, and subjects born elsewhere as 1.

Table 5.13 Results of independent t-tests comparing body/brain variable means for subjects born in South Asia, and subjects born elsewhere

Body/brain variables	Mean (South Asia)	Mean (elsewhere)	*p* value
FM, kg	21.3	19.3	0.22
FFM, kg	37.3	37.8	0.68
SM, kg	15.3	15.3	0.89
Heart volume, cm^3	495.5	501.8	0.77
Liver volume, cm^3	1154	1126	0.58
Kidney volume, cm^3	278.3	275.5	0.81
Spleen volume, cm^3	136.8	127.8	0.44
Gray matter, cm^3	463.7	452.4	0.18
White matter, cm^3	412.4	407.9	0.66
Cerebellum cortex, cm^3	98.5	94.4	0.04
Cerebellum white matter, cm^3	32.0	30.1	0.05
Amygdala, cm^3	2.5	2.4	0.15
Hippocampus, cm^3	7.3	7.2	0.64
Striatum, cm^3	18.3	17.8	0.14

The t-test results are, in general, highly non-significant for differences related to place of birth. The only variables deviating from this pattern are cerebellum cortex and cerebellum white matter: the former test is just significant, and the latter is borderline.

REE is another key variable in this study; Chapter 6 explores associations of body and brain components with REE, and tests the hypothesis that tissues vary in their energy expenditure. I have not discussed REE in the current chapter, however I include it in this section in order to make a determination regarding the inclusion of confounders. A t-test shows that REE is not significantly different by place of birth ($p = 0.97$).

Based on these results, I did not consider place of birth a potential confounder in my analyses.

5.5.2 Physical activity

Physical activity variables (PA1 and PA2) are continuous numeric variables, thus I was able to explore whether they correlated with the study's main outcomes of interest. As described in Chapter 4, Section 4.7.3.2, PA1 was derived after removing an extreme outlier from the dataset. PA2 was derived by removing data from 10 further individuals whose questionnaire responses I considered potentially spurious. Specifically, my concern was over-reporting of physical activity, which has been reported in previous studies where IPAQ findings were compared with accelerometer results (e.g. Boon et al., 2010; Cerin et al., 2016).

Descriptive statistics are given in Table 5.14. Variability in PA1 and PA2 is high, as shown by the CVs. Mean PA in kcal/week is reduced for PA2, relative to PA1.

Table 5.14 Descriptive statistics for PA variables

	n	**Mean**	**SD**	**Range**	**CV %**
PA1 (kcal/week)	68	3458	2218	476-9874	64
PA2 (kcal/week)	58	2897	1727	476-7748	60

Correlation coefficients for PA1 and PA2 with brain and body volumes are given in Table 5.15. REE is added in the table.

Table 5.15 Pearson correlation coefficients for brain and body outcomes with PA variables

Body/brain variables	PA1 r, *p*	PA2 r, *p*
FM, kg	0.34, 0.004	0.41, 0.001
FFM, kg	0.30, 0.01	0.35, 0.006
SM, kg	0.31, 0.01	0.40, 0.002
Heart, cm^3	0.35, 0.004	0.42, 0.001
Liver, cm^3	0.27, 0.02	0.37, 0.004
Kidney, cm^3	0.23, 0.06	0.22, 0.09
Spleen, cm^3	0.35, 0.004	0.23, 0.08
Composite organ, cm^3	0.34, 0.006	0.42, 0.001
Gray matter, cm^3	0.02, 0.85	0.02, 0.89
White matter, cm^3	-0.02, 0.85	-0.03, 0.81
Cerebellum cortex, cm^3	0.01, 0.94	0.07, 0.59
Cerebellum white matter, cm^3	0.08, 0.51	0.02, 0.90
Amygdala, cm^3	-0.06, 0.65	-0.02, 0.87
Hippocampus, cm^3	0.05, 0.70	-0.03, 0.81
Striatum, cm^3	0.18, 0.13	0.08, 0.56
REE, kcal/24hr	0.24, 0.05	0.40, 0.002

PA1 is significantly related to FM, FFM, SM and all body organs except the kidneys. PA2 demonstrates a similar pattern, although this variable shows no relationship with spleen volume. Neither PA variable is related to any of the brain volume outcomes. With regard to REE, PA1 is borderline significant, whilst PA2 demonstrates a stronger relationship.

As I show in Chapter 6, REE is strongly correlated with FFM, and I suspect the correlations between REE and PA may be confounded by variability in FFM. To test this, I take the residuals of REE on FFM to derive a new variable where

variability attributable to FFM has been adjusted out. This REE-residual variable is not associated with PA1 (r = 0.04, p = 0.75) or PA2 (r = 0.21, p = 0.11), which suggests the relationships seen above for REE and the PA variables are artefacts of REE's association with FFM.

Although PA1 and PA2 correlate with several body tissue outcomes, their lack of correlation with REE once FFM is controlled for indicates PA is not a relevant potential confounder in the Chapter 6 analyses of REE and body/brain variables. Likewise, the finding that PA does not relate to any brain measures obviates the need to control for this variable when testing tissue trade-offs in Chapters 7 and 8, where brain measures are set as the outcome in all models.

5.5.3 Menstrual cycle

The three menstrual cycle variables I described in Chapter 4, Section 4.7.3.3 are distinguished by length of cycle, as determined by subjects' reported first day of last menstruation prior to data collection. Correlations among these variables and my primary study outcomes are shown in Table 5.16 below.

MC variables are not related to FM, FFM or SM, but MC1 and MC2 correlate with the liver and composite organ (MC3 is borderline significant for the liver). MC1 is just significant with spleen volume, at p = 0.04. Several additional positive associations are found among MC variables and brain components, including gray matter, white matter, cerebellum cortex, hippocampus, striatum and composite brain. REE is not significantly associated with MC1, MC2 or MC3.

Table 5.16 Pearson correlation coefficients for brain and body outcomes with menstrual cycle variables

Body/brain variables	MC1[1] r, *p*	MC2 r, *p*	MC3 r, *p*
FM, kg	0.01, 0.94	0.12, 0.37	0.09, 0.52
FFM, kg	0.16, 0.26	0.16, 0.23	0.10, 0.48
SM, kg	0.17, 0.24	0.22, 0.11	0.17, 0.20
Heart, cm^3	0.14, 0.34	0.09, 0.52	-0.01, 0.94
Liver, cm^3	0.35, 0.02	0.31, 0.02	0.26, 0.05
Kidney, cm^3	0.25, 0.09	0.25, 0.07	0.17, 0.21
Spleen, cm^3	0.30, 0.04	0.22, 0.12	0.09, 0.50
Composite organ, cm^3	0.39, 0.008	0.33, 0.02	0.23, 0.10
Gray matter, cm^3	0.26, 0.07	0.24, 0.08	0.29, 0.03
White matter, cm^3	0.32, 0.03	0.35, 0.009	0.42, 0.001
Cerebellum cortex, cm^3	0.31, 0.03	0.24, 0.08	0.26, 0.05
Cerebellum white matter, cm^3	0.25, 0.08	0.25, 0.06	0.20, 0.14
Amygdala, cm^3	0.12, 0.43	0.25, 0.07	0.18, 0.18
Hippocampus, cm^3	0.26, 0.07	0.37, 0.006	0.38, 0.003
Striatum, cm^3	0.41, 0.004	0.43, 0.001	0.38, 0.003
Composite brain, cm^3	0.35, 0.01	0.35, 0.01	0.41, 0.001
REE, kcal/24hr	0.22, 0.13	0.24, 0.08	0.24, 0.08

[1]MC1: menstrual cycle range 21-35 days, n = 48; MC2: cycle range 21-41 days, n = 54; MC3: cycle range 21-45 days, n = 58

I initially considered that variation in menstrual cycle phase at data collection might impact on the REE measurement in particular, following reports of intra-individual variation in REE over the course of a cycle in women (Soloman et al., 1982; Henry et al., 2003). My MC variables are a relatively crude index of cycle phase, however their lack of association with REE does suggest REE was not significantly impacted by variability associated with menstruation in my sample. For this reason,

I conclude it is not necessary to control for MC variables as potential confounders in my Chapter 6 analysis of body/brain composition and REE.

MC variables do, however, demonstrate associations with the liver, composite organ and several brain components, including composite brain, which was unexpected. Why day-of-cycle at the data collection visit would correlate with body organ volumes is unclear, however, as noted in Chapter 4, recent studies have suggested hormonal fluctuations over the menstrual cycle are related to brain structural changes, for example in the hippocampus (Lisofsky et al., 2015). As I test for tissue trade-offs in Chapter 7, I treat MC variables as potential confounders where they associate with both the predictor and outcome variable in the model.

5.6 Discussion of findings in this chapter

The results detailed in Sections 5.3 and 5.4 above support part (iv) of my second hypothesis, as set out in Chapter 2. This predicted 1) that organs and tissues in the body are positively related to one another; 2) that specific volumes of the brain are likewise positively related; and 3) that, in contrast, positive associations between brain and body tissues are fewer and weaker in magnitude. With respect to the second prediction, I found support, although the pattern was less consistent.

With respect to body components, findings suggest that an individual with greater weight and taller height has more FFM, including larger organs and greater SM mass. Greater body weight is also strongly associated with increased FM, although FM is relatively independent of height. Indeed, FM demonstrates a high degree of variability, as indicated by a CV of 32.9%. The CV for FFM is just 11.4%, although there is more variability amongst FFM components: for the heart, kidneys and liver the CV is approximately 17%, whilst for the spleen it is even higher than FM at 34.9%. For SM, the CV is somewhat higher than overall FFM (14.2%). Thus, variability in FM as a component of weight is apparently distributed relatively uniformly across the range of stature. FFM scales more closely with stature,

however within the FFM compartment SM and organs appear to vary to a greater extent with body size (see below).

That tissues and organs are generally strongly related to body weight is unsurprising, as the former are components of the latter. At the same time, the associations I observed amongst FFM, FM and height have been shown before. For example, in a multi-ethnic sample of children and adults, Wang and coworkers (2012) also found FM to be highly variable (CV >30%), and not strongly related to height. The opposite was seen for FFM, which increased significantly with increasing height, as I saw in my data. These associations are consistent with the notion that FFM represents the 'body core', wherein components including SM and organs are more stable aspects of physique than FM, which demonstrates greater plasticity over time (Wang et al., 2012).

Further evidence suggests a relatively strong association between height and lean mass is present from birth. For example, birth weight has been widely found to associate with later lean mass, but to a lesser degree with later FM (Wells et al., 2007; Kuzawa et al., 2012), while both birth weight and birth length correlate with adult height (Sørensen et al., 1999). As discussed in Chapter 1, components of lean mass are canalized following sensitive periods in fetal life and/or infancy so that they track into adulthood. Linear growth similarly becomes canalized under the influence of growth hormone, so that organs develop in tandem with stature (Wells, 2016). Autopsy and imaging studies have shown that organ size correlates positively with stature in adults (e.g. de la Grandmaison et al., 2001; Sheikhazadi et al., 2010; Davis et al., 2015).

My findings suggest that measured body organs scale to a variable degree with height, weight and other body tissues. Despite their difference in size, the heart, liver and kidneys scale similarly to height and weight, whilst the spleen's association with body size is weaker. A similar pattern is found with respect to organ correlations with FM, FFM and SM, whereby coefficients for the spleen are

smaller than those observed for the heart, liver and kidneys. In de la Grandmaison et al.'s (2001) autopsy dataset, the liver weight of females correlated most strongly with height, followed by the kidneys, whilst the spleen was more weakly associated (heart weight was significantly correlated with BMI, but not height).

Indeed, in my dataset all lean body outcomes were significantly, positively related to one another. Prior studies have reported similar findings. Using MRI, Gallagher et al. (1998) found strong correlations among heart, liver, kidneys, SM, and a measure of AT-free mass, which is comparable to FFM. Similar results were shown by Illner et al. (2000) using MRI and DXA in a mixed-sex cohort, and also by Bosy-Westphal and colleagues (2004), who used equivalent methods to measure body composition in underweight, normative weight, and obese adults. However, there is some variation between the size of the coefficients I observed in my sample, and those reported in these studies. For example, for associations amongst organs I saw coefficients in the range 0.34 – 0.64. The results of Illner et al. (2000) were similar to my own (0.44 – 0.71), however those of Bosy-Westphal et al. (2004) were higher (0.67 – 0.83). With respect to associations amongst SM and organs, the range of coefficient values reported by Gallagher et al. (1998; 0.75 – 0.85) was higher and did not overlap with my sample range (0.42 – 0.72).

With respect to FM, Gallagher et al. (1998) and Illner et al. (2000) found no significant associations with lean body organs and tissues. In contrast, I found that FM was significantly associated with FFM, SM and organs, although the coefficients were somewhat weaker than those observed among lean components, with a range of 0.36 – 0.44. The variation in findings among studies could potentially be explained by methodological differences, despite the common use of MRI and DXA, or the fact that the above authors' samples were comprised of both men and women of European ethnicity. Variation in findings may owe in part to the fact that the allometric relationship between FM and FFM is complex, not linear; it may also vary among populations, and in association with factors such as nutritional status (Wells and Victora, 2005).

With respect to the brain, the composite brain volume variable demonstrated a different pattern than other non-FM components in my sample. First, the brain was less variable, with a CV of 7.5%. Although it appeared to scale to some extent with height, the brain appeared not to vary in association with body mass. It was not significantly related to FFM, SM, body organs, or FM, thus appearing not to scale with fat or fat-free body tissues. Relatively weak relationships between the brain and components of the body were also shown by Gallagher et al. (1998), Illner et al. (2000), and Bosy-Westphal et al. (2004). In each case, organs, SM and FFM were more closely related to one another than they were to the brain.

Reported associations of brain mass with body weight and height are 'weak and inconsistent,' according to Heymsfield et al. (2012b). These authors have shown that brain mass scales hypoallometrically with both height and FFM, so that brain mass represents a smaller proportion of FFM with increasing height (Heymsfield et al., 2012a,b). This is consistent with the relatively small brain CV: relative to, for example, the heart or liver, the brain is not expected to demonstrate as much variation amongst individuals of differing height or body size. Indeed, Heymsfield et al.'s (2012b) comparative analysis of the brain and liver, which are similar to one another in size and metabolic rate, found that the liver scaled to height with a power similar to FFM, in contrast to the brain's scaling with height at a lower power. Liver mass also correlated more strongly with FFM than brain mass, which I found in the present analysis. In my sample, the FFM-liver coefficient was $r = 0.71$, relative to the FFM-brain coefficient of $r = 0.16$, which was non-significant.

As with the composite brain variable, I found that the brain's component parts were largely uncorrelated with organs and tissues in the body. The only significant relationships, which demonstrated moderate effect sizes, were seen for cerebellum white matter (with FFM and kidney volume) and white matter (with kidney volume). White matter and cerebellum white matter in fact demonstrated a greater degree of variability than most brain components (excepting the amygdala), with CVs of 10.4% and 13.1%, respectively, whilst their gray matter

counterparts had CVs more similar to composite brain (7.5%). It is difficult to interpret the specific findings relating white matter to FFM and kidney volume, however they suggest that testing for somatic trade-offs using a global brain outcome alone might obscure underlying relationships. Overall, however, the results indicate that knowing the size of body organs and tissues has limited utility for predicting the size of specific volumes of the brain, or total brain size.

As I predicted in Hypothesis 2, despite weak links with body tissues, brain components did tend to correlate with one another, which is consistent with the idea that there is a common developmental process underlying their growth (Finlay and Darlington, 1995; Clark et al., 2001). However, the brain component correlations themselves were not particularly strong. An exception was gray and white matter volumes with composite brain ($r > 0.90$), which was expected, as gray and white matter are the largest components of the composite brain variable. However, the r-value for gray and white matter was 0.72, indicating that the variance in one explains only ~50% of the variance in the other. This may be related to the fact that, although individuals with more gray matter tend to have more white matter, the volumes do not scale equally with brain size: as brain size increases, white matter must make longer-range connections between more distant cortical regions, and thus tends to increase in volume more quickly than gray matter (Zhang and Sejnowski, 2000).

Within the brain, correlations were weakest for the cerebellum. For example, cerebellum cortex and cerebellum white matter volumes demonstrated r-values of only ~0.40 with composite brain, and were even more weakly correlated with gray and white matter ($r = 0.21 – 0.33$). They demonstrated largely non-significant relationships with subcortical structures. This may again relate to scaling differences. While the cerebrum and cerebellum are both absolutely larger in larger brains, the cerebrum is relatively large with increasing brain size, while in contrast the cerebellum represents a constant proportion of the total brain (Clark et al., 2001; Herculano-Houzel, 2010). The cerebellum is recognized to play an important

role in cognitive and motor-related brain function, and as noted above, it is highly interconnected with cerebral gray matter (Leiner et al., 1989; Rilling, 2006, 2008; Ramnani, 2006), however this is not reflected by the results of simple correlations in my sample.

Although components within the body and those within the brain generally show significant, positive correlations, as I predicted, it is notable that the relationships are far from direct. The same is true for associations of body and brain components with height and weight, suggesting that a considerable amount of variation in organ and tissue size is unexplained by overall body size. The largest correlation coefficient among body organs and tissues was 0.72 for SM and liver (excepting SM and FFM, which were predictably closely related), which indicates that the variance in the size of one tissue explained by variance in another in my dataset does not exceed ~50%. With respect to brain components, the highest r-value is likewise 0.72 (between gray and white matter, as described above). Correlations were generally somewhat weaker for the brain than those seen for body components, and there were fewer significant findings within the brain.

Species are characterized by a common ‘bauplan’ or ‘blueprint’ for the body’s development (Shingleton, 2010) and common mechanisms are recognized to underlie the growth of the brain and body (Netchine et al., 2011). Nevertheless, the current findings indicate that a given increase in one body or brain variable does not correlate with the same degree of increase in another. This suggests that there is considerable variation in the way the body and brain are built, which may be explained by the influence of environmental factors during development, or the effect of genes on the relative growth of different body components.

6 REE and the metabolic cost of organs and tissues

In Chapter 5 I described patterns of association amongst brain and body composition outcomes in my dataset. In this chapter, I test the hypothesis that the brain, body organs, SM and FM are differentially metabolically costly. This was designated Hypothesis No. 1 in Chapter 2. I briefly introduced REE and the notion that organs and tissues have different SMRs (specific metabolic rates) in Chapter 1. Here, Section 6.1 provides additional background on the assessment of variation in tissue energy expenditure by previous authors.

Section 6.2 describes relevant statistical methods for this chapter, and Section 6.3 demonstrates how REE associates with body and brain variables. Section 6.4 employs two different methods to investigate tissue-specific metabolic rate values, after which these values are used to calculate each tissue's contribution to the overall energy budget. In Section 6.5, I explore whether brain sub-structures appear to differentially contribute to REE. Finally, in Section 6.6 I show the best-fit multivariable model for REE on organ/tissue outcomes, and offer a summary of the chapter's findings in Section 6.7.

6.1 Background: assessing the REE of specific organs and tissues

Accessible measures of height and weight were employed in early studies on the link between body size and metabolism (e.g. Kleiber, 1932; Heusner, 1985; see review of Heymsfield et al., 2012a). With the development of methods that could partition the body into its component parts (several of which I described in Chapter 3), researchers built on the knowledge that body size determines REE, finding that not all components contributed equally to the body's resting energy budget. It was recognized that "there are in the body various kinds of cells, differing in the rate of oxygen consumption" (Brozek and Grande, 1955, pg. 22). Keys and Brozek (1953)

differentiated between metabolically 'active' and 'inert' components of mass, with the former including brain and body organs, and the latter adipose tissue and fat.

Measuring cerebral blood flow using a nitrous oxide method, in 1945 Kety and Schmidt estimated the mass-specific oxygen consumption (i.e. SMR) of the brain in a cohort of adult males. Their results suggested an SMR of 260 kcal/kg/day, which is similar to values reported subsequently (Heymsfield et al., 2012a). In 1950, Drabkin estimated the level of oxygen consumption for additional tissues, including SM, heart, liver, and kidneys. Holliday and colleagues (1967) and Elia (1991, 1992) compiled existing data to further describe variation in tissue SMRs in children across developmental stages, and in adults.

A key finding by the above authors and others was that the brain and a small number of internal organs comprising approximately 5-7% of adult body weight account for >60% of whole-body REE. At the same time, SM and AT, which represent much larger masses in the body, have comparatively low mass-specific energy turnover (Brozek and Grande, 1955; Holliday et al., 1967; Elia, 1991, 1992). Elia's (1992) publication of SMR values for organs, as well as SM, AT and a miscellaneous compartment ('Residual mass'), has been widely cited in subsequent REE-body composition investigations, as developed below.

The SMRs of organs and tissues (referred to as 'K_i' values) can be estimated directly *in vitro* or *in vivo*, however the former may yield poor estimates, and the latter employs methods involving arteriovenous (A-V) oxygen concentration and blood flow measurements, which are technically difficult (Elia, 1991, 1992; Wang et al., 2010). A-V studies were used to establish the K_i values Elia published in 1991 and 1992, and have been used by other authors (e.g. Zurlo et al. (1990) for SM). Subsequently, several researchers have developed REE-body composition models utilizing Elia's values, testing their applicability for estimating whole-body REE from MRI-measured body organs and tissues. With data from MRI, DXA and IC, I follow the methods set out by these authors to assess tissue energy

expenditure in my own sample (described in the following sections), as the more direct estimation of K_i values by A-V methods was not feasible.

One of the first studies to use MRI and REE data alongside Elia's K_i values – which he gave for brain, heart, kidney, liver, SM, AT and residual mass – was that of Gallagher and colleagues (1998). They quantified AT, SM, the liver, kidneys, and brain by MRI, measured the heart by echocardiography, and REE by IC. They derived residual mass by subtracting the sum of the remaining six components from body weight, and multiplied the mass of each organ or tissue by Elia's published K_i values. The sum of these products gave 'calculated' REE, which the authors observed was strongly correlated with measured REE ($r = 0.94$). These findings suggested both that REE could be predicted *in vivo* using organ and tissue measurements, and also that Elia's K_i values were accurately applied in the authors' study cohort.

A subsequent investigation in an independent, larger sample produced consistent findings (Illner et al., 2000). Using very similar methodology, Illner and coworkers (2000) also calculated REE from imaging-derived organ/tissue masses and Elia's values, and likewise found a strong association with measured REE ($r = 0.92$). Similar results were reported for studies carried out by Heymsfield et al. (2002), Bosy-Westphal et al. (2004), and Midorikawa et al. (2007). Altogether, these various findings suggested the applicability of Elia's values across young, healthy men and women (Wang et al., 2010; Heymsfield et al., 2012a), although the range of populations in which such studies have been carried out remains limited. With respect to the ethnic background of the subjects in the studies cited here, the cohort of Heymsfield et al. (2002) was comprised of African American, white, Asian and Hispanic men and women recruited in the United States; Midorikawa et al. (2007) undertook their study in Japanese individuals; and the remainder appear to have recruited individuals of European ethnicity (Gallagher et al., 1998; Illner et al., 2000; Bosy-Westphal et al., 2004; Wang et al., 2010).

Several of the above authors also explored using stepwise multiple regression to explain the variance in measured REE using brain, heart, liver, kidney, AT, SM and residual masses in their samples. Different combinations of organs and tissues emerged as significant predictors in different models, for example brain mass and SM (Gallagher et al., 1998); liver mass and SM (Illner et al., 2000; Bosy-Westphal et al., 2004); and brain, liver, SM, AT and residual mass (Heymsfield et al., 2012a). The outcomes in each case represent a mix of both low and high metabolic rate tissues, as per Elia's (1992) published values.

In a 2010 study, Wang and coworkers measured REE by IC, and organs and tissues by MRI and DXA. They developed a univariable regression method with which they demonstrated further evidence of the applicability of Elia's values across young adults (results were less consistent in individuals > 50 years of age). I describe the method of Wang et al. (2010) in more detail in Section 6.4, where I employ the same approach to assess the applicability of Elia's K_i values in my own sample. First, Section 6.2 describes general statistical methods, and Section 6.3 gives descriptive statistics for REE and describes its association with body and composite brain outcomes.

6.2 Statistical methods

In this chapter, variables were used in their raw form rather than log-transformed following previous authors (e.g. Wang et al., 2000; Heymsfield et al., 2002; Later et al., 2008; Javed et al., 2010; Heymsfield et al., 2012a). Kleiber described a nonlinear relationship between REE and body mass in mature mammals, however Wang and coworkers (2000) demonstrated that while the relationship between REE and FFM is curvilinear, it can be linearly modeled when FFM variation is within the range of 40-80 kilograms. Later and colleagues (2008) found no evidence that K_i values vary in relation to body size in humans across a wide range of body mass, which suggests modelling raw REE and brain/body composition data in the current study is tenable.

Pearson correlation coefficients were calculated for REE with brain and body variables, with plots given to demonstrate associations. REE-body composition regression models were employed to test the hypothesis that organs and tissues are variable in their metabolic expense. For clarity, the specific details of the various regression models are described further in relevant sections below.

6.3 Associations among REE, body composition and brain volume outcomes

As described in previous chapters, REE is the number of kilocalories used over 24 hours to carry out several essential bodily functions, and I used IC to obtain the measurement in my sample. Table 6.1 gives descriptive statistics for REE. Two participants missed the IC measurement due to scheduling conflicts, thus the sample size for this outcome is 68.

