Indian preadolescent girls:

Lifestyle patterns and accumulated risk factors

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ATTESTATION OF AUTHORSHIP

"I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other research institution of higher learning".

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ACKNOWLEDGEMENTS

ABSTRACT

The Indian population is at high risk for obesity and its related diseases. Paradoxically, there is also a high prevalence of low birth weight in this population. Throughout life, factors associated with these abnormalities reflect genetic, environmental and lifestyle patterns.

World-wide, the Indian population is largely non-meat-eating which could compromise the quantity and quality of the diet in macronutrients (proteins) and micronutrients (vitamin B_{12}). Vitamin B_{12} has been suggested to increase the risk for the metabolic syndrome (dyslipidemia, insulin resistance, hypertension and central adiposity). Factors measured in this pilot study designed to examine the differences between meat-eating and non-meat-eating Indian preadolescent girls were body composition, dietary food and nutrient analysis, physical activity patterns and biomarkers of diet and metabolic syndrome.

Six non-meat-eating (9.8±0.9 y) and six meat-eating (10.0±0.6 y) Indian preadolescent girls participated in the two weeks study. Mothers and their daughters in each group had followed the same dietary pattern from birth. Anthropometry, hand-to-foot bioelectrical impedance and resting energy expenditure were measured. Biomarkers associated with one carbon metabolism; serum $B_{12,}$ methylmalonic acid (MMA) and folate were measured. Inflammatory markers; high sensitivity C-reactive protein and ferritin were measured. Serum lipids, fasting glucose and haematological parameters were measured. Time spent in sedentary activities and dietary information was extracted from seven day physical activity and food diaries respectively.

There was an overall trend towards higher values for the non-meat-eaters as compared to the meat-eaters in body fat percent (29.7±6.6 vs. 29.0±6.2%, p = 0.85), and waist to hip ratio (0.89±0.12 vs. 0.84±0.07, p = 0.37) but the meat-eaters weighed more (31.2±5.5 vs. 33.3±9.6kg, p = 0.65). Compared to British reference ranges, girls in both groups had a higher BF% of 29±6% which was 34 percentile points above the British median (McCarthy et al., 2006) adjusted for age.

Both groups spent 21 hours each day in non-moving/sedentary activities. Dietary consumption of vitamin B_{12} was higher in meat-eaters compared to non-meat-eaters (2.5±0.8 vs 1.8±0.6μg.day^{-1}, p = 0.11). Serum vitamin B_{12} was substantially higher in the meat-eaters (543±212 vs. 232±95 pmol/L, p = 0.01) with lower serum concentrations of MMA (0.2 ± 0.1 vs 0.3 ± 0.2 μmol/L, p=0.3). Serum folate was adequate in all girls ranging from 16.5-45.0 pmol/L, which was within the normal reference values. Two non-meat-eating girls were vitamin B_{12} deficient (<170pmol/L). These differences were associated with high fibre and less protein intake in the non-meat-eaters (30±8 vs. 20±7 g day^{-1}; 64±12 vs. 66±11 g.day^{-1}).

The initial findings in this pilot study provide early evidence that risk factors for metabolic disease associated with body composition, diet and activity are accumulating in preadolescent Indian girls. Imbalance in one carbon metabolism is clearly a factor to be considered. In those with a low consumption of meat and/or animal products, B_{12} monitoring, dietary recommendations and if necessary supplementation should be considered and where possible intervention before pregnancy (as for folate) be a priority. New Zealand Indian people would be a priority group.

It is time for serious action in this area of health so that the risk accumulated through an imbalance in nutrition and physical activity is reduced and the health of those as yet unborn is improved.

ABBREVIATIONS

Throughout this thesis standard international units and standard abbreviations have been used. Wherever applicable units used for measurements are stated.