Table 6.1 REE descriptive statistics

	n	**Mean**	**SD**	**Range**	**CV %**
REE (kcal/24hr)	68	1,337	184	993-2034	13.8

REE is significantly correlated with both weight ($r = 0.73$, $p < 0.001$) and height ($r = 0.45$, $p < 0.001$), with a relatively strong association with weight, as shown in Figures 6-1 and 6-2 below.

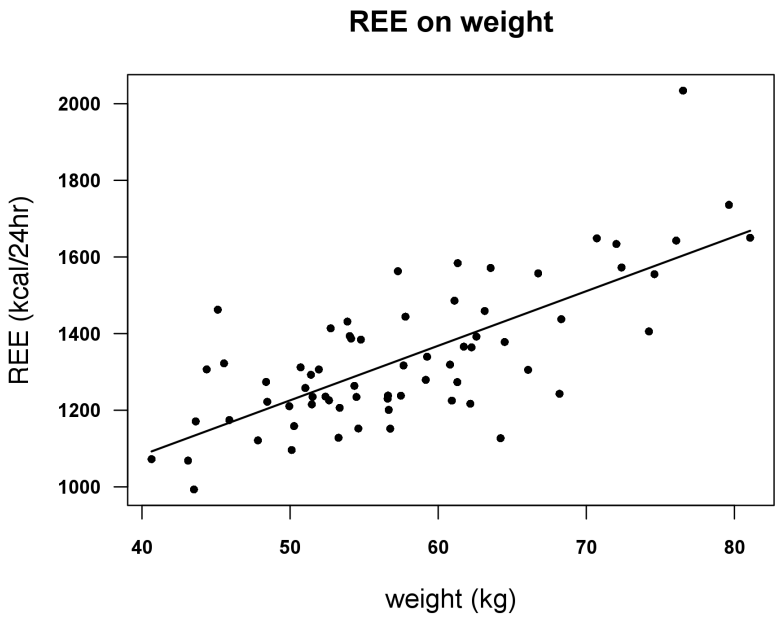


Figure 6-1 REE against weight for 68 subjects.
$r^2 = 0.53$, $p < 0.001$

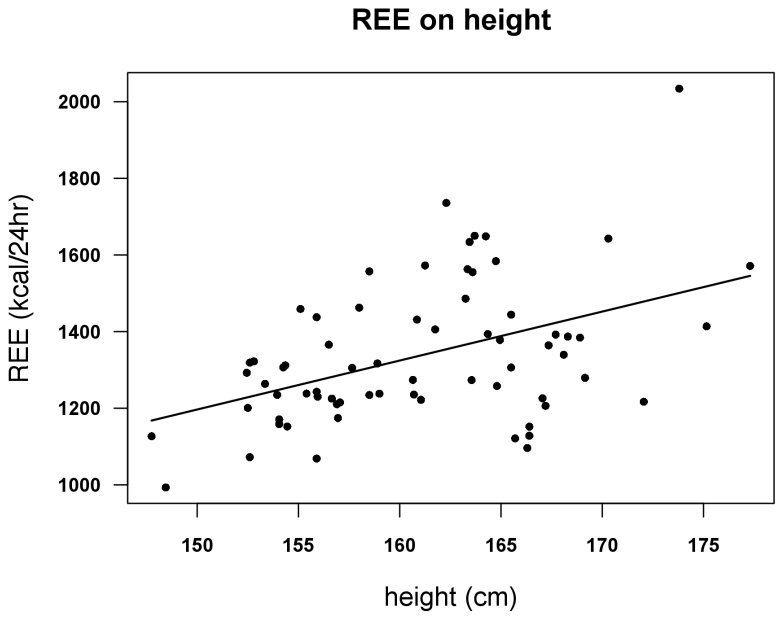


Figure 6-2 REE against height for 68 subjects.
$r^2 = 0.20$, $p < 0.001$

After demonstrating above that REE correlates with weight, Table 6.2 shows more nuanced associations among REE and components of body weight, i.e. organs and tissues of the body, and the composite brain variable. Volumes are converted to mass for analyses later in this chapter, however here correlations and plots with raw organ and tissue volumes are given. The association of REE with specific brain components is explored further in Sections 6.5 and 6.6.

Table 6.2 Pearson correlation coefficients of REE with raw body composition outcomes

Body composition outcomes	**REE, kcal/24hr r, *p***
FM, kg	0.53, <0.001
FFM, kg	0.76, <0.001
SM, kg	0.77, <0.001
Organ volumes, cm^3	
Heart	0.70, <0.001
Liver	0.67, <0.001
Kidney	0.61, <0.001
Spleen	0.32, 0.008
Composite organ	0.74, <0.001
Composite brain	0.21, 0.08

All body outcomes are positively correlated with REE, with highly significant *p*-values. However, composite brain demonstrates no such association, appearing once again to deviate from the pattern found for body tissues, as observed in Chapter 5. Here, the largest coefficients are seen for REE with FFM and SM. Among the single organs, heart volume demonstrates the largest coefficient (although the liver coefficient is similar in size), and spleen volume is the smallest. The correlation of composite organ volume with REE approaches the strength of the correlations seen for REE with FFM and SM. Plots below visualize the relationships among REE and brain/body outcomes detailed in Table 6.2.

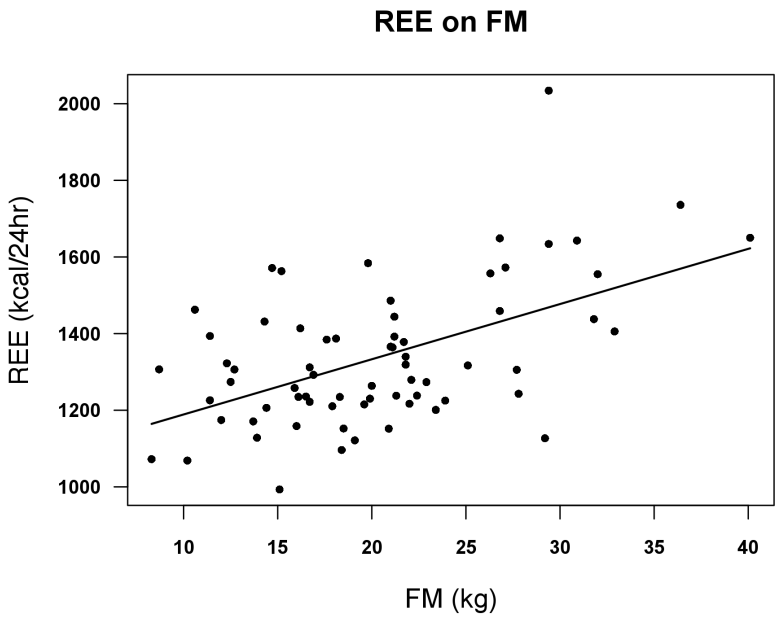


Figure 6-3 REE against FM.
$r^2 = 0.28$, $p < 0.001$; n = 68

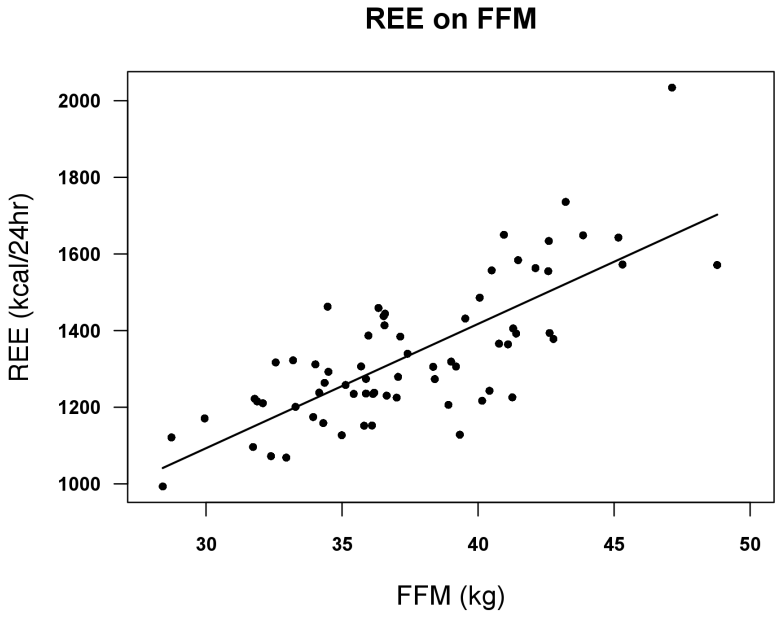


Figure 6-4 REE against FFM.
$r^2 = 0.58$, $p < 0.001$; n = 68

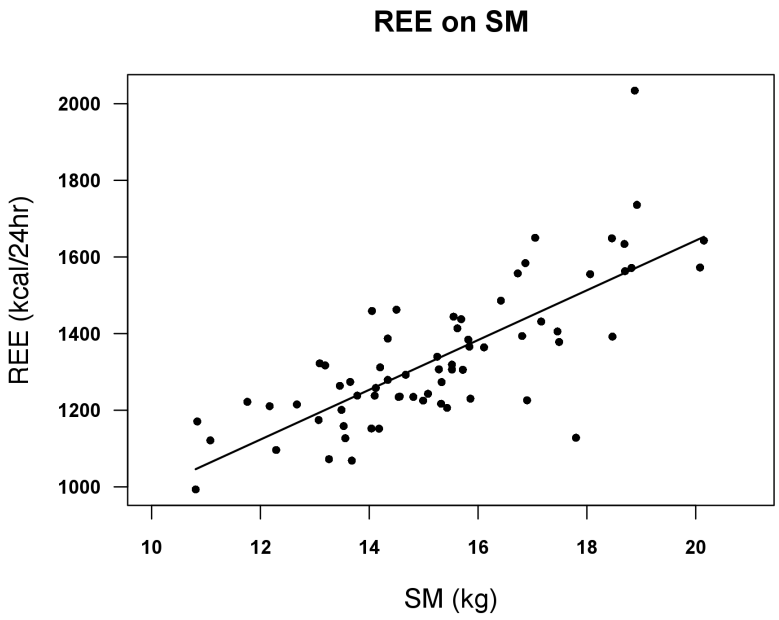


Figure 6-5 REE against SM.
$r^2 = 0.59$, $p < 0.001$; n = 68

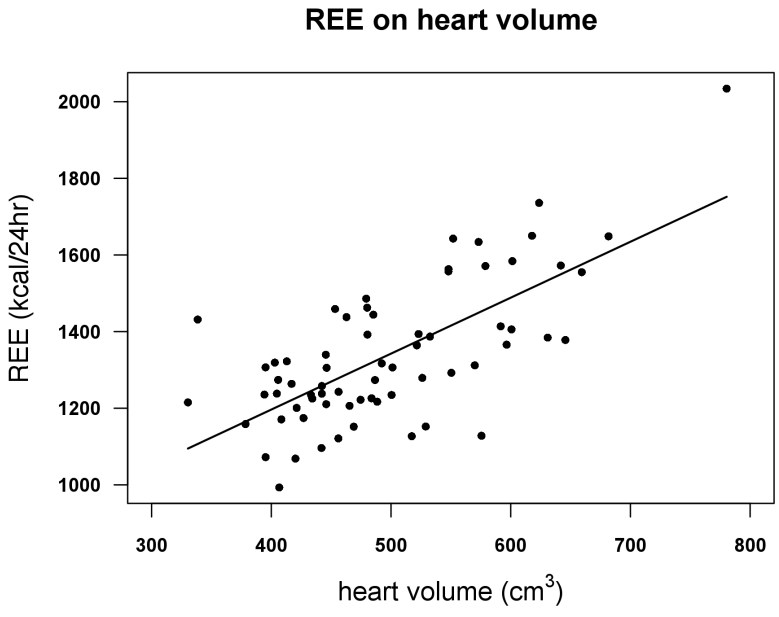


Figure 6-6 REE against heart volume.
$r^2 = 0.49$, $p < 0.001$; n = 67

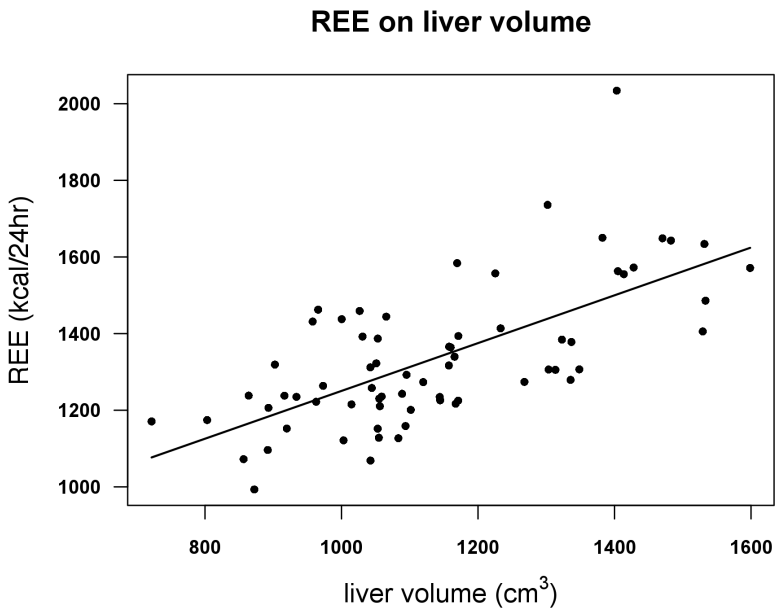


Figure 6-7 REE against liver volume.
$r^2 = 0.45$, $p < 0.001$; n = 68

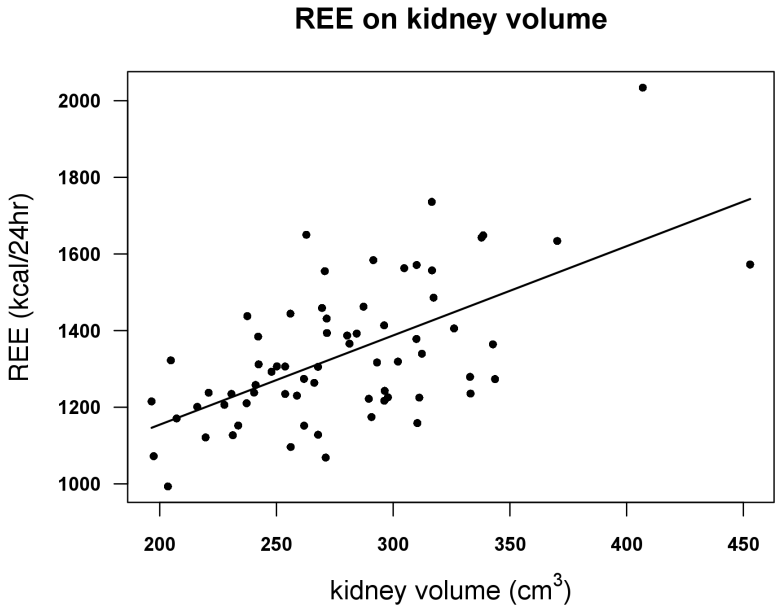


Figure 6-8 REE against kidney volume.
$r^2 = 0.37$, $p < 0.001$; n = 68

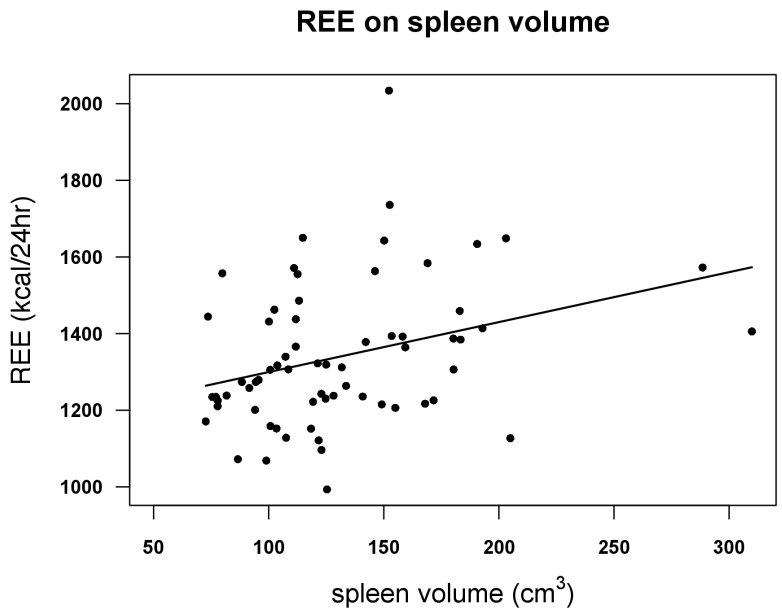


Figure 6-9 REE against spleen volume.
$r^2 = 0.10$, $p = 0.008$; n = 66

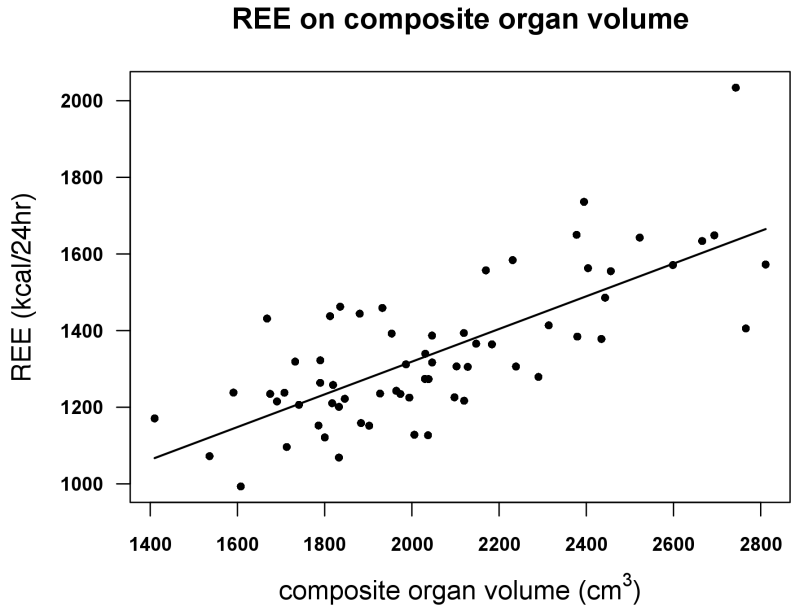


Figure 6-10 REE against composite organ volume.
$r^2 = 0.55$, $p < 0.001$; n = 65

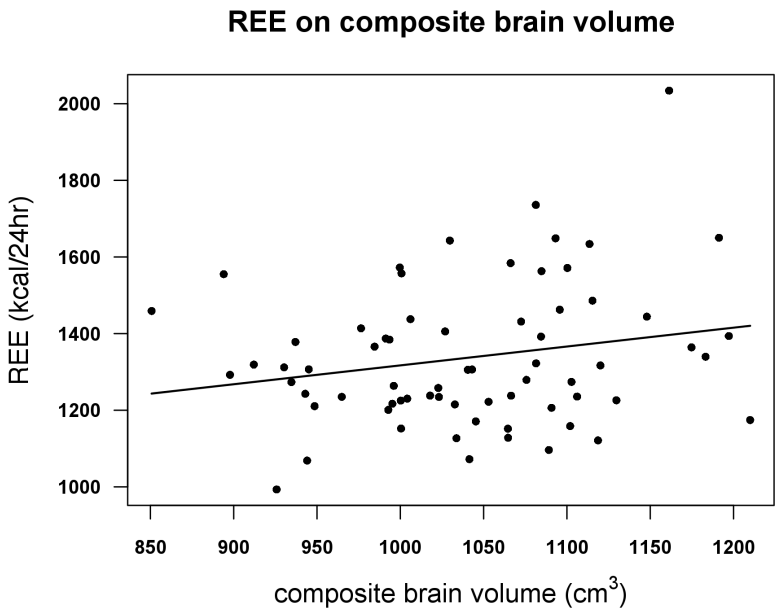


Figure 6-11 REE against composite brain volume.
$r^2 = 0.04$, $p = 0.08$; n = 68

6.4 Assessing variation in tissue-specific metabolic rate

As described, a tissue's SMR, or K_i value, indicates its metabolic expense relative to other tissues in kcal/kg/day. In this section, I explore two methods for assessing the K_i values of organs and tissues measured in my sample. The first is the univariable method employed by Wang and colleagues (2010), which I introduced in Section 6.1, although I employ my measure of FM in place of AT. The second is a multivariable method, whereby the same 7 components used in the univariable method – brain, liver, kidney, heart, SM, FM and residual masses – are entered into a regression model to predict REE. It is recognized from the outset that both methods have limitations, which I discuss further in Section 6.7.

6.4.1 Assessing K_i values with a univariable regression method

In previous research (e.g. Gallagher et al., 1998; Illner et al., 2000; Bosy-Westphal et al., 2004; Midorikawa et al., 2007; Wang et al., 2010), body mass (BM) has been treated as the sum of seven body components:

(6-1) $$\mathrm{BM} = M_{brain} + M_{heart} + M_{kidneys} + M_{liver} + M_{SM} + M_{FM} + M_{residual}$$

where M is mass of the specific body component. Residual mass comprises tissues including blood, bone, skin, stomach and intestines, connective tissue, and the lungs (Gallagher et al., 1998; Wang et al., 2010). It is calculated as total body mass minus summed brain, heart, liver, kidneys, SM and FM.

REE is considered the sum of the products of each body component mass and its corresponding individual resting metabolic rate:

(6-2) $$\mathrm{REE} = \sum(K_i * M_i)$$

where K_i is the specific resting metabolic rate in kcal/kg/day for the individual body component ('i'), and M_i is the mass of the component in kilograms.

As described in Section 6.1, several studies have used K_i values reported by Elia (1992) for the 7 organs and tissues in Equation 6-1 (see Table 6.3 below). The K_i values vary in size; those for brain and liver are about half the size of those estimated for the heart and kidneys. Values for SM and FM are 1/34 and 1/98 the size of the heart and kidneys, respectively.

Table 6.3 Elia's K_i values

Organ/tissue	Elia's K_i value (kcal/kg/day)[1]
Brain	240
Heart	440
Kidneys	440
Liver	200
SM	13
FM	4.5
Residual	12

[1]K_i values from Elia, 1992

Incorporating Elia's tissue-specific metabolic rate values into Equation 6-2, whole-body REE is calculated thus:

(6-3) $$\mathrm{REE} = 240M_{brain} + 440M_{heart} + 440M_{kidneys} + 200M_{liver} + 13M_{SM} + 4.5M_{FM} + 12M_{residual}$$

To evaluate the applicability of Elia's K_i coefficients across adults, Wang et al. (2010) developed a statistical approach which included the following steps:

An REE value for each organ or tissue was calculated by holding the remaining organs and tissues at Elia's K_i values, for example:

(6-4) $$\mathrm{REE}_{liver} = \mathrm{REE} - (240M_{brain} + 440M_{heart} + 440M_{kidneys} + 13M_{SM} + 4.5M_{FM} + 12M_{residual})$$

Least-squares univariable regression was then carried out as:

(6-5) $$\mathrm{REE}_{liver} = K_{liver} * M_{liver}$$

where the regression coefficient of REE_{liver} on liver mass is the K_i value for liver.

These steps were repeated for each of the remaining organs and tissues to derive their K_i values and CIs. Equation 6-5 contains no intercept, indicating Wang and colleagues forced the regression through the origin. Using this method, the authors derived K_i values relatively similar to those of Elia; Elia's values fit within the 95% CIs constructed around Wang et al.'s sample coefficients. Using my dataset, I followed the steps outlined in Equations 6-4 and 6-5 for each of the 7 components to test whether I might similarly find K_i values similar to Elia's.

First, I converted my measured volumes to mass using known tissue density values (Duck, 1990; Table 6.4). These were multiplied by organ volumes, and mass in grams was converted to kilograms. I used the composite brain variable.

Table 6.4 Organ density values and descriptive statistics for organ mass

Organ	Density (g/cm^3)[1]	Mass (kg), Mean±SD	Mass (kg), Range
Brain	1.036	1.1±0.08	0.88-1.25
Heart	1.06	0.5±0.09	0.35-0.83
Kidneys	1.05	0.3±0.05	0.21-0.48
Liver	1.06	1.2±0.21	0.76-1.7

[1]Density values from Duck, 1990

Of the remaining components, FM and SM were originally measured in kilogram units. I calculated residual mass as described above, obtaining a mean of 19.2 kg (SD = 2.13; range 14.7-26.3).

Following Wang et al.'s univariable analysis, I obtained the K_i values shown below. They are accompanied in Table 6.5 by their associated SEs and 95% CIs, and Elia's values for comparison.

Table 6.5 K_i values calculated in the present sample following the univariable method of Wang et al. (2010)

Organ/ tissue	Elia's K_i values	K_i values – this study	SE	95% CI	Absolute difference, mine vs. Elia	% Difference, mine vs. Elia
Heart	440	362	23.8	314.5, 409.3	78	-20
Kidneys	440	295	42.6	209.7, 379.9	145	-40
Brain	240	201	11.7	177.5, 224.3	39	-18
Liver	200	165	10.3	144.1, 185.2	35	-19
SM	13	10	0.8	8.7, 12.0	3	-23
FM	4.5	2.6	0.6	1.4, 3.8	1.9	-54
Residual	12	10	0.7	8.5, 11.1	2	-20

Each of the values derived in my sample are smaller than Elia's, with percentage differences ranging from 17.7 for brain, to 53.5 for FM. The boxplot in Figure 6-12 below shows my coefficients and their associated SEs alongside Elia's values.

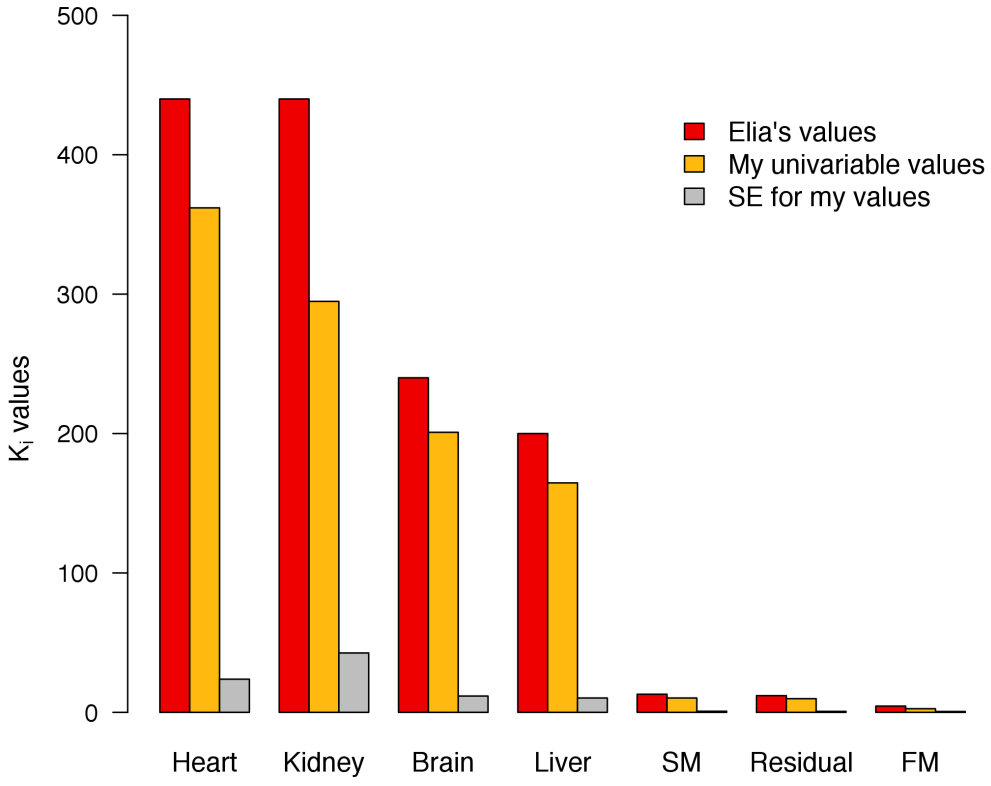


Figure 6-12 Coefficients and their error (gray bars) derived from univariable regression, and Elia's values.

The variation in findings suggests Wang et al.'s (2010) method of setting tissues to Elia's values (see Equation 6-4) may be problematic if these values are not in fact applicable across study samples. Nevertheless, the ranking of tissues by K_i value-size shows consistency: for both Elia's and my derived sample coefficients, the largest values correspond to the heart and kidneys, followed by the brain and liver, and finally SM, residual mass, and FM.

6.4.2 Assessing K_i values with a multivariable regression method

A more straightforward analysis may involve fitting a single multivariable regression model, where measured REE is entered as the dependent variable, the 7 organ/tissue masses are predictors, and the multivariable regression coefficients are interpreted to reflect organ/tissue K_i values. This approach avoids the assumption that tissue-specific metabolic rates in a given sample are in fact equal to Elia's values. I carried out this analysis for comparison with Wang's univariable method (Equations 6-4 and 6-5), and the results are shown in Table 6.6.

Table 6.6 K_i values calculated in the present sample from multivariable regression of REE on the masses of 7 body components

Organ/ tissue	Elia's K_i values	K_i values – this study	SE	95% CI	Absolute difference, mine vs. Elia	% Difference, mine vs. Elia
Heart	440	475	201	73.9, 875.8	35	8
Kidneys	440	196	359	-522.0, 914.2	244	-77
Brain	240	270	97.1	75.3, 463.7	30	12
Liver	200	78	95.4	-113.1, 268.4	122	-88
SM	13	34	11.4	11.4, 57.2	21	90
FM	4.5	5.1	2.2	0.7, 9.6	0.6	13
Residual	12	1.0	9.1	-17.3, 19.3	11	-169

Based on the form of Equation 6-2, and to maintain consistency with the univariable approach, I forced the multivariable regression through the origin. Unlike the univariable method, overall this has a negligible effect on the coefficients and SEs, so that error is generally large and 95% CIs are correspondingly very wide. For liver, kidneys, and residual mass, the error exceeds the size of the coefficient. The boxplot in Figure 6-13 shows the multivariable method-derived coefficients and their associated error, alongside Elia's values for comparison.

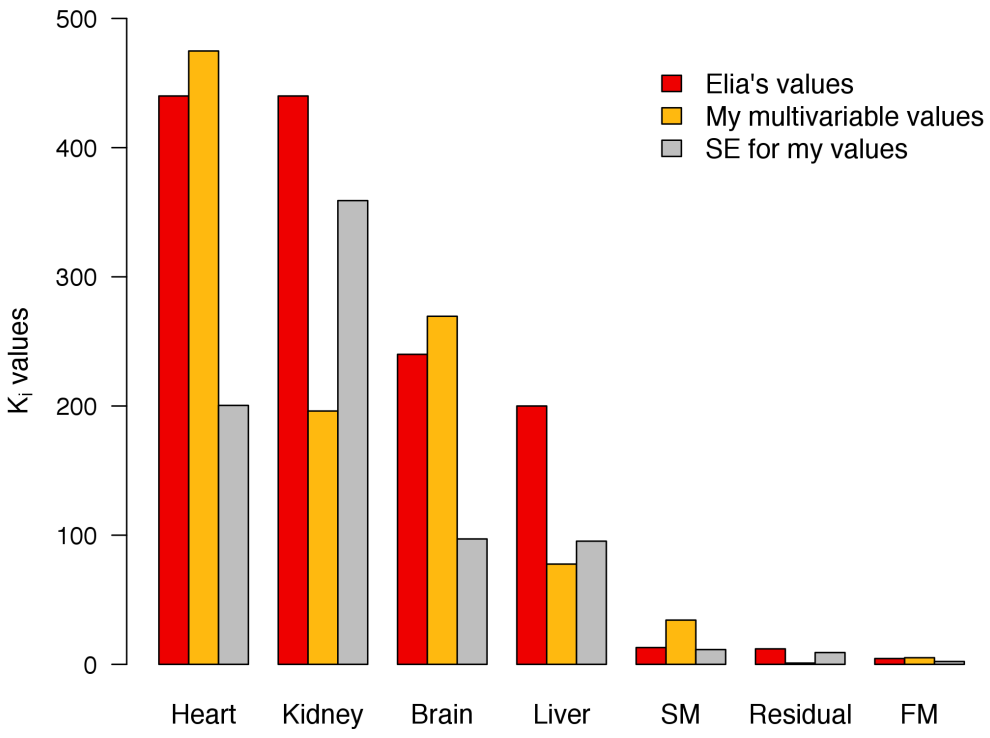


Figure 6-13 Coefficients and their error derived from multivariable regression, and Elia's values.

Given the high degree of error, three of the coefficients are quite close (heart, brain and FM), and ranking by size is generally consistent. However, next to Elia's values my coefficients for the kidneys and liver are much lower, and considering its size, the coefficient for SM is considerably higher.

6.4.3 Proportional contributions of tissues to whole-body REE

Following the analyses described above, I have three sets of K_i values: 1) Elia's values; 2) coefficients derived in my sample following the univariable regression method of Wang et al. (2010); and 3) coefficients derived in my sample using

multivariable regression analysis, where I set 7 tissues as predictors of REE in a single model.

The three sets of values give three different calculations for whole-body REE in my sample. For reference, as described at the beginning of this chapter, average REE measured by IC in my sample was 1,337 (SE = 184, range = 993-2034). I refer to this as REE_m.

Equation 6-6 shows the products of my sample's measured mean organ/tissue masses and Elia's K_i values. Each product is labelled to identify the organ or tissue to which it corresponds. With this model, average REE is 1,382 kcal/day. This differs from REE_m by 45 kcal/day.

(6-6) $$\mathrm{REE} = 259.2_{brain} + 232.76_{heart} + 128.04_{kidneys} + 241.4_{liver} + 198.9_{SM} + 91.35_{FM} + 229.92_{residual}$$

The values shown in Equation 6-7 are the products of average tissue masses in my sample and my univariable regression-obtained K_i coefficients. Again, each value is labelled. Here, average REE is 1,091 kcal/day, which differs from REE_m by 246 kcal/day.

(6-7) $$\mathrm{REE} = 216.97_{brain} + 191.45_{heart} + 85.79_{kidneys} + 198.67_{liver} + 157.59_{SM} + 52.78_{FM} + 187.77_{residual}$$

Finally, Equation 6-8 below shows the products of my sample's average tissue masses and multivariable regression-obtained K_i coefficients. In this model, REE = 1,340 kcal/day. As one would expect, this value is nearly identical to REE_m.

(6-8) $$\mathrm{REE} = 291.06_{brain} + 251.17_{heart} + 57.07_{kidneys} + 93.66_{liver} + 524.79_{SM} + 103.53_{FM} + 18.97_{residual}$$

With whole-body REE calculated using K_i values and mass, it is possible to assess the proportional contribution of each individual organ or tissue to REE (see Figure 6-14). Using the K_i coefficients I obtained following the univariable method, percentage contributions of tissues are similar to those obtained using Elia's coefficients. In contrast, the findings are notably dissimilar for several tissues when the multivariable method-derived values are used.

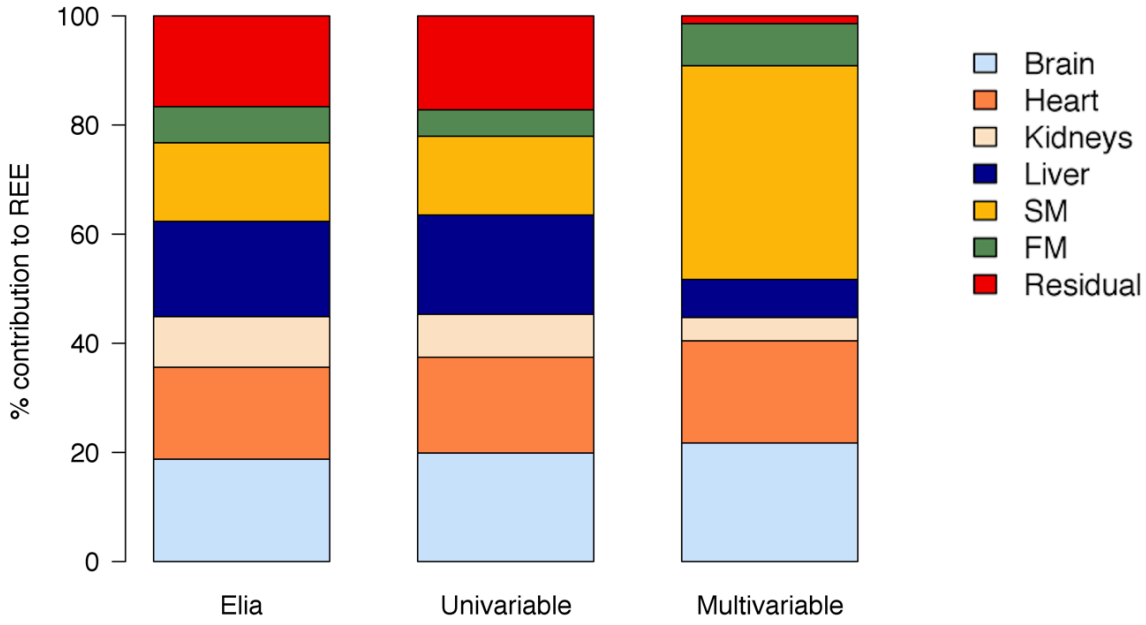


Figure 6-14 Percentage contributions of 7 tissues to REE.

The calculated contributions of the brain and heart to REE demonstrate the most stability across the three models. However, the contribution of SM increases substantially in the multivariable model, where the SM coefficient (34) is more than double Elia's SM K_i value (13) or my univariable value (10). For residual mass the reverse is true, and percentage contributions of kidney and liver masses also

decrease. The increased contribution of FM suggests in the multivariable model that relatively inexpensive fat tissue accounts for a greater proportion of REE than the kidneys or liver, which would contradict the conventional wisdom.

6.5 Exploratory analysis: correlations of brain components with REE

I noted in Section 6.1 that previous authors with similar data to mine have fitted models predicting REE from both brain and body components. I carry out a similar analysis in Section 6.6. It appears, however, that the question of whether brain sub-structures differentially correlate with measured REE has not been tested. I examine this in this section with a small exploratory analysis.

It may be predicted that the components of the brain, a high metabolic rate organ, would correlate significantly with REE (although this prediction is less tenable following the demonstration in Section 6.3 of a non-significant relationship between REE and composite brain). If correlations are observed, it may further be predicted that components demonstrating relatively strong correlations make a greater contribution to REE (although see Brozek and Grande, 1955). First, Table 6.7 shows correlations of REE with raw brain component volumes.

Table 6.7 Pearson correlation coefficients of REE with raw brain volume outcomes

Brain volumes, cm^3	**REE, kcal/24hr** **r, *p***
Gray matter	0.11, 0.37
White matter	0.22, 0.07
Cerebellum cortex	0.27, 0.02
Cerebellum white matter	0.22, 0.07
Amygdala	0.23, 0.06
Hippocampus	0.13, 0.31
Striatum	0.05, 0.71

In contrast to the generally strong associations found among REE and body composition variables (Table 6.2), only the cerebellum cortex is significantly related to REE, with a relatively small coefficient. White matter, cerebellum white matter, and amygdala volumes approach significance. The following selected plots (Figures 6-15 to 6-18) show correlations of REE with raw brain component volumes. I do not show plots of the highly non-significant relationships.

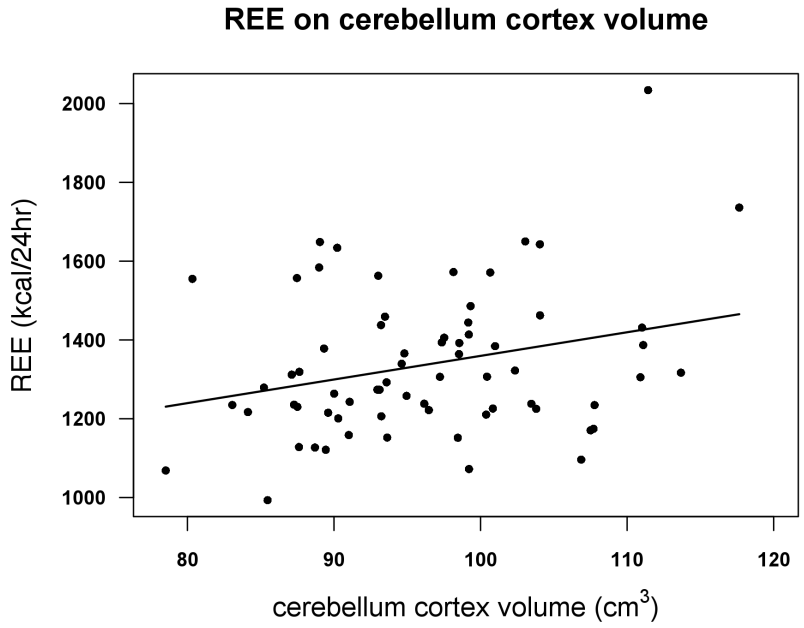


Figure 6-15 REE against cerebellum cortex volume.
$r^2 = 0.07$, $p = 0.02$; n = 68

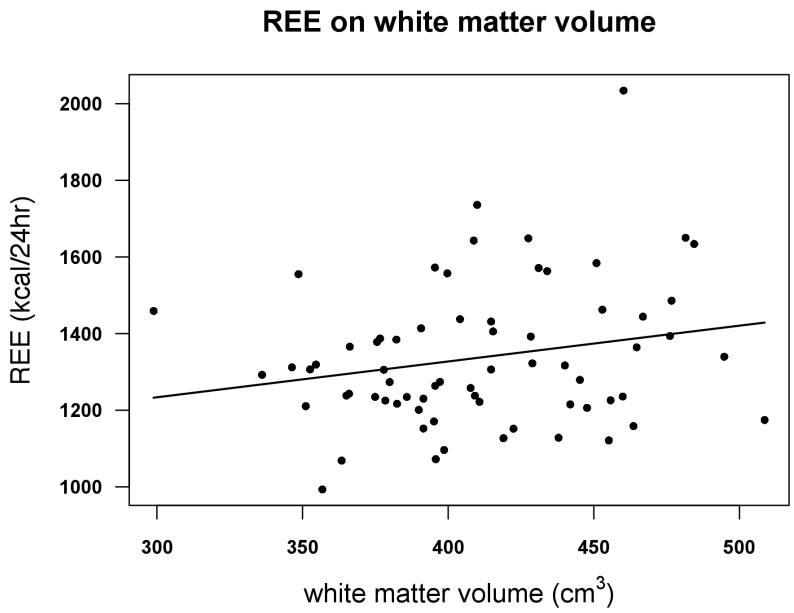


Figure 6-16 REE against white matter volume.
$r^2 = 0.05$, $p = 0.07$; n = 68

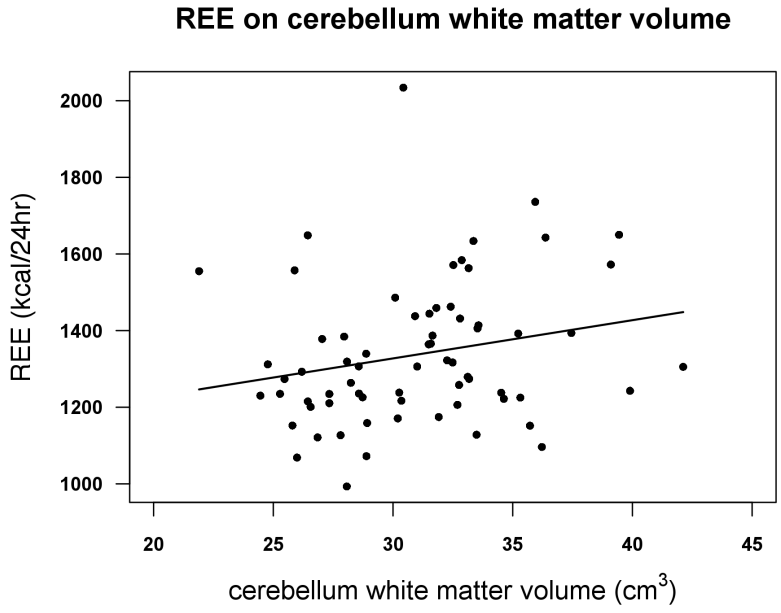


Figure 6-17 REE against cerebellum white matter volume.
$r^2 = 0.05$, $p = 0.07$; n = 68

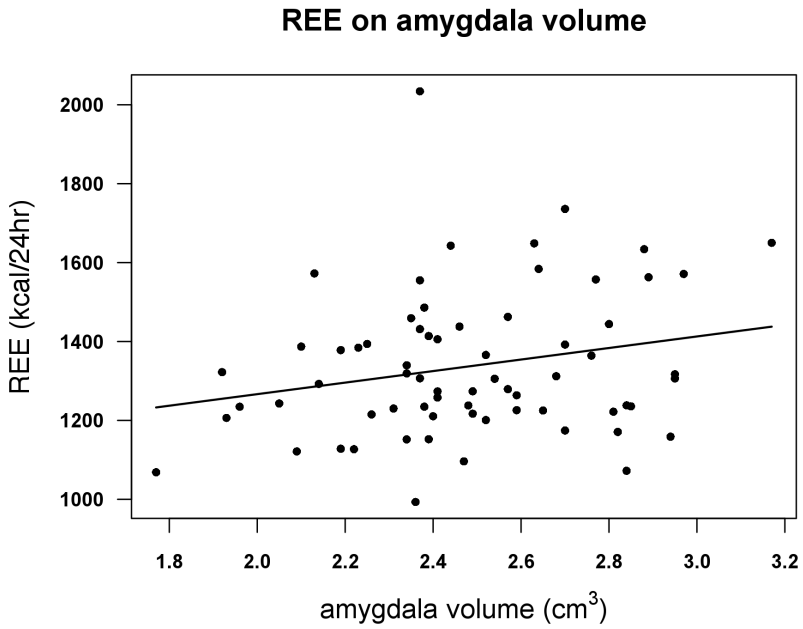


Figure 6-18 REE against amygdala volume.
$r^2 = 0.05$, $p = 0.06$; n = 68

The lack of significant associations between REE and brain volumes suggests the latter will not explain a substantial amount of variance in the former in a larger model. However, to continue the exploratory analysis, I fit a multivariable REE prediction model to investigate the results for each brain volume, holding constant for the others.

In general, tissue mass, not volume, is considered to have a metabolic cost. For this reason, in the rest of this section and the next I again utilize volume-to-mass conversions. I described the conversion for composite brain in Section 6.4. Here, I convert specific brain component volumes to mass using specific density values for gray (1.039) and white matter (1.043; Duck, 1990).

Brain volumes including cerebellum white matter, amygdala, hippocampus and striatum become very small when converted to kilograms, thus I converted all

volumes to grams for this analysis, including composite brain (used in Section 6.4 in kilogram units). Table 6.8 shows descriptive statistics for the composite brain variable and each brain component in grams. For all variables, n = 70.

Table 6.8 Descriptive statistics for composite brain and brain components converted to mass in grams

Brain outcome	Density (g/cm^3)[1]	Mass (g), Mean±SD	Mass (g), Range
Composite brain	1.036	1079±81	881-1254
Gray matter	1.039	476±37	399-556
White matter	1.043	428±44	312-531
Cerebellum cortex	1.039	100±9	82-122
Cerebellum white matter	1.043	32.3±4.2	22.8-43.9
Amygdala	1.039	2.6±0.3	1.8-3.3
Hippocampus	1.039	7.5±0.6	6.0-9.5
Striatum	1.039	18.7±1.5	16.0-22.4

[1]Density values from Duck, 1990

I assume that the gray matter density value is broadly applicable across different gray matter regions (gray matter, cerebellum cortex, amygdala, hippocampus and striatum variables), and that the white matter value applies to white matter and cerebellum white matter. Even if incorrect, the use of these standard values for conversion means that variability in outcomes among subjects will be maintained.

In the model shown in Table 6.9 below, I set REE as the dependent variable, and enter all brain component mass variables as predictors.

Table 6.9 REE = gray matter + white matter + cerebellum cortex + cerebellum white matter + amygdala + hippocampus + striatum

	Coefficient	SE	*p*	R^2
Intercept	788.5	379.9	0.04	0.10
Gray matter	-1.1	0.9	0.25	
White matter	1.6	0.8	0.04	
Cerebellum cortex	5.1	2.9	0.09	
Cerebellum white matter	3.5	6.2	0.57	
Amygdala	173.7	91.4	0.06	
Hippocampus	-22.4	55.4	0.69	
Striatum	-29.4	18.6	0.12	

With all brain variables entered in the model, cerebellum cortex is non-significant (although with $p < 0.1$), whilst amygdala mass remains borderline significant. White matter is now just significant at $p = 0.04$. The model explains 10% of the variance in REE.

In Table 6.10, highly non-significant predictors from the previous model are removed. Here, only cerebellum cortex approaches significance, and model R^2 is slightly reduced.

Table 6.10 REE = white matter + cerebellum cortex + amygdala

	Coefficient	SE	*p*	R^2
Intercept	403.0	312.5	0.20	0.08
White matter	0.53	0.5	0.31	
Cerebellum cortex	4.7	2.5	0.07	
Amygdala	92.5	75.9	0.23	

In both models, few outcomes are significant and R^2 is small. Overall, the preceding exercise suggests that variability in brain components does not significantly explain variability in the body's resting energy budget.

6.6 Explaining REE variability with brain and body components

Although the prediction of REE by brain components alone was largely unsuccessful, I examine in the current section which components may emerge as significant when brain and body variables are entered in an REE-prediction model together. As I noted in Section 6.1, researchers have found variable results using stepwise, multivariable regression to fit models predicting REE from brain and body composition outcomes. The analysis in the current section differs from the multivariable model in Section 6.4, as here I include specific brain components and spleen mass, but not residual mass.

My aim is to make straightforward comparisons of cost amongst different tissues, and for this reason it is important that all variables be expressed in the same units. Incorporating both body and brain masses, a larger range of size necessitated a choice between converting the much larger masses to grams, or the much smaller masses to kilograms. I chose to use all brain/body variables in kilogram units for the present analysis.

As noted above, an additional variable, spleen mass, is incorporated in this section. The spleen is less often tested for its contribution to REE, although some authors have investigated it in this context (Javed et al., 2010; Heymsfield et al., 2012a). Similar to the other organs, MRI-measured spleen volume was converted to mass with the density value given by Duck (1990); descriptive statistics are given in Table 6.11 below. Descriptive statistics for the heart, liver and kidneys were given in Section 6.4 (Table 6.4). Density values for brain components were given in the preceding section (Table 6.8).