ACC	Acetyl CoA carboxylase.
AFA	Upper arm fat area
AMA	Upper arm muscle area
BIA	Bioelectrical impedance analysis
BF%	Body fat percentage
BMI	Body mass index
CβS	Cystathionine β-Synthase
CRP-hs	C-reactive protein high sensitivity
CPT1	Carnitine palmitoyl transferase - 1
DNA	Deoxyribonucleic acid
DEXA	Dual X-ray Absorptiometry
EAR	Estimated average requirement
ES	Effect size
FFM	Fat free mass
FM	Fat mass
HDL cholesterol	High-density lipoprotein cholesterol
HOMA B%	Homeostasis model assessment β- cell function
HOMA S%	Homeostasis model assessment insulin sensitivity
IAAT	Intra- abdominal adipose tissue
IL-1 β	Interleukin- 1β
LDL cholesterol	Low-density lipoprotein cholesterol
MCH	Mean cell haemoglobin

MCM	Methlymalonyl CoA mutase
MCV	Mean cell corpuscular volume
MMA	Methylmalonic acid
MS	Methionine synthase
MUAC	Mid upper arm circumference.
MRI	Magnetic resonance imaging technique
NFHS-1	National family health survey-1
NFHS-2	National family health survey-2
NO	Nitric oxide
NZCNS	New Zealand national Children Nutrition Survey
PCV	Packed cell volume
P:S ratio	Polyunsaturated: Saturated fat
RDI	Recommended Dietary Allowance
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RNA	Ribonucleic acid
SAH	S-adenosylhomocysteine
SAM	S-adenosyl methionine
SES	Socioeconomic status
THF	tetrahydrofolate
TAA	Total arm Area
WHR	Waist-to-hip ratio

GLOSSARY

Apoptosis: Deliberate life relinquishment by a cell in a multicellular organism. One of the main types of programmed cell death. Excessive apoptosis causes cell-lose disease, such as an ischemic damage. Whereas, insufficient apoptosis results in cell proliferation such as cancer.

Acetyl CoA carboxylase: A biotin dependent enzyme that catalyses carboxylation of acetyl CoA to produce malonyl CoA.

Biomarkers: A substance used as an indicator of biological state. A biochemical feature or facet that can be used to measure the progress of disease or the effect of treatment.

Beta- oxidation (β-oxidation): The process by which long carbon chain of fatty acids are broken down to two carbon units of acetic acid (Acetyl CoA) in the mitochondria, the entry molecule for the Krebs Cycle.

Body Mass Index (**BMI**): A useful index to assess overweight and obesity. It is measured by dividing weight by height squared ($kg.m^{-2}$).

Deoxyribonucleic acid (DNA): Nucleic acid that contains genetic instructions for development and function of living organism. The principal role of DNA in the cell is long term storage of information. Parts of the DNA segments carry genetic information, while part if it has structural purposes like regulating the expression of genetic information.

Epigenetic: Reversible changes occurring in gene function or other cell phenotype, without a change in DNA sequence (genotype). These changes can be induced spontaneously in response to environmental factors. These changes could also be carried forward to subsequent generations.

Genotype: Internally coded inheritable information carried by all living organism. The stored information is used as 'blue print' or set of instructions for building and maintaining a living creature.

Indians: In the present thesis, "Indians" refers to people originating from the Indian subcontinent including Bangladesh and Pakistan.

Interleukin- 1β (IL-1β): One of the first cytokine ever described. IL-1β is a pro-inflammatory cytokine involved in immune defense against infection.

Likelihood ratio: Ratio of individuals with and without an adverse risk profile at a specific demarcation range.

Meat-eaters: Preadolescent girls following an omnivores diet depending on their religious and cultural habits.

Non-meat-eaters: Girls following a lactovegetarian or lactovovegetarian diet.

One carbon metabolism: Involves gene-nutrient interactions. The important genes involved are the enzymes that catalyse the process, DNA and RNA. The important nutrients that regulate the mechanism are folate, vitamin B_{12}, B_6 and methionine.