Table 6.11 Descriptive statistics and density value for the spleen

Tissue	*n*	Density (g/cm^3)[1]	Mass (kg), Mean±SD	Mass (kg), Range
Spleen	68	1.054	0.14±0.05	0.08-0.33

[1]Density value from Duck, 1990

Model 1 below demonstrates REE regressed on FFM, described by Heymsfield as the 'traditional REE model' (Heymsfield et al., 2012a). In my sample, FFM is a highly significant, positive predictor of REE. FFM alone explains 57% of the variance.

Table 6.12 Model 1: REE = FFM

	Coefficient	SE	*p*	r^2
Intercept	119.5	129.0	0.36	0.57
FFM	32.4	3.4	<0.001	

In Table 6.13, Model 2 incorporates FM, which also positively predicts REE, although with a smaller coefficient than FFM. FFM's coefficient is somewhat reduced with the addition of FM, although model R^2 has increased so that 62% of the variance is explained.

Table 6.13 Model 2: REE = FFM + FM

	Coefficient	SE	*p*	R^2
Intercept	148.8	121.6	0.23	0.62
FFM	27.9	3.5	<0.001	
FM	7.0	2.3	0.003	

As I have described, FFM is a heterogeneous compartment made up of several distinct tissues including SM and organs, and Model 3 on the following page begins to break FFM down into these constituent parts. With an aim to investigate which

specific fat-free tissues in the body significantly predict REE, the FFM variable is removed, and SM and body organ volumes are added in its place. FM remains in the model.

Model 3 demonstrates a further increase in R^2. FM remains significant, though less so, and its coefficient is smaller. Of the added fat-free tissue components, heart mass shows the largest coefficient, and SM also emerges as a highly significant predictor of REE. The liver and kidneys are not significant. The spleen coefficient is negative and non-significant.

Table 6.14 Model 3: REE = FM + SM + heart mass + liver mass + kidney mass + spleen mass

	Coefficient	**SE**	***p***	**R^2**
Intercept	254.9	97.6	0.01	0.67
FM	4.8	2.3	0.04	
SM	36.5	10.1	<0.001	
Heart mass	524.9	212.3	0.02	
Liver mass	89.9	99.7	0.37	
Kidney mass	366.8	372.1	0.33	
Spleen mass	-463.0	320.7	0.15	

In Model 4 below, non-significant liver, kidney and spleen masses are removed and brain component volumes are added.

Table 6.15 Model 4: REE = FM + SM + heart mass + gray matter + white matter + cerebellum cortex + cerebellum white matter + amygdala + hippocampus + striatum

	Coefficient	SE	*p*	R^2
Intercept	-315.4	236.9	0.19	0.72
FM	5.1	2.2	0.02	
SM	42.2	8.2	<0.001	
Heart mass	541.3	192.1	0.007	
Gray matter	-69.3	531.1	0.90	
White matter	231.0	464.4	0.62	
Cerebellum cortex	4860.8	1675.4	0.005	
Cerebellum white matter	-4232.6	3813.1	0.27	
Amygdala	55660.2	52964.6	0.30	
Hippocampus	33081.3	32186.2	0.31	
Striatum	-10062.4	10709.2	0.35	

Of the brain components, only the cerebellum cortex is significant. This is consistent with Pearson correlations given in the previous section's analysis of brain components and REE, where the cerebellum cortex was the only component which appeared to significantly associate with REE, albeit weakly. Here, the coefficient is large and highly significant.

FM, SM and heart mass remain significant, and model R^2 increases to 0.72. Of the variables which demonstrate significance in the model, the coefficient for cerebellum cortex is the largest, followed by the heart, SM and finally FM.

Model 5 removes non-significant predictors from Model 4, with R^2 decreasing slightly from 0.72 to 0.71. Based on the K_i value analysis in Section 6.4, the final model is observed to include both high and low metabolic rate components.

Table 6.16 Model 5: REE = FM + SM + heart mass + cerebellum cortex volume

	Coefficient	SE	*p*	R^2
Intercept	-112.8	161.1	0.49	0.71
FM	4.9	2.1	0.02	
SM	42.2	7.6	<0.001	
Heart mass	533.5	183.6	0.005	
Cerebellum cortex	4249.9	1415.6	0.004	

6.7 Discussion of findings in this chapter

This chapter addressed my first hypothesis, which predicted that organs and tissues would be seen to demonstrate variability in metabolic expense, assessed by SMR, or K_i, values in kcal/kg/day units. I also hypothesized that I would find K_i values similar to those reported in previous analyses in non-South Asian cohorts, as K_i values are apparently generally conserved across development and across healthy adult individuals (Elia, 1992; Wang et al., 2001). Although there has been some indication that the values reported by Elia (1992) are not applicable in older-aged individuals (Gallagher et al., 2000; Bosy-Westphal et al., 2003; Wang et al., 2010), this was not a concern in the current study of individuals aged 20-28 years.

The results of my K_i value assessment in Section 6.4 suggested that brain and body components are indeed differentially metabolically costly, providing support for the first part of my hypothesis, however there was variation in findings between the two methods I employed. My derived values did not match as closely with Elia's values as did those of Wang et al. (2010) using similar methods; therefore, there was less support for my prediction that the values derived in my sample would match the findings reported in non-South Asian cohorts.

Although it would have been ideal to utilize A-V methods and assess organ/tissue K_i values more directly, such methods were not available in the current study, as I

noted in Section 6.1. The univariable and multivariable methods I described in Section 6.4 allowed me to utilize the data I collected to assess the differential expense of body components, however these statistical methods are indirect and both are arguably flawed. The univariable method of Wang et al. (2010) assumes that Elia's K_i values can be accurately applied to each of the 7 components included in the REE/body composition model. The method is somewhat circular, constraining the possible outcome for a given organ or tissue component as the remaining components are held at Elia's values. This is especially problematic if Elia's values are not in fact universally applicable, for example across groups varying by age, developmental experience (see Criscuolo et al., 2008), or ethnicity. When Wang and coworkers applied this method in their 2010 paper, their derived values agreed with Elia's within very narrow confidence intervals. The values I derived in my dataset with the same method were consistently lower than Elia's or those found by Wang et al. (2010). One possible explanation for these differences is that K_i values are more variable across populations than has previously been recognized. The sample analyzed by Wang et al. (2010), for example, was comprised of Europeans recruited in Germany.

The multivariable method I employed appeared more straightforward and avoided the assumption that Elia's values were in fact 'true' values for each body component. However, the multivariable regression model similarly constrained the outcomes for each component, as each 'K_i value' (i.e. each of the regression coefficients) was calculated holding the remaining variables constant. Furthermore, the use of statistical versus physiological methods (i.e. the measurement of organ oxygen uptake *in vivo*) to estimate K_i values has been criticized previously. In an early paper, Brozek and Grande (1955) argued that it was improper to claim that a statistical regression coefficient reflected the oxygen consumption rate of an organ or tissue. They drew the distinction between direct measures and the R^2 value in a multivariable regression, which if conflated, spuriously switches the argument "from a *statistical* into *physiological* accounting,

from the analysis of covariation in body structure and basal metabolism to the partitioning of basal oxygen consumption" (Brozek and Grande, 1955, pg. 25).

The argument of Brozek and Grande (1955) helps to explain the lack of agreement between the univariable and multivariable method outcomes in my analysis. For example, the coefficient for SM in the multivariable model in Section 6.4 was more than double both Elia's SM K_i value, and the value I derived for SM using the univariable method. SM significantly explains a portion of the variance in REE, a finding which similarly emerged from my analysis in Section 6.6, however this does not accurately reflect its K_i value in kcal/kg/day. In the multivariable model there are several additional predictor variables, e.g. organs, with which SM correlates positively, so that the SM coefficient may reflect effects of these variables. Considering this, it is surprising that there in fact was some consistency in the coefficients estimated with the univariable and multivariable methods. There was considerable error associated with the estimates in the multivariable model, however coefficients were similar between methods for the heart, brain and FM, and in both models the brain and organs demonstrated the larger coefficients.

Multivariable regression is thus most profitably used to examine which brain or body components, when they vary, contribute the most to variation in REE. Although imperfect, the univariable method of Wang and coworkers (2010) is preferable to the multivariable method if one's intention is to statistically assess relative organ/tissue metabolism. Indeed, although the K_i values derived in my sample following Wang's method did not match Elia's, they followed a very similar pattern. The current analysis thus demonstrates, consistent with prior literature, that the brain, heart, liver and kidneys have higher energy expenditure per kilogram of tissue than SM or FM. Values are higher for the heart and kidneys than they are for the brain and liver, and the value for SM is larger than that seen for FM. Classically, the brain, heart, kidneys and liver have been designated high metabolic rate organs in comparison to low metabolic rate SM and FM, yet it appears they could be further delineated: based on the K_i values, the heart and

kidneys are the most expensive per kilogram of tissue, followed by the brain and liver, which are intermediate, and finally, SM and FM, where each kilogram of tissue is relatively cheap.

However, a different pattern is observed for the percentage contributions of organs and tissues to whole-body REE, as this depends both on the K_i value and tissue/organ mass. In Chapter 1 (Figure 1-4, Section 1.1.5), I included a figure which showed the percentage contributions of the brain, heart, kidneys, liver, SM, AT and residual mass to REE based on data from the reference female, as reported by Elia (1992). Contributions of the brain and liver (the 'intermediately' expensive tissues) to REE were both 21%, whilst those of the heart and kidneys were just 8% and 9%, respectively, due to their relatively smaller masses. Due to its relatively large mass, the proportional contribution of SM to REE was 16%, which is larger than the figures seen for the heart and kidneys, despite muscle being one of the relatively cheap body tissues.

In my sample, in the current chapter (see Figure 6-14 in Section 6.4 above), I used Elia's values and my univariable method-derived K_i values to calculate the proportional contribution of body components to total REE. Comparing my results with the calculations based on data for the reference female illustrates both similarities and differences. For example, in my cohort, the brain accounts for ~20% of the resting energy budget, which is close to Elia's (1992) calculation for the reference female (21%), and which has been cited by various authors as the proportional contribution of brain metabolism to overall REE in adult humans (e.g. Clarke and Sokoloff, 1999; Raichle, 2006). I calculated the percentage contribution of liver metabolism to total REE as ~17%, somewhat lower than the 21% Elia (1992) reported for the reference female. However, my results for the heart were also ~17%, which is much higher than the 8% reported for the reference female (Elia, 1992).

This difference owes to the larger average heart mass in my study sample, which is 0.53 kilograms compared to 0.24 kilograms in the reference female (Snyder et al., 1975; Elia, 1992). This discrepancy in average size may be explained by a number of factors, including that the reference female data used by Elia in 1992 is relatively old, having been published in 1975 by Snyder and colleagues. Additionally, the dataset was comprised of numerous studies undertaken in various populations at different times, wherein methodology likely differed. However, it appears unlikely that the difference in average heart mass is explained by differing age or body size; in fact, the average age, height, weight, and BMI of my cohort is virtually the same as that reported for the reference female (Later et al., 2010).

The apparently large average heart size in my sample of South Asian women is a surprising finding, as previous autopsy evidence showed that heart mass was reduced by ~30% in Indians, relative to French individuals (Wells et al., 2016). A more recent autopsy study in Indians reported an average heart mass of 0.24 kilograms for females similar in age to my study cohort (Singh et al., 2004). It is possible that imprecision in separating the heart from surrounding blood vessels or pericardial fat during segmentation from MR images led to error in my estimate. A recent imaging study reported an average heart mass of 0.42 kilograms in women of small body size (Davis et al., 2015), which is closer to my results.

Beyond the K_i value analysis, brain and body variables appeared to demonstrate different patterns of association, similar to the results seen in Chapter 5. Correlation analyses showed that REE was associated with all body composition variables, but not composite brain. Within the brain, REE was only weakly correlated with cerebellum cortex volume. A recurring finding in the literature is that FFM is the main determinant of REE (e.g. Zurlo et al., 1990; Sparti et al., 1997; Gallagher et al., 1998; Illner et al., 2000; Müller et al., 2002; Javed et al., 2010). Indeed, FFM, SM and the composite organ variable showed the strongest relationships with REE in my sample (all $r > 0.70$), and I found that FFM alone explained 57% of the variance in REE. With respect to the brain, Gallagher et al.

(1998) found a correlation between brain mass and REE in their sample, however Illner et al. (2000) reported a non-significant correlation between the brain and REE in the female portion of their study cohort.

Finally, my exploratory analysis in Section 6.5 suggested that brain volumes alone are largely unable to account for variance in whole-body REE. This may owe in part to the brain's relatively low variability in comparison to both fat and lean body components, as shown in Chapter 5 by the relative size of CVs. In a regression model, the brain and its component volumes are thus better able to account for the variance in REE once additional variability is accounted for by body outcomes. I observed this in Section 6.6, where the previously weak relationship of cerebellum cortex volume with REE was strengthened in a multivariable model with FM, SM and organs. This analysis showed that FM, SM, the heart, and the cerebellum cortex were significant predictors of REE.

As I described in Section 6.1, other authors have reported differing results of multivariable REE-body composition prediction models, however most have found that both high- and low-metabolic rate tissues predict REE (e.g. Gallagher et al., 1998; Illner et al., 2000; Bosy-Westphal et al., 2004; Heymsfield et al., 2012a), which is consistent with my findings. In each of the previously published studies cited here, SM explained a portion of the variance in REE, whilst the brain or other body organs were not significant in the model. SM has a much lower K_i value than the brain and organs, and is not classically considered an 'active' tissue in the resting state (Brozek and Grande, 1955). In my own model, FM, which has the lowest K_i value relative to the other tissues, significantly predicted REE whilst kidney and liver volumes did not. As I noted above for SM, positive correlations of SM or FM with other model variables could result in SM or FM reflecting effects of the other variables, or potentially displacing them in the model.

7 Trade-offs I. Brain and lean body components

In Chapter 5, I described a pattern of moderate, positive associations amongst most components within the body, and a somewhat weaker pattern amongst comparatively fewer components within the brain. I found virtually no significant associations between brain and body variables, and overall there was no evidence for significant negative correlations. In Chapter 6, I found evidence to suggest that organs and tissues in the body are indeed variably costly.

In this chapter, I test the hypothesis that negative correlations, indicating trade-offs, are observable between the brain and metabolically expensive organs, and between the brain and less-metabolically expensive SM, once body size and head size are taken into account. (I also test for trade-offs between the brain and the FFM variable, as a more global measure of lean mass.) Beyond composite brain volume, I test whether specific brain volumes trade off with organs and/or SM. I predicted in Chapter 2 that gray matter may be more likely than white matter to demonstrate trade-offs, owing to its relatively high energy expenditure.

This chapter's sections are organized with respect to the lean body component being tested against the brain. After I describe statistical methods in Section 7.1, Section 7.2 gives results for brain volume trade-offs with FFM, Section 7.3 gives results for the brain against SM, and Section 7.4 shows findings relating to the brain and body organs. In Section 7.5 I offer a discussion of the findings. I describe results of analyses testing for a trade-off between the brain and FM in Chapter 8.

7.1 Statistical methods

All variables were natural log-transformed to capture allometric relationships and a series of multivariable regression models were fitted. My overall approach was to set a brain component as the dependent variable in each model, and a body organ or tissue variable as the predictor of interest. I included height and TIV (total

intracranial volume) in each model. TIV was described in Chapter 4; it is part of the standard FreeSurfer output. The addition of height served to control for body size, and TIV was added to each model to control for head size. As described in Chapter 2, controlling for body and head size was predicted to allow for the detection of more subtle relationships among traits that otherwise may demonstrate a significant positive correlation, or no correlation.

Height was chosen rather than weight to control for body size because FM, FFM and SM masses are components of weight, which would have resulted in their appearing twice in a given model. TIV is commonly used in volumetric imaging studies to control for variation in head size, which represents a significant source of variation otherwise unaccounted for (Buckner et al., 2004; Sanfilipo et al., 2004; Bickart et al., 2011; Malone et al., 2015).

7.2 Trade-offs among brain components and FFM

In my first model, shown in Table 7.1, I set composite brain volume as the dependent variable. FFM was added as the predictor of interest along with height and TIV. Controlling for height and TIV, FFM is significantly, negatively related to composite brain volume.

Table 7.1 Model 1: Composite brain = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.36	0.59	0.54
FFM	-0.11	0.04	0.016
Height	0.35	0.13	0.006
TIV	0.82	0.06	<0.001

In order to visualize the trade-off by plotting composite brain against FFM, I sought to make new variables where FFM was adjusted for height, and where composite brain volume was adjusted for TIV. This can be carried out using log-log regression

(see e.g. Wells et al., 2002; Wells and Victora, 2005). Regressing natural log-transformed variable X on a similarly transformed variable Y, the slope represents the power (*P*) by which Y should be raised to calculate X/Y^{p} – this is an index which is uncorrelated with the denominator Y (Wells et al., 2002). Following these methods, I regressed log FFM on log height, and then calculated FFM/(height$^{1.53}$), where 1.53 is the coefficient of log FFM on log height. I then regressed log composite brain on log TIV, and calculated the index as composite brain/(TIV$^{0.85}$). This allowed me to completely adjust FFM for height, and composite brain for TIV. Figure 7-1 shows the plot of these adjusted variables.

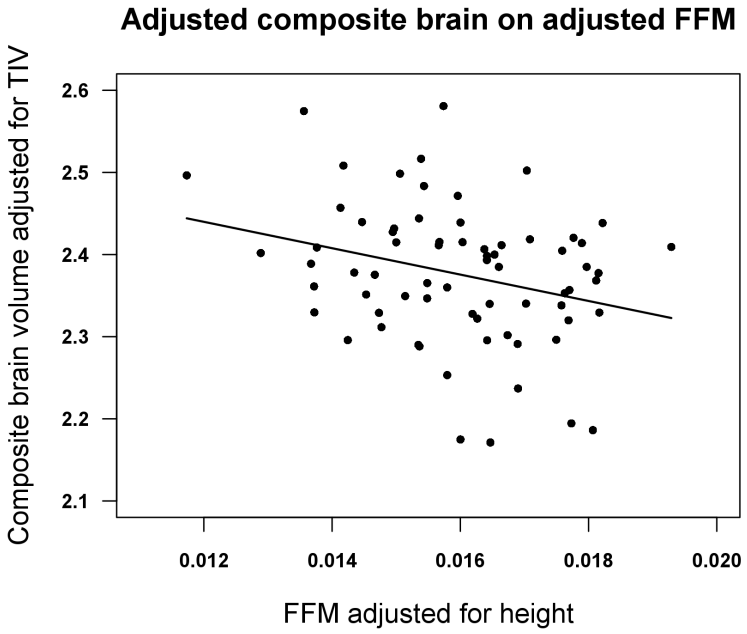


Figure 7-1 Trade-off between adjusted composite brain and adjusted FFM.
$r^2 = 0.07$, $p = 0.016$

A principal goal of my analysis is to look in more detail at how brain components may trade off with body composition variables. For example, is there evidence that specific parts of the brain underlie the brain-FFM trade-off suggested in Model 1?

Previous investigations of brain-body relationships have largely focused on a global brain measure, i.e. overall volume or mass.

Tables 7.2 – 7.5 show the next set of models. These were fitted identically to Model 1, except that I included more specific brain components – gray matter, white matter, cerebellum cortex and cerebellum white matter – rather than composite brain volume as the dependent variable.

Table 7.2 Model 2: Gray matter = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.66	0.72	0.37
FFM	-0.14	0.05	0.010
Height	0.30	0.15	0.056
TIV	0.80	0.07	<0.001

Table 7.3 Model 3: White matter = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-3.07	1.10	0.01
FFM	-0.10	0.08	0.24
Height	0.45	0.23	0.06
TIV	0.99	0.10	<0.001

Table 7.4 Model 4: Cerebellum cortex = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	0.39	1.38	0.78
FFM	-0.08	0.10	0.42
Height	0.28	0.29	0.34
TIV	0.42	0.13	0.002

Table 7.5 Model 5: Cerebellum white matter = FFM + height + TIV

	Coefficient	SE	*p*
Intercept	-2.87	2.06	0.17
FFM	0.14	0.15	0.37
Height	0.41	0.44	0.35
TIV	0.52	0.20	0.01

Consistent with the findings in Model 1, FFM demonstrates a negative coefficient for gray matter, white matter, and cerebellum cortex, although FFM is only significant in predicting gray matter volume. Figure 7-2 shows the gray matter-FFM relationship. I made a new variable where gray matter volume was adjusted for TIV using the log-log regression method described above. Log gray matter was regressed on log TIV, and the index was calculated as gray matter/($TIV^{0.81}$).

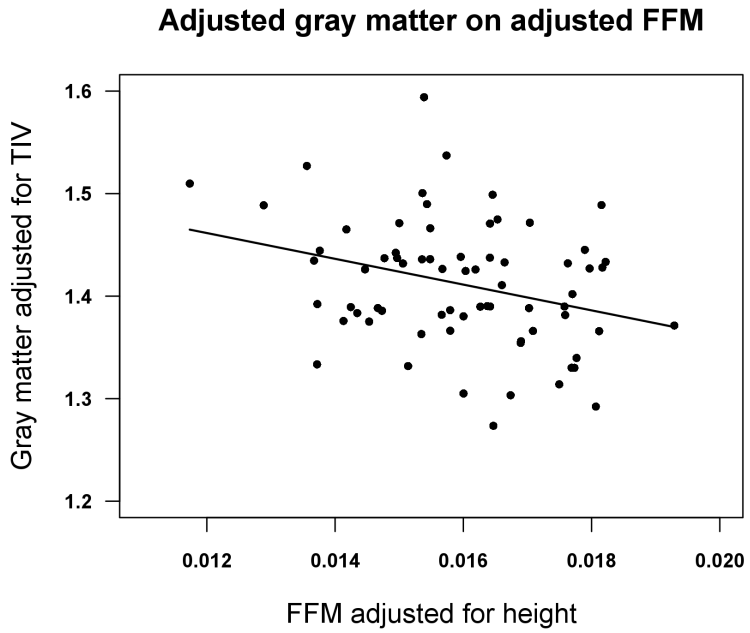


Figure 7-2 Trade-off between adjusted gray matter volume and adjusted FFM.

$r^2 = 0.08$, $p = 0.01$

Tables 7.6 – 7.8 show further models where the amygdala, hippocampus and striatum are set as dependent variables. Relationships among these structures and FFM are not significant, although the *p*-value associated with the FFM coefficient in the hippocampus model is <0.1.

Table 7.6 Model 6: Amygdala = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-1.45	2.03	0.48
FFM	0.04	0.15	0.78
Height	-0.04	0.43	0.92
TIV	0.34	0.19	0.09

Table 7.7 Model 7: Hippocampus = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-1.41	1.06	0.19
FFM	-0.14	0.08	0.088
Height	-0.0003	0.23	0.999
TIV	0.54	0.10	<0.001

Table 7.8 Model 8: Striatum = FFM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-1.44	1.25	0.25
FFM	-0.09	0.09	0.33
Height	0.18	0.27	0.50
TIV	0.52	0.12	<0.001

Findings thus far demonstrate that somatic trade-offs are observable, with a global measure of fat-free tissue appearing to trade off with both composite brain and

gray matter variables. In the next two sections I test the hypothesis, set out in Chapter 2, that the brain trades off with SM and body organs.

7.3 Trade-offs among brain components and SM

In this section, I fit models similar to those in Section 7.2, however now SM is entered as the predictor of interest. A given brain variable is once again added as the dependent variable in each model. Height and TIV are again included to control for body and head size, respectively.

Tables 7.9 and 7.10 show that SM is significantly, negatively related to composite brain volume and gray matter volume.

Table 7.9 Model 9: Composite brain = SM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.41	0.60	0.50
SM	-0.08	0.03	0.017
Height	0.33	0.12	0.008
TIV	0.82	0.06	<0.001

Table 7.10 Model 10: Gray matter = SM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.81	0.72	0.27
SM	-0.12	0.04	0.004
Height	0.30	0.15	0.05
TIV	0.80	0.07	<0.001

Figures 7-3 and 7-4 below show plots to visualize the above relationships. I once again used the log-log regression method to make a new variable for use in plotting

these relationships. Here, I adjusted SM for height by regressing log SM on log height, deriving the index SM/(height$^{1.74}$).

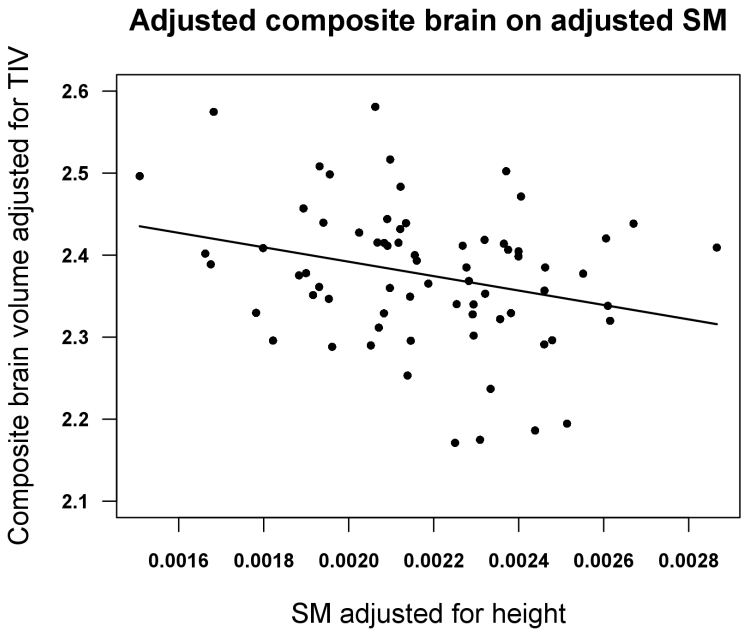


Figure 7-3 Trade-off between adjusted composite brain volume and adjusted SM.

$r^2 = 0.06$, $p = 0.017$

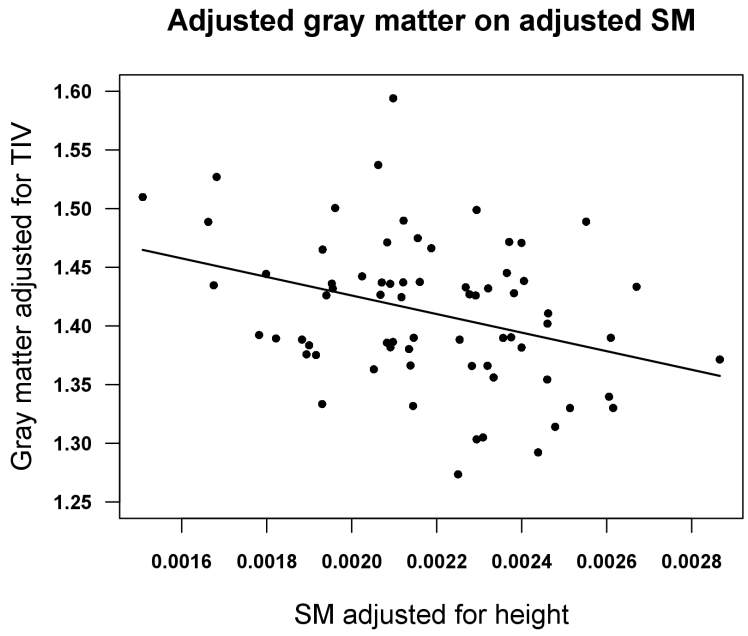


Figure 7-4 Trade-off between adjusted gray matter volume and adjusted SM.

$r^2 = 0.10$, $p = 0.004$

SM was not significant in predicting the remaining brain volume outcomes. Table 7.11 consolidates the results from these models, demonstrating coefficients, SEs and *p*-values for the SM predictor variable.

Table 7.11 Non-significant findings for SM as predictor of brain volume outcomes

Brain variable	SM coefficient	SE	*p*
White matter	-0.06	0.06	0.38
Cerebellum cortex	-0.06	0.08	0.47
Cerebellum white matter	0.07	0.12	0.56
Amygdala	0.02	0.11	0.89
Hippocampus	-0.07	0.06	0.28
Striatum	-0.10	0.07	0.17

7.4 Trade-offs among brain components and organs

Finally, in this section I test whether body organs trade-off with composite brain volume and/or brain components.

Table 7.12 shows an initial model where composite brain is regressed on the composite organ variable, controlling for height and TIV.

Table 7.12 Model 11: Composite brain volume = composite organ volume + height + TIV

	Coefficient	**SE**	***p***
Intercept	0.14	0.55	0.81
Composite organ volume	-0.07	0.03	0.034
Height	0.33	0.12	0.010
TIV	0.79	0.06	<0.001

Consistent with results seen for FFM and SM in preceding sections, composite organ volume is negatively related to composite brain volume, however the *p*-value is somewhat larger. Figure 7-5 below shows the relationship between composite brain volume adjusted for TIV, and composite organ volume adjusted for height. The calculation to derive composite organ volume adjusted for height was: composite organ/(height$^{2.03}$).

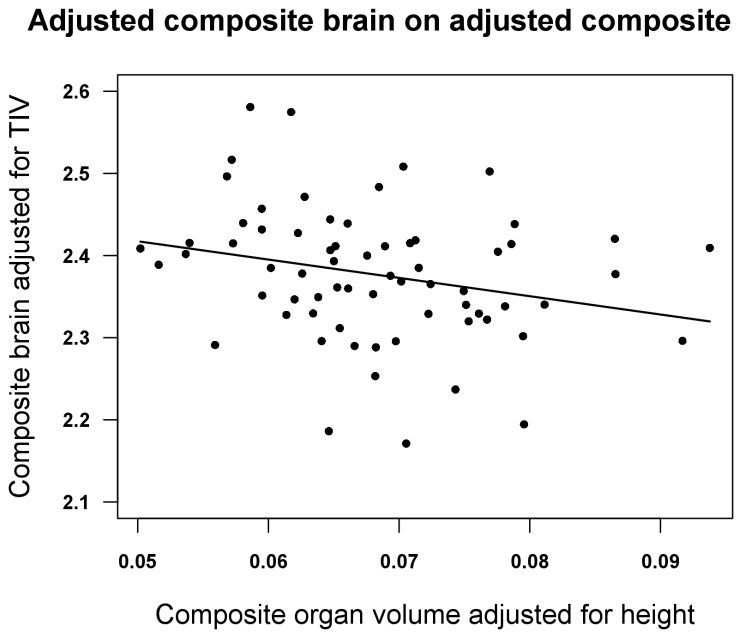


Figure 7-5 Trade-off between adjusted composite brain volume and adjusted composite organ volume.

$r^2 = 0.05$, $p = 0.034$

Composite brain and composite organ variables were both significantly related to potentially confounding menstrual cycle variables (MC1 and MC2) in Chapter 5, Section 5.5.3. Thus, I added MC1 and MC2 variables independently to Model 11 to assess their effect on the composite brain-composite organ association. MC1 and MC2 were highly non-significant in the models (data not shown).

Below, Table 7.13 shows gray matter regressed on composite organ, controlling for height and TIV. The observed trade-off is shown in Figure 7-6.

Table 7.13 Model 12: Gray matter volume = composite organ volume + height + TIV

	Coefficient	SE	*p*
Intercept	-0.21	0.70	0.76
Composite organ volume	-0.11	0.04	0.007
Height	0.30	0.16	0.059
TIV	0.79	0.07	<0.001

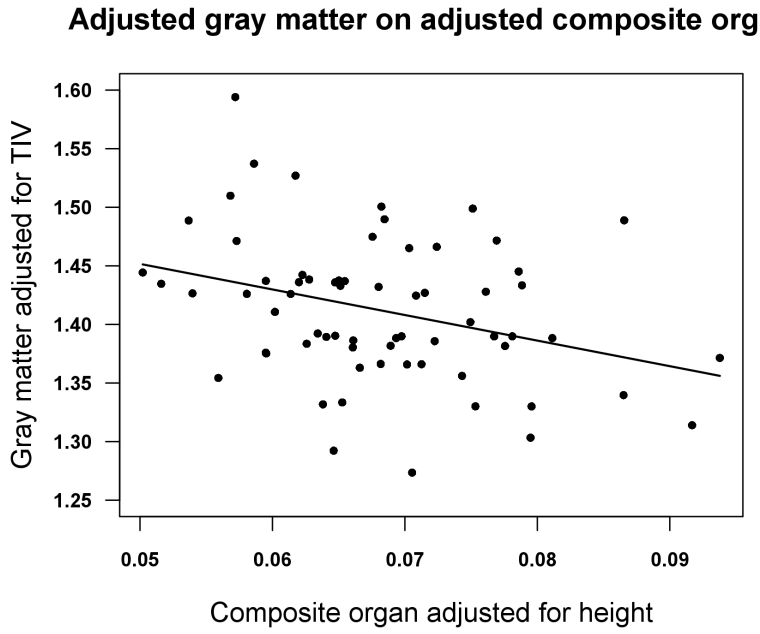


Figure 7-6 Trade-off between adjusted gray matter volume and adjusted composite organ volume.
$r^2 = 0.09$, $p = 0.007$

Once again, I consolidate findings from the remaining models. Table 7.14 shows coefficients, SEs and *p*-values for the composite organ volume predictor variable where results were non-significant. Coefficients for height and TIV are not shown.

Table 7.14 Non-significant findings for composite organ volume as predictor of brain volume outcomes

Brain variable	Composite organ volume coefficient	SE	*p*
White matter	-0.03	0.06	0.60
Cerebellum cortex	-0.07	0.08	0.35
Cerebellum white matter	0.02	0.11	0.86
Amygdala	0.02	0.11	0.85
Hippocampus	-0.04	0.06	0.46
Striatum	-0.05	0.07	0.49

Models which demonstrate significant relationships between brain outcomes and specific organs are given below. All remaining regressions of brain outcomes on organ volumes were non-significant.

First, Table 7.15 demonstrates a significant, negative relationship between composite brain and liver volume (also see Figure 7-7; the calculation to derive liver volume adjusted for height was liver volume/(height$^{1.99}$). Composite brain and liver were associated with menstrual cycle variables MC1 and MC2, therefore I fitted additional models to test the impact of these potential confounders on the liver volume coefficient. Once again, MC1 and MC2 coefficients were very small and highly non-significant in the models, and the data are not shown.

Table 7.15 Model 13: Composite brain volume = liver volume + height + TIV

	Coefficient	SE	*p*
Intercept	-0.16	0.55	0.77
Liver volume	-0.08	0.03	0.004
Height	0.35	0.12	0.004
TIV	0.82	0.05	<0.001

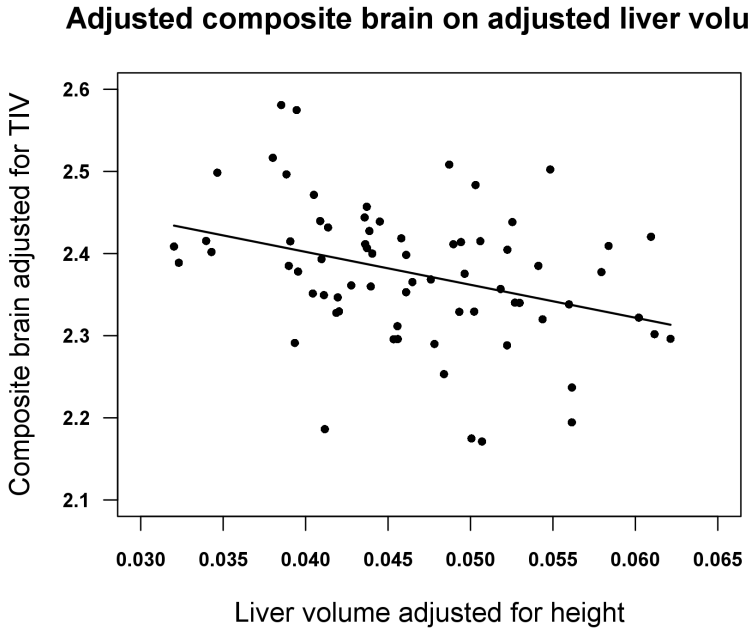


Figure 7-7 Trade-off between adjusted composite brain volume and adjusted liver volume.

$r^2 = 0.10$, $p = 0.004$

Of the remaining organs and brain components, only gray matter on liver and gray matter on kidney volume demonstrated significant relationships. Consistent with results shown so far, the relationships are negative. The models are given below in Tables 7.16 and 7.17, followed by corresponding plots (Figures 7-8 and 7-9). Using the log-log regression method, the calculation to derive kidney volume adjusted for height was kidney volume/(height$^{1.74}$).

Table 7.16 Model 14: Gray matter = liver volume + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.38	0.68	0.58
Liver volume	-0.10	0.03	0.004
Height	0.28	0.15	0.058
TIV	0.80	0.07	<0.001

Table 7.17 Model 15: Gray matter = kidney volume + height + TIV

	Coefficient	SE	*p*
Intercept	-0.41	0.69	0.55
Kidney volume	-0.10	0.03	0.004
Height	0.25	0.14	0.08
TIV	0.81	0.07	<0.001

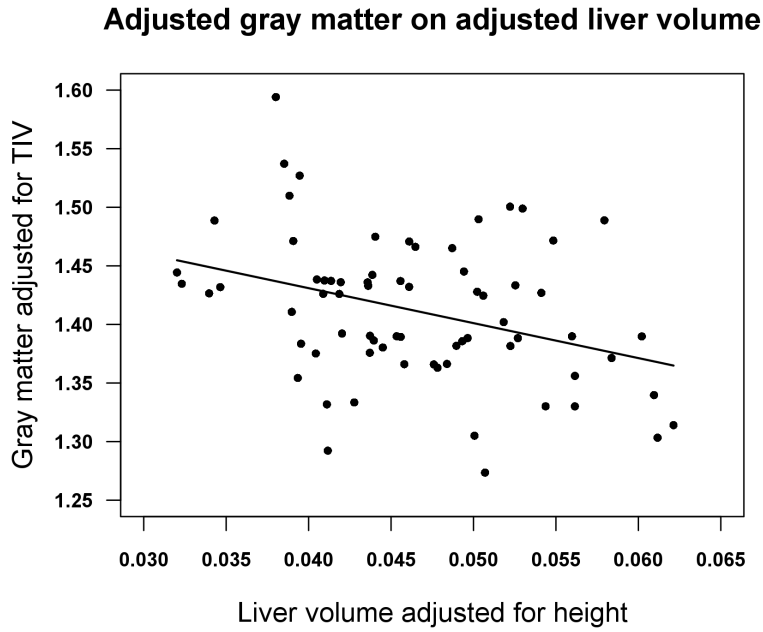


Figure 7-8 Trade-off between adjusted gray matter volume and adjusted liver volume.

$r^2 = 0.11$, $p = 0.003$

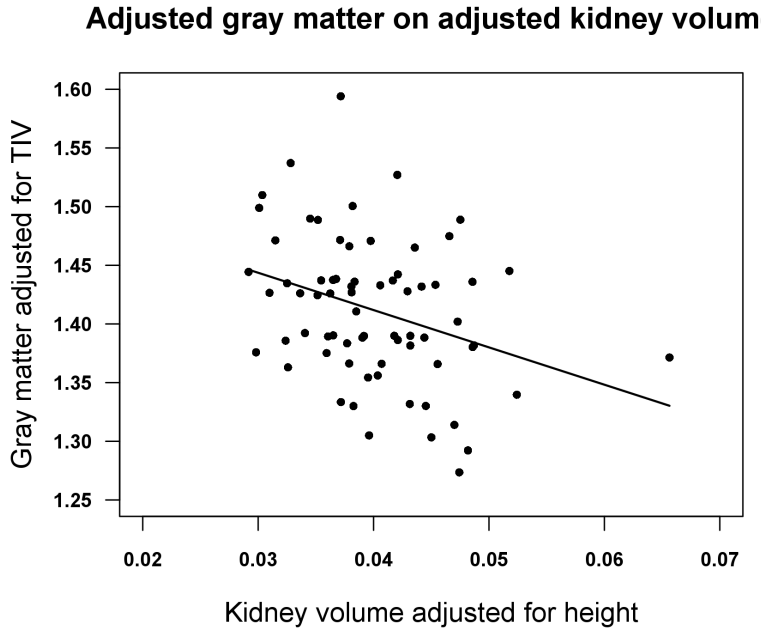


Figure 7-9 Trade-off between adjusted gray matter volume and adjusted kidney volume.
$r^2 = 0.10$, $p = 0.004$

7.5 Discussion of findings in this chapter

The results detailed above provide support for parts (i), (ii), and (iii) of my second hypothesis, as described in Chapter 2. I found that trade-offs were observable between composite brain and organs, between composite brain and SM, and also between composite brain and FFM, controlling for body and head/skull size. With respect to organs, composite brain demonstrated a negative relationship with composite organ and liver volume variables.

When specific brain components were tested against body variables, gray matter was significantly, negatively related to FFM, SM, composite organ, liver volume, and kidney volume. As predicted, trade-offs were not observed for white matter brain components, however they were also not observed for other components of gray matter in the brain, i.e. cerebellum cortex or subcortical structures. Overall,

the results demonstrated a pattern whereby composite brain and gray matter both appeared to trade off against lean organs and tissues in the body, however the associations for gray matter were stronger. This suggests that gray matter may be the principal brain component driving the composite brain-lean tissue trade-offs. The implication is that in my sample, having more or less gray matter occurs in association with having less or more of FFM, SM, and certain organs. These findings are the first, to my knowledge, to demonstrate such relationships in humans at the level of detail provided by this study's methods of measurement.

I discussed earlier in the thesis that Aiello and Wheeler's ETH proposed an evolutionary gut-brain trade-off, whereby the brain came to account for a greater proportion of the energy budget as it increased in size over time, concomitant with the gut becoming smaller and less complex. Hales and Barker's TPH proposed that during development the brain may compete for energy resources with other organs such as the pancreas, resulting in differential growth of these organs. Although I was not able to test these ideas directly without data on the size of the gastrointestinal tract and pancreas, my results provide broad support for the prediction that the brain and metabolically expensive organs in the body compete for resources, and thus may exhibit trade-offs. My findings in Chapter 6 were consistent with the notion that organs are more energetically expensive per kilogram tissue mass than SM or FM.

As I detailed in Chapter 1, support had previously been found for the ETH, however this was largely in non-human animals. The brain and the gut were observed to trade off against one another in fish (Kaufman, 2003; Kotrschal et al., 2013; Tsuboi et al., 2015) and amphibians (Liao et al., 2016), but not in bats (Jones and MacLarnon, 2004). Despite organs being recognized to have relatively high energy expenditure, evidence for trade-offs between the brain and organs other than the gut is lacking in non-humans. The study of bird species carried out by Isler and van Schaik (2006), which I return to below in discussing SM, detected no negative relationships between the brain and organs, including the heart, liver, and kidneys.

In their later test of somatic trade-offs in mammal species, the same authors similarly reported that negative relationships were not detected between brain size and organs including the heart, liver, kidneys and spleen (Navarette et al., 2011). These findings stand in contrast to mine, as I found that the liver and kidneys do appear to trade-off against the brain. However, the cross-species analyses carried out by Isler, van Schaik and colleagues in birds and mammals tested for evolved, genetic trade-offs. The trade-offs I observed in my sample might have a genetic contribution, but it is equally, if not more, likely that my within-species analysis has elucidated trade-offs shaped through life course plasticity.

I also described in Chapter 1 several studies in both humans and non-humans which provided support for the prediction that internal organs including, but not limited to, the pancreas may be reduced in the face of early-life energy stress within the life course. However, several of these studies in humans provided more indirect support, for example using external anthropometric methods of measurement which served as a proxy for organ size (e.g. Campbell and Thoms, 1977; Barker et al., 1993; Yajnik et al., 2003) rather than quantifying organ size with the use of imaging. The study I described by Latini and colleagues (2004) used ultrasonography to measure and compare organ size in SGA and AGA infants, finding that organs including the liver and kidneys were comparatively reduced in the former group. However, they did not investigate potential relationships between the organs and brain. Here, I show that the size of the brain is negatively related to the size of the kidneys and liver in adults, however my data cannot determine which tissues are relatively reduced or increased.

It is difficult to determine the direction of causality in these trade-offs, but it is arguably more likely that the kidneys and liver, rather than the brain, are the components that respond by varying in size. Several lines of evidence suggest that non-brain tissues demonstrate a greater degree of plasticity than the relatively canalized brain. First, I showed in Chapter 5 that in my dataset the CV for composite brain is 7.5%, whilst that for the liver and kidneys is ~17.5%, indicating

the comparatively greater variability of the organs. Second, and consistent with the brain's relatively low CV, head size, a proxy for brain size, has been shown to be highly heritable (Smit et al., 2010; Silventoinen et al., 2011). Third, I reviewed in Chapter 1 evidence for brain-sparing growth; for example, asymmetric IUGR, the most common type of IUGR, is characterized by decreased abdominal size and relatively preserved head size (Brodsky and Christou, 2004). Fourth, head circumference appears to be the least variable body component across populations, as suggested by a large study including individuals from the UK, India, Sri Lanka, and Jamaica (Leary et al., 2006). Thus, my data are not inconsistent with the suggestion by previous authors that organ size is reduced whilst brain size is relatively preserved in South Asians facing competition for energy resources in early life (Yajnik et al., 2003; Yajnik, 2004).

The results I have presented similarly support the prediction that, in addition to organs, SM is traded off against the brain in South Asians (Yajnik et al., 2003; Wells et al., 2016). As I overviewed in Chapter 1, it is well-established that South Asian individuals demonstrate decreased muscle mass, with evidence largely coming from less-comprehensive body composition assessments and comparisons with other populations (e.g. Chowdhury et al., 1996; Rush et al., 2007; Unni et al., 2009). Using simpler techniques, Baker and coworkers demonstrated evidence for the preservation of head girth (a proxy for brain growth) at the expense of an anthropometric proxy for arm muscle growth in US children with low birth weight (Baker et al., 2010). However, the current study is the first to my knowledge to measure brain and muscle tissue in South Asians and test for a brain-SM trade-off relationship directly. With respect to non-human animal studies, Isler and van Schaik (2006) found in birds a negative relationship between brain mass and pectoral muscle mass, which powers flight, however these results were observed across rather than within species, suggesting a genetic basis.

Muscle mass is relatively metabolically inexpensive compared to organs, owing to a relatively low degree of energy turnover per kilogram of tissue at rest. This

knowledge led Aiello and Wheeler (1995) to argue that a decrease in muscle mass would not have provided enough additional energy to fund the larger hominin brain. However other authors, noting that humans are relatively 'undermuscled' compared to non-human primates of similar body size, suggested that systematically building less muscle may have facilitated hominin brain enlargement (Leonard et al., 2003; Snodgrass et al., 2009; Muchlinski et al., 2012). My results suggest somatic trade-offs involving tissues of both greater and lesser metabolic expense exist, and may occur concomitantly, within the same individuals. Although with my dataset I cannot distinguish genetic from environmental/developmental mechanisms underlying observed trade-offs, my findings are nevertheless consistent with the predictions of both Aiello and Wheeler, and Leonard, Snodgrass and colleagues, that trade-offs between the brain and organs, or the brain and SM, may represent evolved aspects of human body composition.

With respect to part (iii) of my second hypothesis, I showed that among brain component volumes gray matter exhibited detectable trade-off relationships with lean body tissues. As noted above, the negative relationships between gray matter and organs, SM and FFM were stronger than those seen between composite brain and body variables. In setting out my hypothesis, I predicted that trade-offs would be observed involving gray matter, but not white matter. This prediction was based on research which has shown that gray matter has a higher energy expenditure than white matter (Hofman, 1983; Karbowski, 2007; Zhu et al., 2012), which I suggested may render it more likely to trade off against other tissues due to increased competition for energy resources. Specifically, gray matter metabolism is greater than that of white matter by a factor of three (Karbowski, 2007), and indeed, I found no trade-off relationships involving white matter volumes.

However, considering the findings for gray matter, it is somewhat surprising that I did not observe trade-offs between the cerebellum cortex, which is the gray matter portion of the cerebellum, and lean body variables. The cerebral cortex is relatively

large in volume, but the cerebellum contains a considerable proportion of the human brain's neurons: roughly half by one estimate (Ramnani, 2006), and as much as 80% by another (Herculano-Houzel 2010). This is notable as considerable energy is expended related to neuronal signaling, and also in the maintenance of neurons at rest (Niven and Laughlin, 2008). As I discussed in Chapter 5, the cerebellum is recognized to play an essential role in myriad functions via its connections with the cerebral cortex (Leiner et al., 1989; Rilling, 2006; Ramnani, 2006; Bostan et al., 2013), and these brain components have been proposed to have experienced coordinated evolution (Barton and Harvey, 2000; Herculano-Houzel, 2010; Balsters et al., 2010). If this is the case, an interesting proposition is that the cerebellum cortex is indeed the more energetically expensive brain component, however it is the cerebral gray matter that trades off a portion of its larger mass against body tissues in the face of somatic competition for energy resources. In Chapter 6, the cerebellum cortex was the only brain component found to be significant in explaining a portion of the variance in REE, although as I discussed, this may not directly reflect the relative size of the tissue's K_i value.

Few previous studies have looked within the brain in the course of investigating somatic trade-offs. However, in line with my findings, a recent imaging study found that regional gray matter brain volumes were negatively associated with FFM (Weise et al., 2013). The authors were in fact investigating relationships between brain and body composition in the context of obesity, although they reported that their sample was comprised of healthy adults. They found negative associations among gray matter brain regions and FM, however these were not significant with FFM adjustment. In the next chapter, I investigate whether negative associations are observable between the brain and FM in my study sample.

8 Trade-offs II. Brain and FM

In Chapter 7, I showed that composite brain and gray matter volumes traded off against lean body components including FFM, SM and organs. Here, I test if a similar trade-off is observed amongst brain volumes and FM. The motivation to test for this largely came from results reported by Navarette and coworkers (2011). The authors' investigation of somatic trade-offs across mammal species identified a negative relationship between adipose depots and brain volume (i.e. the capacity to store energy, or leverage increased cognition to acquire it; Kaplan et al., 2000; Wells, 2016). However, this association was largely found in the non-primate mammal portion of their sample. Results for primates were more equivocal, and the notion of an evolutionary brain-fat trade-off remains untested within a species, or more specifically, within humans.