Phenotype: Outward physical manifestation of organism. They are physical parts, sum of atoms, molecules, macromolecules, cell structure, metabolism, energy utilization and anything that is a part of the observable structure, function or behaviour of living organism.

Ponderal Index (PI): A means of characterizing the relationship of height to mass for an individual. Also refers to the ratio of the cube root of mass (in kilograms) divided by the height in centimetres. The fraction is then multiplied by 1000.

Preadolescence: The period of childhood just before the onset of puberty, often a designated as between the age of 10 and 12 in girls and 11 and 13 in boys.

Prepubertal: Before puberty, the period during which secondary characteristics start to develop and the capability for sexual reproduction is attained.

Puberty: Refers to the process of physical changes by which a child's body becomes an adult's body capable of reproduction.

Tanner stages: Stages of physical development in children, adolescents and adults. The stages define physical: measurements of development based on external, primary and secondary sex characteristics such as the size of the breast, genitalia and development of pubic hair.

Tanner stage 1 (female): There is no pubic hair growth, no glandular tissue development of the breast and areola skin of the breast follows the skin contour of the chest.

CHAPTER 1: INTRODUCTION

Obesity or excess body fat is not "just a simple" imbalance between energy intake and energy output. It is a symptom or manifestation of imbalance between various nutritional and activity factors that play a role in maintaining the health of an individual from their conception to death. Obesity may have been programmed *in-utero* and the onset may be influenced by persistent adaptations to prenatal and postnatal environment (breastfeeding) in relation to maternal nutrient intake. Nutrient intake refers to macronutrients: carbohydrate, proteins and fats and micronutrients: vitamins, minerals, phytonutrients, fibre, antioxidants and flavonoids. The risk factors for obesity and its adverse effects may be accumulated due to an unfavourable maternal environment during foetal life and childhood and are expressed in adult life. It is difficult to correct these risks factors in adulthood where they have manifested themselves as irreversible changes. Therefore it is preferable to examine and address these problems at an earlier stage in life i.e childhood or at the prepubertal stage before adolescent growth without the confounding hormonal and behavioural effects of puberty. For limiting the confounding effects of metabolic changes due to puberty, studies in prepubertal children are preferable.

The Indian population is a high risk population for obesity and its related diseases in New Zealand. A recent report, called the "Asian Health Chart Book 2006" by The Ministry of Health (2006) was based on a number of New Zealand data sets. In this report Asian data was split into three groups; Chinese, Indian and Other Asian. Indians had a higher prevalence of obesity, diabetes, cardiovascular diseases related hospitalization, mortality and deprivation than Chinese, Other Asian and European.

Indian women appeared to be at more risk of ischemic heart disease than Indian men. They were more likely to be physically active than their other Asian counterparts. However, Indian neonates in New Zealand had a 70% higher risk of low birth weight compared to the total population of New Zealand. The question of why Indian babies are so small, yet Indian population is at such high risk of chronic disease underpinned the direction of this body of work. In addition, previous work conducted by Dr Chittranjan Yajnik in India, and guidance by Professor Elaine Rush confirmed thesis direction.

Indians are at risk because of their non-meat-eating habits which could compromise the quantity of micronutrients (vitamin B_{12}) and macronutrients (protein) in the diet. Vitamin B_{12} and protein have a synergistic role in driving a metabolic imbalance responsible for insulin resistance and adiposity (James, 2006; Merezak et al., 2001). This thesis will specifically explore the present literature and address the significance of vitamin B_{12} and protein intake with respect to the Indian dietary pattern of a high risk population and the role of these two nutrients as a possible driving force accelerating increase in obesity in a vulnerable population. In this thesis the chosen population is migrant Indians. Throughout the thesis the term Indian refers to people originating from the Indian subcontinent including Bangladesh and Pakistan.

1.1 Review of literature

This section will explore the following problems in relation to the development of accumulated risks and lifestyle factors in Indian preadolescent girls with different dietary patterns.