Section 8.1 describes statistical methods for the chapter, Section 8.2 gives results of brain-FM regression models, and I discuss the findings in Section 8.3.

8.1 Statistical methods

Once again, all variables were natural log-transformed to capture allometric relationships. A series of multivariable regression models were fitted where a given brain component was entered as the dependent variable, and FM was added as the predictor of interest. In each model, height and TIV are entered to control for body and head size, respectively.

8.2 Trade-offs among brain components and FM

Table 8.1 below shows the first model. Composite brain volume is entered as the dependent variable, with FM as predictor. The FM coefficient is negative, but not significant.

Table 8.1 Model 1: Composite brain volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	0.12	0.58	0.83
FM	-0.002	0.01	0.87
Height	0.20	0.11	0.08
TIV	0.81	0.06	<0.001

As in Chapter 7, I next look in more detail within the brain. The highly non-significant relationship between FM and composite brain seen in Model 1 suggests that the brain's component volumes will likewise be uncorrelated with FM. I demonstrate the results for gray matter, white matter, cerebellum, and subcortical structures against FM in Tables 8.2 – 8.8 below.

Table 8.2 Model 2: Gray matter volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.02	0.71	0.98
FM	-0.02	0.02	0.22
Height	0.13	0.14	0.38
TIV	0.78	0.07	<0.001

Table 8.3 Model 3: White matter volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-2.64	1.05	0.01
FM	0.01	0.02	0.77
Height	0.30	0.21	0.16
TIV	0.99	0.11	<0.001

Table 8.4 Model 4: Cerebellum cortex volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	0.76	1.31	0.56
FM	0.01	0.03	0.71
Height	0.15	0.26	0.57
TIV	0.42	0.13	0.002

Table 8.5 Model 5: Cerebellum white matter = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-3.52	1.93	0.07
FM	0.07	0.04	0.13
Height	0.52	0.38	0.18
TIV	0.57	0.20	0.005

Table 8.6 Model 6: Amygdala volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-1.66	1.89	0.39
FM	0.05	0.04	0.23
Height	-0.06	0.38	0.88
TIV	0.38	0.19	0.06

Table 8.7 Model 7: Hippocampus volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-0.80	1.03	0.44
FM	0.01	0.02	0.79
Height	-0.21	0.20	0.32
TIV	0.53	0.10	<0.001

Table 8.8 Model 8: Striatum volume = FM + height + TIV

	Coefficient	**SE**	***p***
Intercept	-1.04	1.18	0.38
FM	0.005	0.03	0.87
Height	0.04	0.24	0.85
TIV	0.52	0.12	<0.001

Consistent with the results in Model 1, the remaining models demonstrate that no brain component volumes are significantly related to FM. With the exception of composite brain and gray matter, all coefficients are positive. All *p*-values are highly non-significant.

8.3 Discussion of findings in this chapter

The findings above are in line with my prediction (set out in Hypothesis No. 2, part (ii)) that FM would not be observed to trade off against the brain in my sample of human subjects. Thus, the current results do not support in humans the findings reported by Navarette et al. (2011) across mammal species.

Navarette and coworkers (2011) suggested that the mammals in their sample traded off fat against the brain, rather than investing in both starvation-buffering strategies. As I described in Chapter 1, Heldstab et al. (2016) found in a follow-up study that a body fat proxy measure was negatively correlated with brain size, although this was apparent in arboreal, not terrestrial species.

An evolutionary trade-off between brain volume and FM in humans appears unlikely as, in addition to a pattern of terrestrial locomotion, *Homo sapiens* are positive outliers for both adiposity and brain size relative to body size (Wells, 2012d; Heldstab et al., 2016). Adult males and females from non-western populations are fatter than other primates (Heldstab et al., 2016). For example, Hadza female foragers have been found to demonstrate on average 20% of weight

as fat (Sherry and Marlowe, 2007; Pontzer et al., 2012) in comparison to *Pan paniscus* females (<10% fat; Zihlman and Bolter, 2015), and wild toque macaque (*Macaca sinica)* females (~3% fat on average; Dittus, 2013). Human infants are likewise fatter than infants of most other ape species, which has been argued to have facilitated increased brain size (e.g. Kuzawa, 1998; Cunnane and Crawford, 2003), although increased infant adiposity may have evolved first (Wells, 2012a).

Taking a life course perspective, the brain and FM may be less likely to trade off due to differences in their developmental trajectories. Normative structural brain changes are documented into adolescence and adulthood (Sowell et al., 2003; Gogtay et al., 2004), however similar to body organs, fetal life and early infancy are recognized as critical periods for brain development (Fox et al., 2010; Stiles and Jernigan, 2010). As noted above, human infants typically display high adiposity at birth, however several studies have found inconsistent correlations between birth weight and later fatness (Wells et al., 2007). During puberty, hormones are a key driver of fat deposition (Wells, 2007b), and as FM remains relatively plastic across the life course, factors such as diet and physical activity also impact adiposity. Thus, the brain and FM may not experience a high degree of direct competition for energy in relation to tissue accretion, whilst the maintenance costs of fat are also very low, suggesting that a trade-off between these two organs over ongoing 'running costs' is similarly unlikely. Alternatively, pubertal and/or adult lifestyle effects on FM might render a trade-off less likely to be observed.

In fact, prior imaging studies investigating the impact of obesity and visceral fat on brain structure and function have demonstrated negative brain-adiposity relationships in humans. For example, BMI and measures of visceral fat were associated with reduced gray matter volume and reduced integrity of the brain's white matter tracts (Debette et al., 2010; Stanek et al., 2011; Veit et al., 2014). However, a negative correlation between brain volume and adiposity in this context indicates brain tissue atrophy associated with a pathological level of fat

accumulation (Willette and Kapogiannis, 2015), rather than an evolved trade-off between fat stores and brain tissue in competition for energy resources. The negative associations observed in these imaging studies apparently emerge within the life course, however I did not detect evidence of such relationships in the current analysis.

9 Does early-life development shape trade-offs?

Several trade-offs among brain and lean body components were demonstrated in Chapter 7. In this chapter, I use the variables birth weight and tibia length to investigate whether I can detect an influence of early-life development on the observed trade-off relationships. First, Section 9.1 describes statistical methods for the chapter. Next, Section 9.2 gives descriptive statistics for birth weight and tibia length, and describes their relationships to brain and body composition variables.

In Section 9.3, I revisit the regression models from Chapter 7 where significant, negative correlations were identified. I add birth weight and tibia length to the models to assess their potential impact on the trade-off relationships therein. Finally, I discuss the findings in Section 9.4.

9.1 Statistical methods

As described in Chapter 4, I obtained data on birth weight and gestational age from study participants. This allowed me to adjust birth weight for gestational age by calculating birth weight SDS. Birth weight SDS were derived using UK-WHO reference birth centiles (Cole et al., 2011).

Brain and body variables, including tibia length, were natural log-transformed. Pearson correlations were utilized to explore relationships among birth weight SDS, tibia length, height-residual (see below), and brain and body components. Data were plotted to visualize significant associations.

Birth weight SDS and tibia length were added to the regression models from Chapter 7 which demonstrated significant brain-body trade-offs. Birth weight SDS was uncorrelated with tibia length ($r = 0.04$, $p = 0.72$), thus these variables

functioned as independent proxies of fetal weight gain and linear growth, respectively, with previous research emphasizing that the majority of variability in limb proportions develops in postnatal life (e.g. Jantz and Jantz, 1999; Cole, 2000; Bogin and Varela-Silva, 2010; Pomeroy et al., 2014).

In Chapter 7, height was added to each model to control for variability in body size, and I also sought to control for the effects of body size in the current analysis. However, adding both height and tibia length variables to a given model would result in tibia length appearing twice, as tibia length contributes to total height. This could potentially upset the models and generate spurious results.

Thus, I derived a new variable for use in regression analyses in the current chapter. Specifically, height was regressed on tibia length and the residuals taken to obtain a 'height-residual' variable that is independent of variability in tibia length. This allowed me to utilize tibia length as a developmental marker, and also control for body size without entering tibia length into the model twice.

9.2 Associations of developmental variables with brain/body outcomes

Table 9.1 below shows descriptive statistics for birth weight, gestational age, birth weight SDS, and tibia length. One subject did not report her birth weight, whilst the same subject plus two others were not able to obtain their gestational age. I am thus missing birth weight SDS data for three subjects, as reflected in the table.

As noted in my inclusion criteria in Chapter 4, I aimed to include in the study only women who had been born at term (gestational age >37 weeks). Early in recruitment a miscommunication regarding this information led to one subject being recruited and measured who subsequently reported a gestational age of 34 weeks. All other subjects ranged from 37-42 weeks' gestation.

Table 9.1 Raw values for developmental variables

	n	Mean±SD	Range
Birth weight, kg	69	3.2±0.5	2.0-4.5
Gestational age, wks	67	39.3±1.5	34.0-42.0
Birth weight SDS	67	-0.3±1.1	-2.9-2.6
Tibia length, mm	70	368±25	300-429

Table 9.2 below shows correlations of birth weight SDS, tibia length, and also height-residual with brain and body composition outcomes. Birth weight SDS is not significantly correlated with weight or height, nor any brain or body components.

In contrast, tibia length is positively correlated with all body organs/tissues except FM, and also with gray matter, white matter, cerebellum white matter, and composite brain volumes. Tibia and height are predictably strongly related, as tibia is a component of height. Beyond this, with respect to body variables, the largest coefficients are found for tibia length with FFM, SM, heart and composite organ. For brain outcomes, the relationship between tibia and composite brain has the largest coefficient, followed by that seen with white matter. However, coefficients are broadly similar in size for significant outcomes within the body, and for those within the brain. Although the correlations appear somewhat stronger for body relative to brain outcomes with tibia, there is overlap in the size of the coefficients. The pattern of significant findings and the size of the tibia coefficients (i.e. their ranking in size across the different outcomes) are consistent with results shown in Chapter 5 (Tables 5.7 and 5.10) for brain and body variables against total height.

With respect to the height-residual variable, no findings are significant with brain volumes, and significant coefficients with body variables are fewer. Of the latter, coefficients are generally smaller than those seen with tibia length, however weight and FM are positively associated with height-residual. Overall, tibia length as a component of height may better explain variability in brain and body outcomes.

Table 9.2 Pearson correlation coefficients of birth weight SDS, tibia length and height-residual with brain and body composition outcomes

	Birth weight SDS r, *p*	Tibia length r, *p*	Height-residual r, *p*
Weight, kg	0.04, 0.76	0.18, 0.14	0.38, 0.001
Height, cm	0.11, 0.36	0.88, <0.001	--
FM, kg	-0.01, 0.96	-0.01, 0.96	0.26, 0.03
FFM, kg	0.09, 0.47	0.41, <0.001	0.39, <0.001
SM, kg	0.02, 0.87	0.40, <0.001	0.29, 0.01
Organ volumes, cm^3			
Heart	0.11, 0.40	0.43, <0.001	0.26, 0.03
Liver	0.08, 0.51	0.36, 0.002	0.29, 0.02
Kidney	0.11, 0.37	0.37, 0.002	0.19, 0.11
Spleen	0.06, 0.65	0.30, 0.01	0.11, 0.39
Composite organ	0.14, 0.28	0.43, <0.001	0.30, 0.01
Brain volumes, cm^3			
Gray matter	0.06, 0.62	0.31, 0.008	0.13, 0.27
White matter	0.07, 0.60	0.35, 0.003	0.17, 0.16
Cerebellum cortex	-0.10, 0.41	0.18, 0.13	0.11, 0.38
Cerebellum white matter	0.03, 0.84	0.28, 0.02	0.12, 0.33
Amygdala	-0.05, 0.71	0.06, 0.63	0.08, 0.53
Hippocampus	-0.07, 0.55	0.13, 0.28	-0.04, 0.72
Striatum	0.05, 0.67	0.20, 0.09	0.05, 0.71
Composite brain	0.06, 0.64	0.37, 0.001	0.17, 0.17

Plots below demonstrate the significant relationships found between the tibia and brain/body variables.

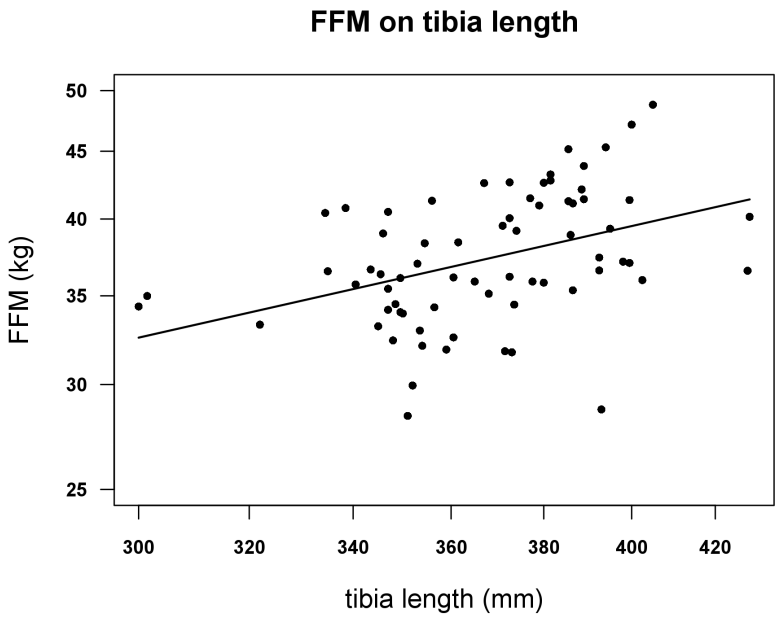


Figure 9-1 FFM against tibia length.
$r^2 = 0.17$, $p < 0.001$; n = 70

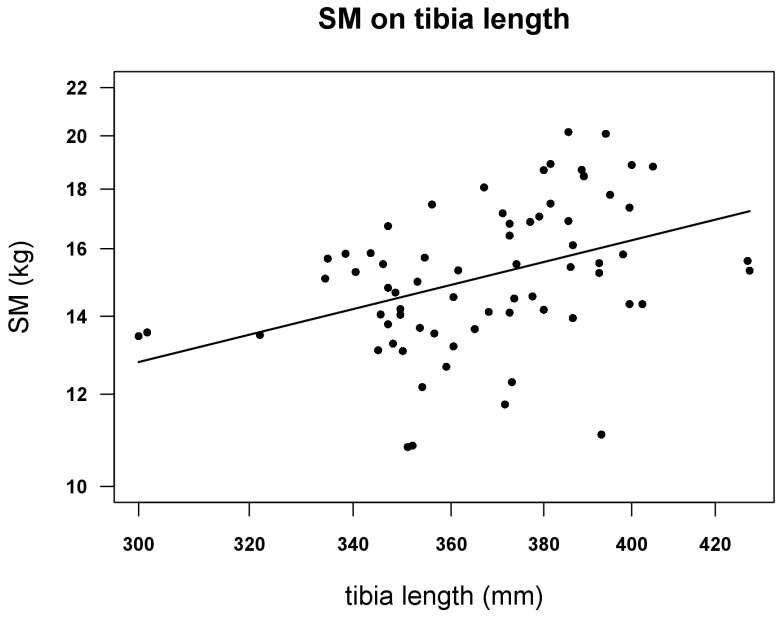


Figure 9-2 SM against tibia length.
$r^2 = 0.16$, $p < 0.001$; n = 70

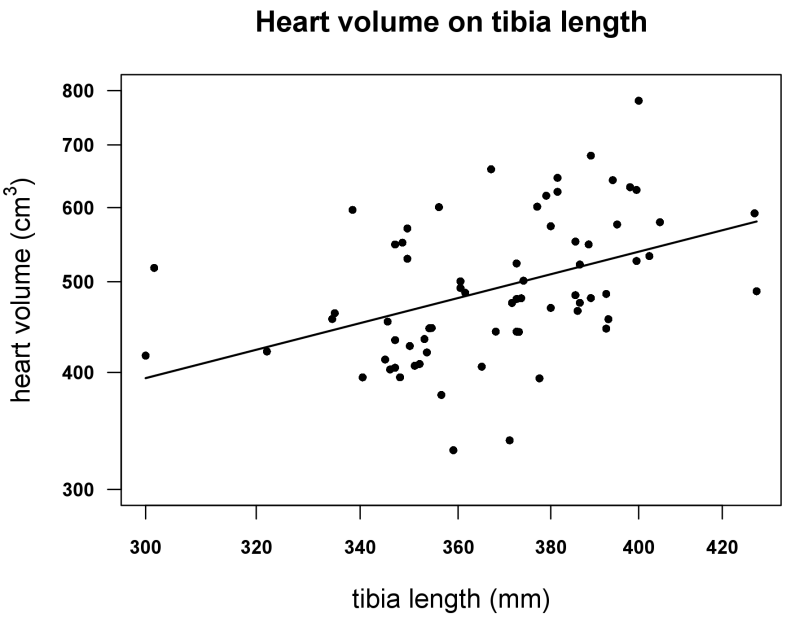


Figure 9-3 Heart volume against tibia length.
$r^2 = 0.18$, $p < 0.001$; n = 69

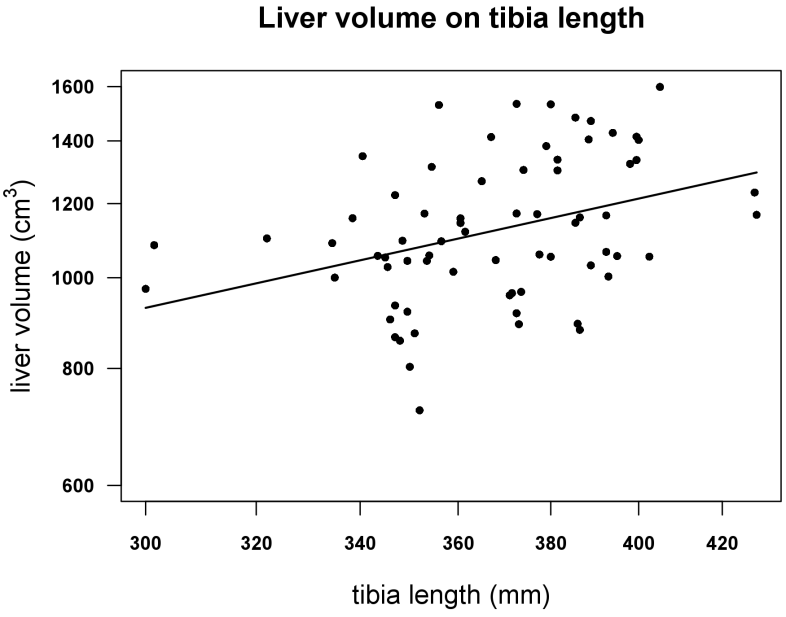


Figure 9-4 Liver volume against tibia length.
$r^2 = 0.13$, $p = 0.002$; n = 70

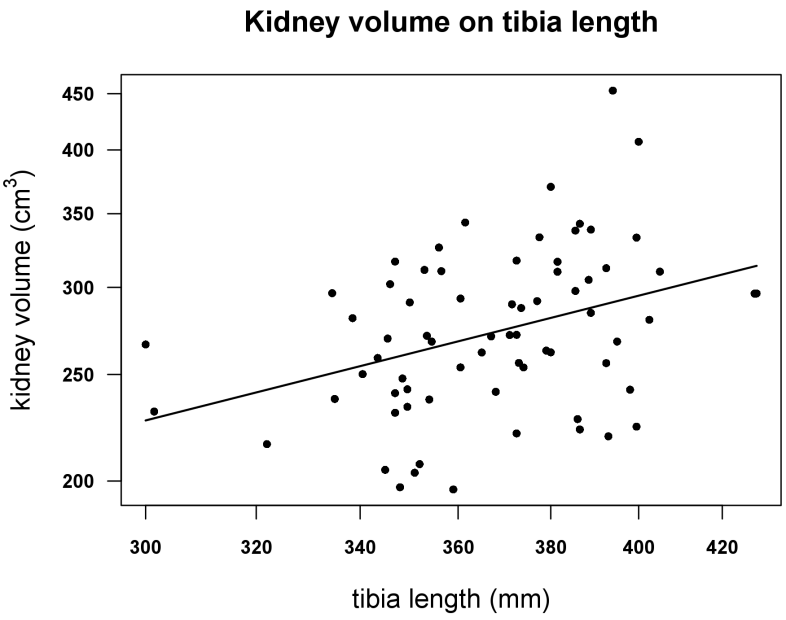


Figure 9-5 Kidney volume against tibia length.
$r^2 = 0.14$, $p = 0.002$; n = 70

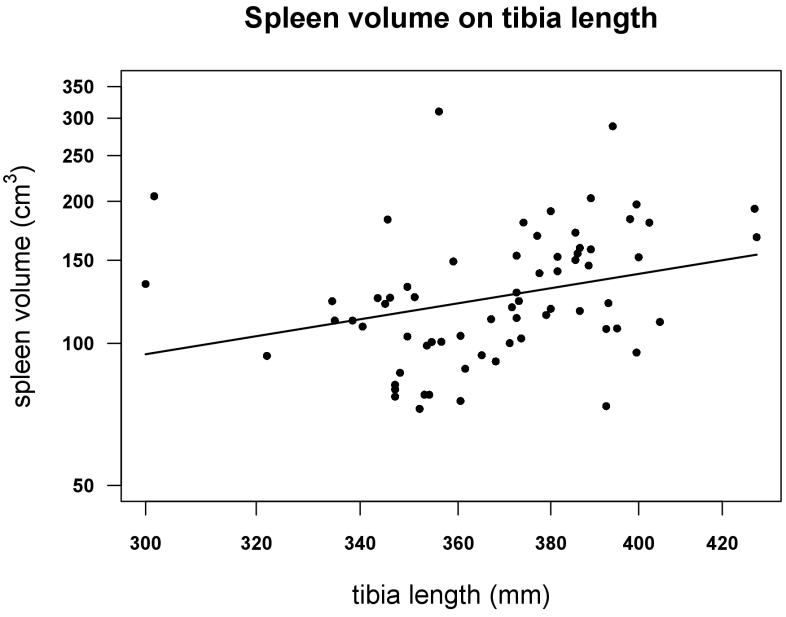


Figure 9-6 Spleen volume against tibia length.
$r^2 = 0.09$, $p = 0.01$; n = 68

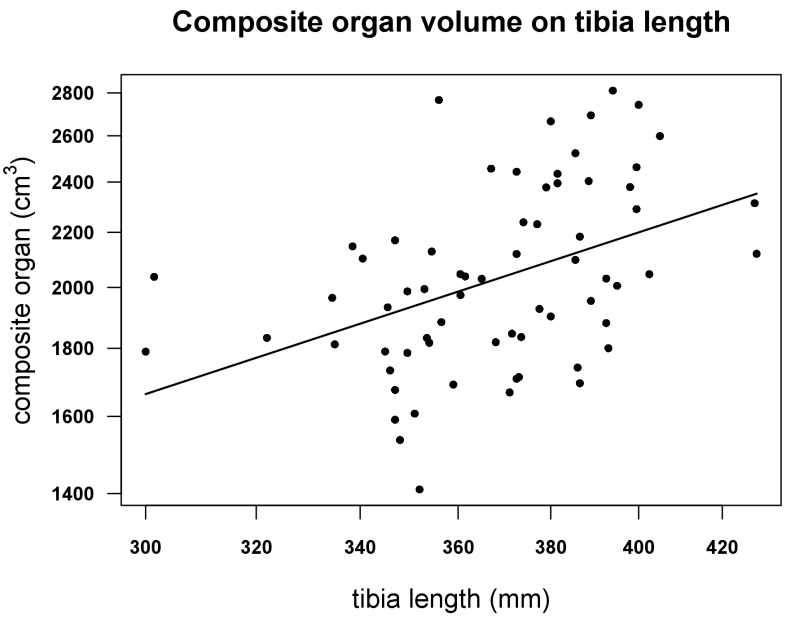


Figure 9-7 Composite organ volume against tibia length.
$r^2 = 0.18$, $p < 0.001$; n = 67

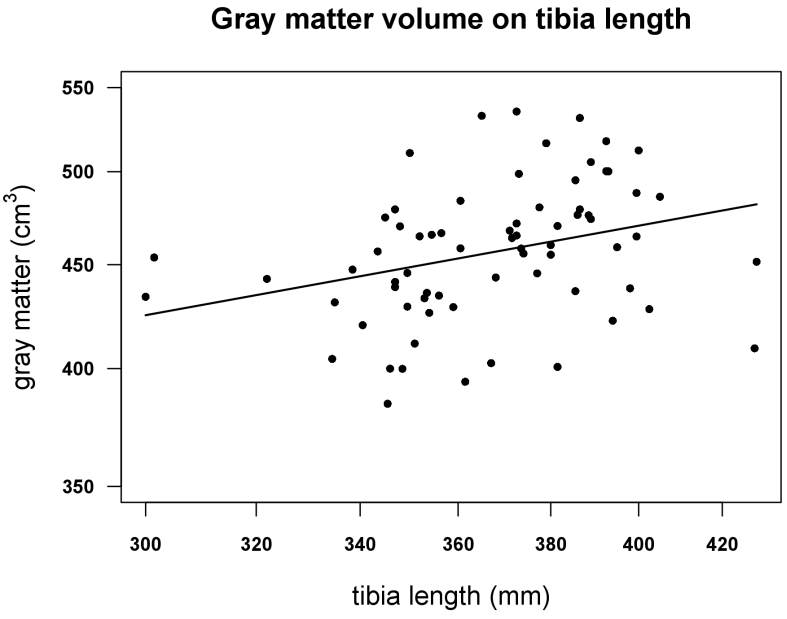


Figure 9-8 Gray matter volume against tibia length.
$r^2 = 0.10$, $p = 0.008$; n = 70

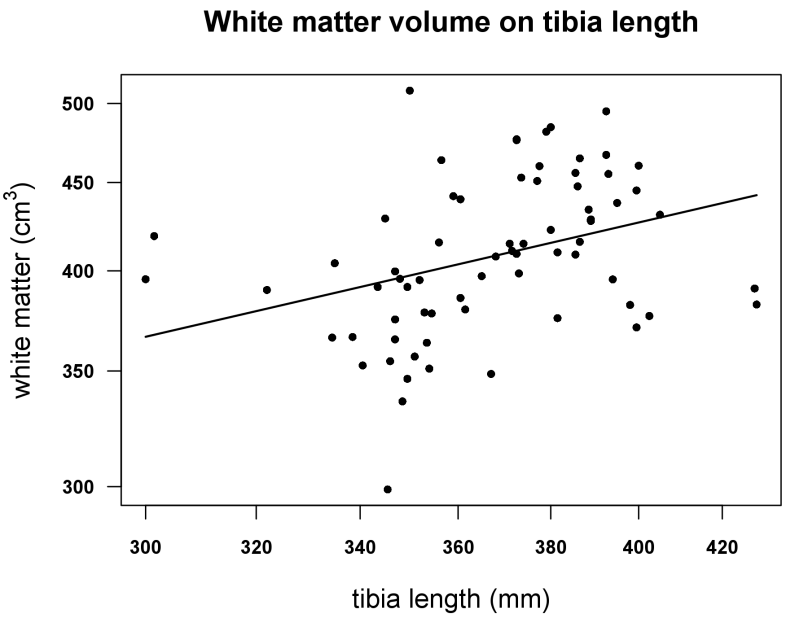


Figure 9-9 White matter volume against tibia length.
$r^2 = 0.12$, $p = 0.003$; n = 70

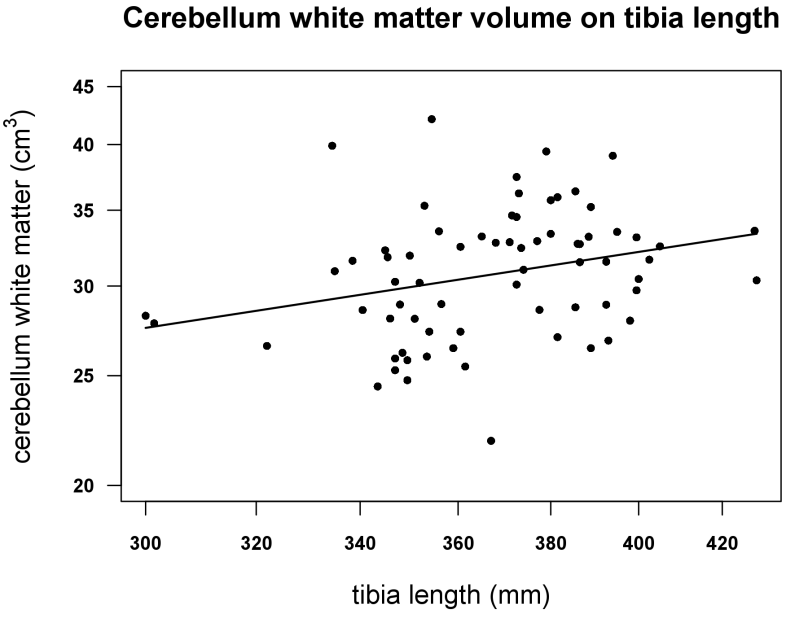


Figure 9-10 Cerebellum white matter volume against tibia length.
$r^2 = 0.08$, $p = 0.02$; n = 70

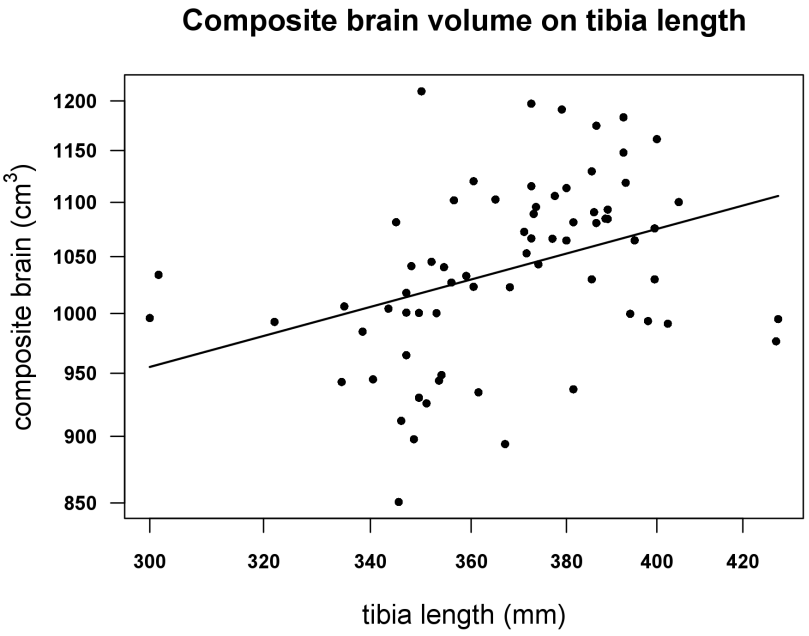


Figure 9-11 Composite brain volume against tibia length.
$r^2 = 0.14$, $p = 0.001$; n = 70

9.3 Testing for a developmental signature

In this section, I add birth weight SDS and tibia length variables to the regression models from Chapter 7 where trade-offs between brain and lean body components were observed. It would suggest that early developmental periods shaped the observed trade-offs if birth weight SDS and tibia length are shown to be significant in the models and the coefficient for the predictor of interest is attenuated. This effect for birth weight would suggest trade-offs were shaped *in utero*, whilst for tibia length, the effect would suggest an impact of postnatal growth, indexed by variability in the lower leg.

TIV remains in the models to control for variation in head size, however as I discussed above, height-residual is entered to control for body size, in place of the raw height variable.

Models 1 and 2 below correspond to Models 1 and 2 (Tables 7.1 and 7.2) in Chapter 7. The results from the Chapter 7 models are included underneath the results for the current model. This is done throughout this section so that results are more easily comparable.

In Model 1, composite brain volume is entered as the dependent variable, and FFM as the main variable of interest. In Model 2 at the top of the following page, gray matter volume is entered as the dependent variable against FFM.

Table 9.3 Model 1: Composite brain = FFM + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	0.29	0.46	0.54
FFM	-0.11	0.04	0.017
Height-residual	0.40	0.25	0.11
TIV	0.83	0.06	<0.001
Birth weight SDS	-0.001	0.004	0.78
Tibia length	0.19	0.07	0.01

	Chapter 7 model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	-0.36	0.59	0.54
FFM	-0.11	0.04	0.016
Height	0.35	0.13	0.006
TIV	0.82	0.06	<0.001

Table 9.4 Model 2: Gray matter volume = FFM + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	-0.03	0.58	0.95
FFM	-0.15	0.06	0.012
Height-residual	0.38	0.31	0.22
TIV	0.81	0.07	<0.001
Birth weight SDS	-0.0001	0.005	0.98
Tibia length	0.14	0.09	0.12

	Chapter 7 model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	-0.66	0.72	0.37
FFM	-0.14	0.05	0.010
Height	0.30	0.15	0.056
TIV	0.80	0.07	<0.001

Model 1 demonstrates that the addition of birth weight SDS and tibia length have little impact on the results for the FFM predictor. Birth weight SDS is not significant in the model. Although tibia length is significant, its addition does not change the results for FFM. In Model 2, birth weight SDS and tibia length are both non-significant.

Below, Models 3 and 4 correspond to Models 9 and 10 (Tables 7.9 and 7.10) in Chapter 7, where significant negative relationships were found for composite brain and gray matter volumes with SM.

Table 9.5 Model 3: Composite brain volume = SM + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	0.14	0.47	0.77
SM	-0.09	0.03	0.014
Height-residual	0.33	0.24	0.17
TIV	0.82	0.06	<0.001
Birth weight SDS	-0.002	0.004	0.66
Tibia length	0.19	0.07	0.01

	Chapter 7 model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	-0.41	0.60	0.50
SM	-0.08	0.03	0.017
Height	0.33	0.12	0.008
TIV	0.82	0.06	<0.001

Table 9.6 Model 4: Gray matter volume = SM + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	-0.25	0.59	0.67
SM	-0.12	0.04	0.005
Height-residual	0.31	0.29	0.29
TIV	0.81	0.07	<0.001
Birth weight SDS	-0.001	0.005	0.84
Tibia length	0.16	0.09	0.09

	Chapter 7 model (R^2 = 0.71)		
	Coefficient	SE	*p*
Intercept	-0.81	0.72	0.27
SM	-0.12	0.04	0.004
Height	0.30	0.15	0.05
TIV	0.80	0.07	<0.001

Tibia length emerges as a significant, positive predictor of composite brain volume in Model 3, although the results for SM remain largely unchanged. In Model 4, neither birth weight SDS nor tibia length are significant.

Models 5 and 6 below correspond to Models 11 and 12 (Tables 7.12 and 7.13) in Chapter 7, where significant negative relationships were identified for composite brain and gray matter volumes against composite organ volume.

Table 9.7 Model 5: Composite brain volume = composite organ volume + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	0.64	0.45	0.16
Composite organ volume	-0.06	0.03	0.04
Height-residual	0.24	0.23	0.30
TIV	0.79	0.06	<0.001
Birth weight SDS	-0.003	0.004	0.52
Tibia length	0.20	0.07	0.01

	Chapter 7 model (R^2 = 0.80)		
	Coefficient	SE	*p*
Intercept	0.14	0.55	0.81
Composite organ volume	-0.07	0.03	0.034
Height	0.33	0.12	0.010
TIV	0.79	0.06	<0.001

Table 9.8 Model 6: Gray matter volume = composite organ volume + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.69)		
	Coefficient	SE	*p*
Intercept	0.30	0.58	0.61
Composite organ volume	-0.11	0.04	0.01
Height-residual	0.21	0.30	0.47
TIV	0.79	0.07	<0.001
Birth weight SDS	0.001	0.005	0.80
Tibia length	0.16	0.09	0.09

	Chapter 7 model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	-0.21	0.70	0.76
Composite organ volume	-0.11	0.04	0.007
Height	0.30	0.16	0.059
TIV	0.79	0.07	<0.001

The results follow the same pattern seen in the models above. In Model 5, the tibia coefficient is significant, but the results for the composite organ coefficient are only somewhat attenuated. Neither tibia length nor birth weight SDS are significant in Model 6, and results for the composite organ coefficient are again similar to the original model.

Finally, Models 7-9 below correspond to Models 13-15 (Tables 7.15 to 7.17) in Chapter 7, which demonstrated trade-offs among brain, liver and kidney volumes.

Table 9.9 Model 7: Composite brain volume = liver volume + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.81)		
	Coefficient	SE	*p*
Intercept	0.46	0.45	0.32
Liver volume	-0.08	0.03	0.005
Height-residual	0.34	0.23	0.14
TIV	0.82	0.06	<0.001
Birth weight SDS	-0.001	0.004	0.81
Tibia length	0.19	0.07	0.01

	Chapter 7 model (R^2 = 0.81)		
	Coefficient	SE	*p*
Intercept	-0.16	0.55	0.77
Liver volume	-0.08	0.03	0.004
Height	0.35	0.12	0.004
TIV	0.82	0.05	<0.001

Table 9.10 Model 8: Gray matter volume = liver volume + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	0.19	0.58	0.75
Liver volume	-0.10	0.03	0.006
Height-residual	0.29	0.29	0.32
TIV	0.81	0.07	<0.001
Birth weight SDS	<0.001	0.005	0.99
Tibia length	0.14	0.09	0.13

	Chapter 7 model (R^2 = 0.71)		
	Coefficient	SE	*p*
Intercept	-0.38	0.68	0.58
Liver volume	-0.10	0.03	0.003
Height	0.28	0.15	0.057
TIV	0.80	0.07	<0.001

Table 9.11 Model 9: Gray matter volume = kidney volume + height-residual + TIV + birth weight SDS + tibia length

	Current model (R^2 = 0.70)		
	Coefficient	SE	*p*
Intercept	0.04	0.57	0.95
Kidney volume	-0.10	0.03	0.006
Height-residual	0.19	0.28	0.49
TIV	0.82	0.07	<0.001
Birth weight SDS	<0.001	0.005	0.88
Tibia length	0.13	0.09	0.14

	Chapter 7 model (R^2 = 0.71)		
	Coefficient	SE	*p*
Intercept	-0.41	0.69	0.55
Kidney volume	-0.10	0.03	0.004
Height	0.25	0.14	0.08
TIV	0.81	0.07	<0.001

Consistent with the previous results in this chapter, the tibia length variable is significant in predicting composite brain volume, but not gray matter volume. Also consistent with the prior results, the addition of tibia length does not appear to impact the results for the main predictor of interest. In Model 7, results for liver volume remain virtually unchanged from the original model.

9.4 Discussion of findings in this chapter

My third hypothesis predicted that markers of fetal and infant growth variability would be observed to mediate brain-body trade-off relationships. Overall, the results described above did not support this hypothesis. Birth weight SDS was not significant in any of the models, thus failing to offer evidence for a fetal growth

effect on trade-offs, and where tibia length was significant, the coefficient for the predictor of interest was not attenuated. However, the tibia's significance in the composite brain models, taking into account the association of FFM, SM, or organs with the brain, suggests that tibia length makes a further predictive contribution to explaining variability in brain size. As described, evidence indicates the tibia is the part of the leg most sensitive to environmental circumstances in early life (Jantz and Jantz, 1999; Pomeroy et al., 2012, 2013). Therefore, the positive tibia-composite brain associations suggest growing well in early life is correlated with growing a larger brain, however this appears in turn to be associated with decreased FFM, SM, kidneys or liver in my sample. Indeed, the tibia appears to be the part of the leg demonstrating the strongest association with the brain, as tibia length and overall height correlated with several brain outcomes, but height-residual (the component of height minus the tibia) did not.

Nevertheless, as noted above, there was no evidence per se that fetal or postnatal experience mediated the trade-offs observed among the brain, FFM, SM and organs. This is surprising, as organs and tissues measured in adult individuals have passed through earlier developmental phases, each of which must have contributed to their current size. As discussed, fetal life and infancy are recognized as particularly critical growth periods, after which many organs and tissues demonstrate decreased plasticity so that aspects of their size and composition track on into adulthood (Lucas, 1991; Barker, 2007; Gluckman et al., 2008; Wells, 2016). A lack of support for the impact of early growth on somatic trade-offs observed in my adult subjects could plausibly be explained by poor sensitivity of the growth markers used.

For example, relying on subject recall to collect data on birth weight and gestational age is not ideal, and the ability of the birth weight SDS variable to index fetal experience in the current study may have suffered from poor data recall. Although not definitive, the limitations of the birth weight variable are suggested by the results of correlation analyses, shown above in Section 9.2. The highly non-

significant associations of birth weight SDS with all brain and body variables are unexpected, as many previous studies have reported significant links between birth weight and later body composition (Wells et al., 2007; Kuzawa et al., 2012). Several studies, for example, have identified associations of birth weight with later lean mass and adult height (Sørensen et al., 1999; Gale et al., 2001; Loos et al., 2002; Li et al., 2003; Wells et al., 2005; Sachdev et al., 2005), suggesting that growth *in utero* plays an important role in shaping later phenotype. At the same time, FM has been shown to vary considerably as a component of birth weight (Catalano and Kirwan, 2001), so that any given birth weight potentially encompasses a large range of lean mass. This could engender difficulty in using birth weight to index lean mass, and perhaps particularly in South Asian populations, wherein increased adiposity has been identified at birth (Yajnik et al., 2003; Modi et al., 2009).

This may help to explain the lack of a signal for birth weight in the present models, even as the South Asian 'thin-fat' phenotype (i.e. increased fat mass alongside decreased lean) has been shown to emerge in fetal life (Yajnik et al., 2003; Modi et al., 2009; Stanfield et al., 2012). I discussed the notion that the thin-fat phenotype may represent a population-level 'thrifty phenotype' which has developed over time in association with chronic nutritional stress in South Asia (Hardikar et al., 2015; Wells et al., 2016). Underlying mechanisms might extend beyond physiological plasticity; for example, inter-generational epigenetic effects may play a role. This was suggested by the recent study of Hardikar and coworkers (2015), wherein body composition changes over 50 generations in nutritionally-stressed rodents involved epigenetic modifications that were not reversed upon nutritional recuperation. The findings of this preliminary investigation in rodents can only be extended to humans with caution. However, epigenetic influences may add to the complexity of the establishment of phenotype in early life and render it more difficult to detect developmental signals using relatively crude indices of growth variability.

Beyond the associations demonstrated between the tibia and composite brain, which I discussed above, the tibia length variable may have been insufficiently sensitive to index early-life effects on brain-body trade-off relationships. For example, it may have lacked specificity to index the postnatal infant period. Alternatively, it is possible that the key period(s) in which observed somatic trade-off relationships developed occurred following infancy. It appears less likely that brain-organ or brain-SM trade-offs would develop post-infancy, when the growth of these tissues is less sensitive to environmental and nutritional stimuli, however this possibility cannot be discarded definitively. At the same time, genetic effects cannot be ruled out, which presents another potential explanation for the lack of findings associated with birth weight and tibia length variables in the current study. Long-term environmental factors influencing body composition variability in South Asian populations may have led to genetic adaptation that researchers have yet to elucidate. The recent study of Wells et al. (2016b) on Indian body composition, for example, described a trend for decreasing stature in India over a 10,000-year time span. Human genetic evolution within this time frame has indeed been documented, such as the classic example of genetic adaptation enabling lactose digestion post-weaning (Tishkoff et al., 2007).

10 General discussion

In this thesis, I set out to test the notion that tissues in the body are in competition for energy resources, an idea put forward by the influential hypotheses of Aiello and Wheeler (1995) and Hales and Barker (1992). I initially overviewed the predictions of the expensive-tissue hypothesis and the thrifty phenotype hypothesis, and demonstrated that they are each consistent with one of the central tenets of evolutionary life history theory: namely, that the finite nature of energy resources necessitates differential allocation to competing traits, or trade-offs.

The review of the literature in Chapter 1 showed that there is evidence for somatic trade-offs in both humans and non-human animals. The evidence base in humans has largely come from tests of Hales and Barker's TPH, where studies have supported the prediction that organs and tissues experience differential growth under adverse environmental circumstances in early development (e.g. Barker et al., 1993; Baker et al., 2010; Pomeroy et al., 2012). In the anthropological literature, support for Aiello and Wheeler's (1995) ETH has largely emerged from studies in fish and amphibians, although trade-offs between tissues beyond the brain and gut have been supported in other animals (e.g. Isler and van Schaik, 2006; Pitnick et al., 2006; Navarette et al., 2011).

The recognition that somatic trade-offs had yet to be directly tested in humans stimulated the central aim of this thesis: to collect comprehensive, high-quality brain and body composition data in a human cohort and investigate whether negative statistical relationships were observable between brain and body organs and tissues. The use of MRI, DXA, and the 4C model of body composition assessment allowed for a higher degree of resolution than external measures such as anthropometry, and in the case of the 4C model, increased accuracy over simpler 2C models.

Despite the high quality of the dataset, the segmentation of the gastrointestinal tract and pancreas from MR images was unfeasible; this meant that the original, specific predictions of the ETH and TPH – trade-offs between the brain and gut, and between the brain and pancreas, respectively – could not be tested. However, relationships with additional organs and tissues were tested, including FM, SM, the heart, kidneys, liver and spleen, and for the first time, relationships with specific brain components were explored.

The results provided broad support for the prediction that somatic trade-offs occur in humans, and that such trade-offs may occur between the brain and visceral organs such as the liver and kidneys, but also between the brain and SM. To my knowledge, this study is both the first direct test of the general predictions of the ETH and TPH in humans, and the first to demonstrate evidence in humans in support of somatic trade-offs at a high level of brain-body composition detail.

10.1 Main study findings

Prior to testing for trade-offs, I tested for associations amongst tissues within the body, and within the brain, which I predicted would be positive. The results showed that associations were indeed positive, however they demonstrated moderate to low effect sizes, with no body or brain component explaining more than ~50% of the variation in another. The indication is that there is substantial variability in the way bodies and brains are built, rather than a common scenario whereby an increase in one component is directly related to an increase in another component. Genetics may contribute to the relative growth of various brain and body components. It is similarly likely, however, that environmental influences during development interact with genes to produce a range of brain and body composition phenotypes, so that the size of components recognized to be linked structurally and functionally, such as the cerebellum and cerebral gray matter, may not be strongly statistically correlated.

Despite some variation between my results and those of previous authors, the REE analysis in Chapter 6 confirmed that organs and tissues are differentially metabolically costly. The brain, predicted by the ETH and TPH to be augmented or preserved at the expense of other organs in the face of finite energy resources, indeed demonstrated one of the largest K_i values, however those of the kidneys and heart were even larger. Nevertheless, in calculating the proportional contributions to total REE for these organs, the brain's contribution was larger than the kidneys and heart owing to its relatively larger mass. On a per kilogram tissue basis the brain may be designated an 'intermediately' expensive organ, however the focus on its cost in the literature is understandable given its increase in size in the human lineage and apparent importance for human survival, which is suggested by its tendency to be relatively 'spared' when resources are constrained in early development (Barker, 2004; Giussani, 2011). Indeed, there is evidence that decreased brain growth is associated with substantial penalties, such as adverse cognitive outcomes. For example, MacKay and colleagues (2010) showed that children born early (with optimal prenatal brain growth potentially truncated) had a higher risk of developing learning difficulties, and this presented as a dose-dependent association across a wide range of gestational age.

As I described in Chapter 1, Aiello and Wheeler (1995) were not the only researchers to approach the question of human brain evolution from an energetics perspective, however their proposition that the larger hominin brain was funded by a reorganization of energy use within the body was novel. Beyond the gut, the authors were less persuaded that other organs or tissues may have traded off with the brain. They considered, for example, that evolutionary changes in the size of the liver or kidneys may have been constrained relative to the gut, rendering it less likely that either of these organs traded off against the brain and facilitated its expansion. This argument centered on the important role of the liver in supplying the brain with glucose, and the similarly essential role of the kidneys in filtering blood and concentrating urine (Aiello and Wheeler, 1995).

The results of the current study, however, suggest that somatic trade-offs between the brain and liver, and between the brain and kidneys, are indeed possible among individual humans. As discussed in Chapter 7, the current study methods could not distinguish between the contribution of genetics and developmental plasticity to these trade-offs, although it is likely that they were shaped at least in part by developmental plasticity in my study cohort, and thus are likely indicative of life course adaptation. If such trade-offs were to persist over multiple generations and incorporate genetic effects over time, organ-size ratios could potentially reach a new optimum, representing an evolutionary adaptation. Indeed, it is likely that any long-term physiological or biological trade-off which was eventually fixed genetically in hominins (e.g. a trade-off between the gut and brain, which remains a possibility) first arose through plasticity as organs and tissues competed for energy within life course development.

My trade-off analysis similarly lent support to the prediction of Leonard, Robertson, Snodgrass and colleagues, that a systematic reduction in average muscle mass in humans over time may have played a role in facilitating brain expansion (Leonard et al., 2003; Snodgrass et al., 2009; Muchlinski et al., 2012). This suggests that brain-liver, brain-kidney, or brain-SM trade-offs may have been 'options' for funding an increasingly large and expensive brain within the constraints of the hominin energy budget. Although it is not inevitable that trade-offs arising via phenotypic plasticity would be the same as evolutionary trade-offs, they arguably represent viable somatic energy allocation scenarios. This is indicated by the fact that I detected such trade-offs in healthy, normally-functioning adults, which further suggests that similar phenotypes could have been stabilized over time through genetic adaptation.

Considering the trade-off and REE analyses together highlights that both high- and low-metabolic rate tissues may trade off against the brain. As I showed in Chapter 6, the K_i value of SM is relatively low. However, SM is relatively large in mass and therefore accounts for a larger percentage of the total energy budget than some

internal organs. Considering the increased cost of SM beyond the basal state (i.e. during physical activity), it is possible that SM would, like the higher-K_i value organs, be a target for energy competition with the brain. Due to its lower K_i value, a decrease in SM mass alone may not be expected to have freed up a considerable amount of energy to fund the brain, however I have shown that it is possible for trade-offs amongst different tissues to occur simultaneously, within the same individuals. It thus appears feasible that trade-offs between the brain and both low- and high-metabolic rate tissues in the body may have constituted concomitant adaptations in the energetic strategy of hominins.

Previous studies which tested for somatic trade-offs involving the brain have reported using only a whole-brain volume or mass measurement. This study took a novel approach by segmenting the brain into several component volumes. It was thus a novel finding of the current study that the brain's gray matter volume, beyond the composite brain measure, was seen to demonstrate trade-offs with lean body components. In testing the hypothesis of a brain-fat trade-off, however, neither composite brain, gray matter, nor any of the remaining brain components showed negative relationships with FM. These results suggested that the findings of Navarette and coworkers (2011) describing such trade-offs across 100 mammal species do not manifest within the human species, and that a brain-fat trade-off is unlikely to help explain the evolution of the human brain. Indeed, it has been argued that the increased adiposity of humans is an important component of an evolved life history strategy which facilitated hominin brain expansion (e.g. Kuzawa, 1998; Wells, 2016).

As acknowledged, Aiello and Wheeler's (1995) prediction that hominin brain expansion was facilitated by a somatic trade-off is one of several hypotheses which sought to explain human brain evolution from an energetics perspective. The ETH highlighted the importance of dietary changes, which, as I described in Chapter 1, were similarly proposed by others to have been integral to changes in brain size (e.g. Foley and Lee, 1991; Leonard and Robertson, 1992). Cooperative breeding

is argued to have been a prerequisite for the evolution of a larger, more expensive brain (Hrdy, 2009; Isler and van Schaik, 2012). The 'expensive brain' framework of Isler and van Schaik (2009) predicts that energy allocation may have been redistributed from tissues such as the gut and SM, and also from functional areas of growth and reproduction. Aiello and Wells (2002) similarly favored the idea that the increased energy costs faced by hominins were covered by not one, but several adaptations. The findings of this study – trade-offs which were statistically significant, but not of substantial magnitude; and which were identified between the brain and various tissues – are consistent with the idea that a strategy for funding the brain involved shifts in energy allocation among several areas. If trade-offs similar to those I have identified occurred in hominins, they appear likely to have represented just one component of a more complex energetic strategy. Importantly, moderate trade-offs such as those observed here may have represented the most plausible scenario to maintain the brain and body organ function of individuals, with selection acting on relatively small differences to potentially drive phenotypic change over time.

Shifting from an evolutionary to a life course, population-level perspective, the brain-tissue trade-offs I observed are consistent with prior studies showing reduced average muscle mass and visceral organ size in South Asian individuals (Rush et al., 2007; Unni et al., 2009; Wells et al., 2016). As described, these aspects of the characteristic South Asian body composition phenotype were predicted by Yajnik and coworkers (Yajnik et al., 2003; Yajnik, 2004) to arise as a result of brain-sparing growth in a nutritionally-poor environment. For the first time, this study provides direct evidence that brain size is negatively related to SM and organ size in South Asian women, thus supporting Yajnik and the general brain-sparing prediction of Hales and Barker's (1992) TPH. Although the direction of causality cannot be confirmed, I discussed in Chapter 7 that several lines of evidence suggest the kidneys, liver or SM would be more likely to vary in size than the relatively canalized brain. Nevertheless, the lack of evidence for a developmental signature underlying the observed trade-offs, as described in

Chapter 9, precludes the current study from offering stronger support for the TPH; namely, its prediction that the differential growth of organs and tissues occurs in fetal and/or infant life. It remains a possibility that the trade-offs identified in my sample developed outside of these specific periods.

10.2 Strengths, limitations and future work

The principal strengths of this study are associated with its methods of recruitment and measurement. Potentially confounding factors were limited by recruiting individuals of one sex, of a relatively focused ethnic background, and within a narrow age range situated after the pubertal growth period, but likely before the start of brain/body composition changes associated with aging. Further variability in body composition was limited by including nulliparity as an inclusion criterion, and avoiding the recruitment of individuals at the extremes of the BMI range. This meant that it was unnecessary to add a large number of variables to statistical models to control for extra sources of variability.

The majority of the measurement methods used to gather data were of the highest possible quality. MRI allowed for accurate *in vivo* determinations of brain gray and white matter volumes and organ volumes, whilst the 4C body composition model is the criterion for quantifying FFM and FM. Using MRI, DXA and the 4C model to measure different outcomes allowed me to derive independent measures of FFM, FM, SM and brain/body organs, and avoid correlated error. This is not the first study to combine MRI and the 4C model, but to my knowledge it is the first to do so in South Asian women, and it is the first to do so in order to test for somatic trade-offs, thus addressing an interesting and influential evolutionary question with several advanced biomedical techniques. The sample size of 70 may seem small by epidemiological standards (for example, the studies of Barker and colleagues routinely involved hundreds or thousands of subjects), however a sample size of 70 is larger than that found in many MRI studies.

I was not able to measure tissue-specific metabolic rates directly in my sample; the technical difficulty of the methods required to do so is indicated by the continued use of Elia's (1992) K_i values in the literature, and no new reports emerging from more recent studies. However, I was able to measure REE in my subjects and assess both the question of relative organ/tissue cost, and whether tissue trade-offs were observable among more or less 'expensive' tissues, within the same cohort.

There were some limitations associated with my methodology as well. As described in Chapter 4, I dealt with missing body organ data by measuring organs on different sets of MR images, and correcting for this with generated regression equations. This may have introduced error, although the process was carried out for a small number of subjects, and it did avoid further missing data for body organs, which were an important study outcome. Error may also have been introduced more generally through the manual measurement of organs using the OsiriX workstation. Although I received instruction from an experienced radiologist prior to undertaking body organ segmentation in my dataset, I do not have extensive prior experience extracting organs and tissues from MR images. A benefit, however, in carrying out all segmentations myself is that any error is likely to be similar across subjects, so that the ranking of organ size is unlikely to have been affected in the final dataset.

The selection of control variables in brain-organ trade-off models was less than straightforward, particularly involving the question of whether it was appropriate to control for head/skull size using TIV. A literature search revealed that in imaging studies, individual variation in head size is recognized as a source of variability for inter-subject whole and regional brain volume outcomes. It is thus common practice to obtain a measure of the cranial cavity to control for overall head size in MRI volumetric analyses (Whitwell et al., 2001; Buckner et al., 2004; Sanfilipo et al., 2004; Malone et al., 2015). These methods have been employed to investigate brain volume change over the adolescent period (Herting et al., 2014),

associations of brain volume with physical activity (Jochem et al., 2017), cortical changes in schizophrenia (Goldstein et al., 1999), the relationship between social network size and amygdala size in humans (Bickart et al., 2011) and brain volume differences between the sexes (Lüders et al., 2002). According to Mathalon et al. (1993, p. 122), variability in head size inserts "'noise' into quantitative brain data," and without adjustment, this source of variability remains unexplained in statistical models and may decrease power (Barnes et al., 2010; Peelle et al., 2012). Furthermore, if TIV is associated with the main variable(s) of interest (composite brain and brain component volumes in my study), it represents a potentially important confounder (Malone et al., 2015). Beyond this, in consultation with a statistician, I was advised to include both height and TIV as control variables in my trade-off analyses. However, the novel nature of the current PhD analysis makes it difficult to determine whether the logic of including TIV as a control variable in previous imaging studies extends to my study. Therefore, the appropriateness of including TIV in trade-off models remains an open question.

As I noted in Chapter 9, the developmental variables I employed may not have been sensitive enough to detect an early-life developmental effect on tissue trade-offs. The birth weight variable may have been particularly problematic, as birth weight data (and also data on gestational age, which were used to generate SDS) were obtained from subjects by recall.

Finally, I was able to test both Aiello and Wheeler's (1995) ETH, which predicts an evolutionary somatic trade-off, and Hales and Barker's (1992) TPH, which predicts somatic trade-offs within the life course. However, my methods were not able to distinguish between the effects of genetics and phenotypic plasticity, both of which may contribute to the development of observed trade-offs, as I noted in Chapter 7, and also above. At the same time, I cannot conclude with certainty the direction of causality in the brain-organ and brain-SM trade-off relationships identified, nor whether competition between tissues for energy resources occurs in relation to

tissue accretion, or the metabolic costs associated with the long-term functioning of organs and tissues.

Some of the limitations of the present study suggest potential avenues for future research. For example, a future study could employ methods to more directly test the possible contribution of genetic variation to body and brain composition, and potential trade-off relationships therein. One possible avenue for testing this would be a twin study, where variability associated with genetics and maternal environment can be controlled (e.g. Loos et al., 2002). Similarly in need of further exploration in humans is the potential role of epigenetic modifications in shaping body composition phenotypes, in particular following the recent work of Hardikar et al. (2015), which suggested that epigenetic changes are associated with differential tissue growth across generations in a rodent model.

With respect to the present dataset, further, more refined statistical analyses could potentially yield additional information. For example, the use of structural equation modeling (SEM), also referred to as causal modeling or causal analysis, could be explored. SEM allows for the examination of complex relationships, wherein “the phenomena of interest are complex and multidimensional” (Ullman and Bentler, 2013, p. 663). This characterization clearly applies to organs and tissues in the body and their relationships to one another, and SEM could allow for causality in tissue/organ relationships to be elucidated more fully. Similarly, hierarchical or multi-level modeling could possibly be employed. These analyses are typically utilized when datasets contain variables with a nested structure, e.g. specific organs or brain components nested within fat-free mass or composite brain, respectively. Sub-models allow for relationships and residual variability to be accounted for at different levels in the overall model (Raudenbush et al., 2004), which may better fit the data and result in more robust conclusions.

Following the largely negative results in the current study in relation to early-life development, the question of whether trade-offs between the brain and lean body

tissues arise in fetal and/or infant life is in need of additional investigation. Further research could employ similar body composition methods to those used here in adults, but with more sensitive developmental proxy measures. Alternatively, and perhaps more robustly, brain and body composition could be measured in infants or children at a level of detail similar to that employed here, although it is recognized that such a study would be practically difficult, perhaps especially in healthy individuals. To limit the number of measurements necessary, however, imaging could be used alone to collect data on the brain, body organs and SM.

Trade-offs identified in this study were not large in magnitude, however a clear pattern was seen whereby either composite brain or gray matter volume was negatively correlated with FFM, SM, or organs. It would be interesting to carry out a similar study in individuals from a different population, or in male subjects, and investigate whether the same pattern of trade-off relationships might be observed. Finally, a future imaging study might measure the brain, the gastrointestinal tract, and the pancreas in humans to test the specific predictions of the ETH and TPH.

This study set out to test whether competition between organs and tissues in the body, as predicted by Aiello and Wheeler's ETH and Hales and Barker's TPH, could be observed using gold-standard measurements in adult humans. Although results were more equivocal for the TPH, my findings constitute the first direct evidence for the general predictions of the ETH within humans. It is hoped that the current results will stimulate the field, both to carry out further studies of somatic trade-offs, and also to recognize the utility of body composition and biomedical techniques for addressing evolutionary questions.

Appendix: Recruitment/data collection materials

Appendix Figure 1: Study recruitment poster

UCL INSTITUTE OF CHILD HEALTH

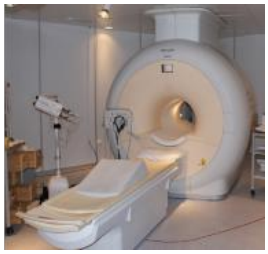
Great Ormond Street Hospital for Children NHS Foundation Trust

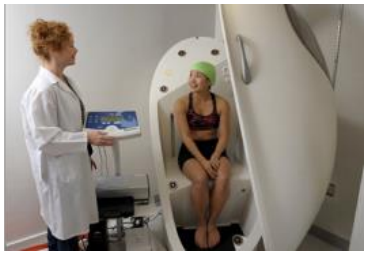
We would like to invite women of **Indian, Pakistani, Bangladeshi and Sri Lankan** ancestry between the **ages of 20-28** to take part in a research study on body composition

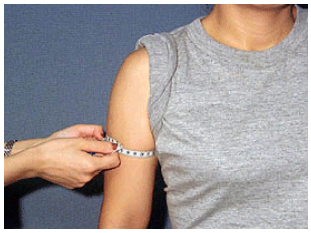
If you're interested, please contact us for further information (contact details found below)

The aim of the study: to gain an understanding of the relationships between lean and fat tissues, and whether these relationships are influenced by growth and nutrition in early life – this will shed light on factors which may make individuals susceptible to certain conditions and diseases (e.g. obesity, type II diabetes, cardiovascular disease)

If you agree to take part, we will conduct a series of measurements as you visit the UCL Institute of Child Health/Great Ormond Street Hospital for Children for one half day (approx. 4.5 hours with breaks for food and drink, which will be provided to you) or on two separate, shorter visits at your convenience. We will offer you £30 for your time and any travel costs will be paid back to you. Weekends and bank holidays are a possibility if preferable to a weekday.







Researcher contact details:

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Professor Jonathan Wells
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30 Guilford Street
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Tel: 02079052104
Email: jonathan.wells@ucl.ac.uk

Version 1.01, dated 04.08.14

Appendix Figure 2: Study consent form

UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

PARTICIPANT CONSENT FORM v1.02, dated 7.10.14

Assessing relationships between organs and tissues in women of South Asian ancestry

Sponsor Protocol No: 14NT03 Investigators: Miss Meghan Shirley, Professor Jonathan Wells

Contact details: Tel: 07923691733 Email: meghan.shirley.13@ucl.ac.uk

Subject Identification No for this study: _____

*Please carefully read each of the following statements and **place your initials** in the boxes on the right to indicate agreement before signing the form.*

1	I confirm that I have read and understood the information sheet (v1.02 dated 03.10.14) pertaining to the above study. I have had the opportunity to consider the information, and have had any and all questions answered.	
2	I confirm that I am not currently pregnant.	
3	I consent to have an MRI scan and a DXA scan, and also to have saliva samples taken.	
4	I understand that my participation in this study is completely voluntary and that I am free to withdraw at any time, and without giving any reason.	
5	In the event of incidental findings, I consent for my GP to be informed of my participation in this study and the results of the MRI.	
6	I agree to take part in this research study.	

_____ _____ _____

Name of Participant (Print) Date Signature of Participant

_____ _____ _____

Name of Researcher Date Signature of Researcher

_____ _____ _____

Name of GP and/or GP practice Phone Address

Only the researchers will have access to the data collected during this study. Information regarding measurements and results will be completely confidential, identified by a number only.

Appendix Figure 3: Study information sheet

UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

Participant Information Sheet v.1.02 – October 2014

Assessing relationships between organs and tissues in women of South Asian ancestry

You are being invited to take part in a research study which will take place at the University College London Institute of Child Health and Great Ormond Street Hospital for Children.

Before you agree to participate, however, we would ask that you carefully read the following information (and discuss it with others if you wish) so that you understand why the study is being done and what it will involve for you. If you would like information additional to what is included here, or if anything is unclear, please do contact the researchers.

To find out more about the research being conducted at the UCL Institute of Child Health, please visit www.ucl.ac.uk/ich/research.

What is the aim of the study?

The aim of this study is to gain an understanding of the relationships between three distinct parts of the body: the brain, lean tissue (i.e. organs and skeletal muscle) and fat tissue. Also, the study aims to find out whether these organ/tissue relationships are influenced by growth and nutrition in early life.

Why is the study being done?

Human health is affected by the size and composition of organs and tissues in the body. How the brain, lean tissue and fat relate to one another, however, is largely unknown; research which considers how tissues are different both within and between people most often looks at the brain and other organs of the body separately. Knowledge of tissue relationships within individuals – and how they might have been shaped during development – will add to our understanding of how the size and composition of tissues could render some people more susceptible to certain diseases.

Why am I being invited to take part?

For this study we are recruiting women of South Asian (Indian, Pakistani, Bangladeshi, Sri Lankan) ancestry who are healthy, aged 20-28 years, have a body mass index (BMI) in the range 17 – 28, were not born pre-term, have not previously been pregnant, and who have not been diagnosed with any conditions that could affect brain/body composition or metabolism (e.g. polycystic ovary syndrome, diabetes, mental health disorders, hypothyroidism). More research is needed to understand why, for a given BMI, individuals of South Asian ancestry tend to have more central fat and lower muscle mass compared to those in other populations; this information will be important in addressing the increasing epidemic of type II diabetes and cardiovascular disease impacting Asian men and women.

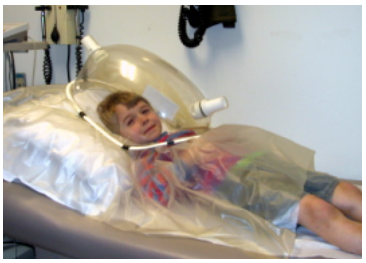
Do I have to take part?

It is fully up to you to decide whether or not you would like to take part in this study. If you do decide to join the study, we will ask you to sign a consent form: one copy will be given to you and the other we will keep for our records. If you do decide to take part, please be aware that you will remain absolutely free to withdraw at any time and without giving a reason.

What will I experience if I take part in the study?

If you agree to take part, we will ask you to fill out two short questionnaires at home with information on your health, physical activity, birth weight, birth order, education and profession. We will ask you to bring these questionnaires with you to an appointment that we will schedule at your convenience. *You will be asked to fast overnight prior to the appointment*, so that you will arrive at the Institute of Child Health (ICH) early the following morning having not eaten breakfast. After we address any questions you have, we will ask you to sign a consent form in the office at ICH. Once you have signed the consent form, we will walk across to Great Ormond Street Hospital (GOSH), which is connected to ICH. We will then do the following measurements:

Resting energy expenditure (REE): It is for this measurement that we will ask you to fast overnight, therefore we will complete this before any of the other measures. REE is the number of kilocalories the body uses over a 24-hour period for basic functions like breathing and maintaining its temperature. We will ask you to lie on a bed under a ventilated plastic canopy for 25 minutes (see photo at right). Following this measurement, a light breakfast will be provided to you. If you have any special dietary requirements please let us know when you book your appointment.



Total body water: To measure the amount of water in your body we will ask you to drink some water containing heavy hydrogen molecules, which occur naturally in all of us in small amounts. These molecules are not radioactive, they simply weigh more than most of the hydrogen molecules found in our bodies. The drink is clear, tasteless, and is not harmful to you. We will collect two saliva samples with a cotton swab, one before the drink and another ~4 hours later. These samples will be analysed by a member of the study team at ICH, after which they will be disposed of.

Hand symmetry: We will make images of your hands using a standard photocopy machine in order to measure the area of your hand, the symmetry of your fingers and the ratio of your ring to your index finger.

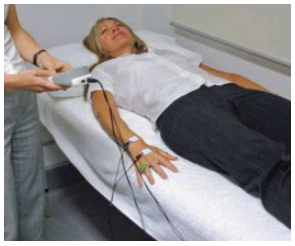
Body volume: For this we use a machine called the BodPod (see photo at right). We will ask you to change into close-fitting underwear or a swimming costume, wear a swimming hat that we will provide, and sit inside the BodPod whilst room air is blown gently around you. The door is shut for less than a minute at a time and usually takes between 4-6 cycles.

Anthropometric measures: We will measure your height, sitting height, and the lengths of your shinbone and foot. We will also collect data on your weight, the circumferences of your arm, leg and head, and the size of skinfolds at four points on your upper body. Skinfolds are measured by gently raising the fat on the front and back of your upper arm, lower shoulder and at a spot above your hipbone, and do not hurt.

Grip strength: We will ask you to squeeze a handheld device called a dynamometer.

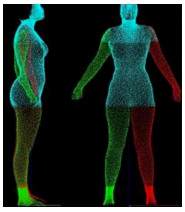
Lung function test: This test entails taking a deep breath in and then blowing through a mouthpiece as hard and fast as possible.

Bioelectrical impedance: We will ask you to lie on a bed, and we will place electrodes on one of your hands and one of your feet (see right). A small electrical signal (far too weak to be felt) will be passed through your body to measure your body composition. The test is harmless and painless.

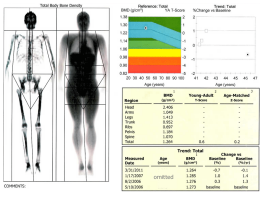


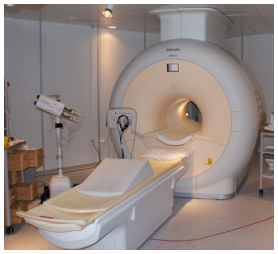
Blood pressure: We will ask you to sit up for this measure, which will be taken three times. We will place a cuff around your upper arm, and there may briefly be a little discomfort due to the pressure when it inflates.

Body shape: We will ask you to wear close-fitting underwear or a swimming costume as you stand inside a 3D body scanner, which looks like a large photo booth and projects light onto the surface of your body. This takes a matter of seconds, you will not experience any physiological risk, and the images acquired (example at right) will be stored securely to ensure your privacy.



Bone scan: For this we will ask you to lie on a bed for about 5 minutes as an arm passes above you taking a picture of your skeleton (below right). The DXA machine measures the amount of bone, fat and lean tissue in your body. It involves a very small amount of radiation, which is less than 1/10 the radiation you would encounter during a flight across the Atlantic, and less than one day's background radiation in the UK.





Magnetic resonance imaging (MRI): During the scan you will lie on a padded table which will be moved to the centre of the MRI magnet (see photo at left). This space is a narrow tube open at both ends. If you are bothered by the confined space or have any other concerns during the scan you can stop at any time by letting the MRI staff know. The scan will take approximately 60 minutes. MRI will let us see detailed images of your brain and the organs of your body, such as the heart, liver and kidneys. To create the images MRI uses radio signals and magnetic fields, which you cannot feel, but which will cause the machine to make loud noises.

We expect these measurements to take approximately 4 hours altogether. This includes breaks for food and drink, as well as factoring in time to walk between ICH and GOSH, and between a few different rooms in the hospital where different measurements will be taken. If you find it more convenient, we could arrange to complete the measurements over two separate, shorter visits to ICH/GOSH.

What are the possible disadvantages and risks of taking part?

None of the measurements will cause you any pain or harm. As mentioned, the radiation involved in the DXA scan is minimal compared with daily background levels of exposure and that which is experienced on a trans-Atlantic flight. However, if there is any possibility you might be pregnant we will not do the scan.

A great deal of research has been done on the potential effects of the radio signals and magnetic fields associated with an MRI scanner: there are no known significant risks in having an MRI scan. However, the scanner is a strong magnet, therefore a risk of accidental injury does exist relating to the sudden movement of metallic objects. Considerable precaution is taken to prevent this from occurring as every person is screened and checked before entering the scanner room. It is important that you tell the researcher and MRI staff if you have had any surgery or if there is any metal in your body, such as piercings. You will not be able to participate in this study if you have implanted electronic or metallic devices.

Additionally, you may be disturbed by the magnet's noise (though ear protection will be provided). Other risks are that you may feel confined (claustrophobic) or uncomfortable due to the length of the MRI scan.

What are the potential benefits of taking part?

As a participant in this study you will receive, if you would like, information about your BMI, blood pressure, % body fat and % non-fat lean mass. *However, it is important you're aware that taking part in this study is not a clinical assessment, and it is important to consult your GP if you have any specific health concerns.*

In the long-term, we hope that the data collected in this study will add important, novel information to our understanding of human anatomy and biology, and also potentially inform public health interventions with regard to chronic disease, obesity and other disorders relating to metabolism. The generation of such information could be beneficial to both science and the general public.

What happens at the end of the study?

Once you have undergone data collection at ICH/GOSH (including body composition measurements and MRI), and completed the two short questionnaires, your commitment to the study will have ended and you will be reimbursed £30 for the time you've dedicated to the study. Once the analysis is complete, we will send you a newsletter to tell you the results of the whole group.

Who will have access to the research data?

All of the data collected during this study will be anonymised with an assigned participant number, so that your name will not appear in the same place as the measurements we've recorded. In this way, all information will remain completely confidential, and only the researchers on the study will have access to it. Any materials containing your personal identifiers will be stored in locked filing cabinets within a secure building (ICH) and/or on password-protected computers and memory sticks. Only members of the research team will have access to the data, and they will analyse it at UCL, and possibly the University of Cambridge.

We will ask you for your GP details. We will not routinely inform them of your participation in this study, but we will ask for your permission to contact them in the first instance in the unlikely event of incidental MRI findings. If your blood pressure is high we will encourage you to inform your GP and have it rechecked.

What will happen to the results of the research study?

The data collected in this study will be published in scientific journals and presented at scientific meetings or conferences. The information may also be used for educational purposes. However, all data will remain anonymised so that no one reading the research will be able to identify you in any way.

What if something goes wrong?

This study has been reviewed and approved by an Independent Research Ethics Committee. This means that a group of individuals whose priority is your safety and well-being believe the research is of minimal risk to you. However, no research project is completely immune to unforeseen risks. It is important for you to know that you have the right to claim damages in a court of law in the unlikely event any harm should occur as a result of you taking part in this study. In this instance, you would be required to prove fault on the part of ICH/GOSH.

Who is funding the research study?

This study has funding from the Wenner-Gren Foundation for Anthropological Research, and the study sponsor is the UCL Institute of Child Health.

UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

Who do I speak to if problems arise, or if I have further questions about the study?

Please contact, in the first instance, the researchers on this study if you have any questions or indeed any complaints about the way the research is conducted. If the issues are not resolved, or if you wish to ask questions or comment in any other way, please contact research-incidents@ucl.ac.uk, or the Patient Advice and Liaison Service (PALS) at GOSH via telephone (02078297862) or email (pals@gosh.nhs.uk).

Details of how to contact the researchers:

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Professor Jonathan Wells
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30 Guilford Street
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Tel: 02079052104
Email: jonathan.wells@ucl.ac.uk

Appendix Figure 4: IPAQ

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

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