- The obesity epidemic and its related diseases in Indians
- Genetic factors and associated metabolic risks
- The nutritional status of Indians
- Dietary patterns in India.
- Vegetarian diets
- Vitamin B_{12} status and metabolic risks factors
- Protein status of non-meat eating and meat-eating Indians
- Iron status of non-meat eating and meat-eating Indians
- Maternal nutrition and epigenetics
- Evolution and disease patterns

The explanation to the above problems will form the aim, hypothesis and significance of the study.

1.1.1 Obesity and related diseases in Indians

The rising epidemic of obesity and its related diseases in Indians is well documented (Yajnik, 2004). A frequently cited study by, Mckeigue et al.,(1991) which compared 1515 European, 1421 Asians and 209 Afro- Caribbean men and 246 European and 291 Asian women aged 40-64y showed 4.3 times higher prevalence of diabetes in Asians

compared to Europeans. Mean serum insulin levels were 1.4 times higher in the fasting state and 2.1 times higher 2 hours (2h) after a glucose load in Asians compared to Europeans. Waist-to-hip ratio (WHR) was higher in Asian men and women compared to Europeans. Asian men had a higher waist-to-hip ratio compared to Asian women. Women in all the groups showed a stronger association for insulin and triglycerides with waist-to-hip ratio ($p<0.001$, for all) compared to body mass index (BMI). Asian men and women showed a strong association between 2h triglyceride and fasting insulin ($p<0.001$), both of which are metabolic variables of insulin resistance syndrome. Thus, insulin resistance associated with central adiposity was striking feature of Asians observed in this study.

Similarly in native Indians, Shelgikar et al., (1991) compared 389 Indian subjects living in India, classified as non diabetics (131 women and 73 men), subjects with impaired glucose tolerance (74 women and 42 men) and diabetics (184 women and 118 men). It was observed that, in both the sexes, subjects with impaired glucose tolerance were most obese based on BMI classification (for obese men BMI ≥ 27 $kg.m^{-2}$ and ≥ 25 $kg.m^{-2}$ for obese women). Non- diabetic men and women had smaller waist-to- hip ratio compared to subjects of the same sex with impaired glucose tolerance and diabetes ($p <0.01$, for all). Waist-to-hip ratio showed a clear shift to the right, in both, impaired glucose tolerance and diabetic subjects. For both the sexes, waist-to-hip ratio and not BMI, was related to fasting and 2-h plasma glucose $r = 0.26$ and $r = 0.25$ respectively ($p < 0.001$ for both). Overall, men showed a significant positive association of 2-h plasma glucose with waist-to-hip ratio ($p<0.001$) and women showed a significant association between 2-h plasma glucose and subscapular skinfold thickness ($p<0.01$). This study revealed that waist-to-hip ratio was greater in subjects with impaired glucose tolerance and diabetes compared to non diabetic subjects of the same sex. Thus increase in central obesity is more influential on metabolism. Individuals with central obesity (increased waist-to-hip ratio) are prone to develop insulin insensitivity and glucose intolerance.

Further work by Knight et al., (1992), showed that, compared to Caucasian men, Indian men had double the serum insulin concentration ($p<0.001$), lower high density lipoprotein cholesterol;HDL cholesterol ($p<0.001$), higher plasma triglycerides ($p<0.072$) and higher waist-to-hip ratio ($p<0.004$). Insulin positively correlated with waist-to-hip ratio in Indian men ($r = 0.46$). They also reported information on physical activity obtained using questionnaires that included recall of light, moderate or strenuous physical activity in the two groups. The self reported intensity of strenuous physical activity was 16% and 9% in the Caucasian and Indian men respectively. The intensity of regular physical activity was 44% in Caucasian men and Indian men respectively. The common observation by Knight et al., (1992) and Mckeigue et al., (1991), was that, for the same body mass index (BMI) Indians had a higher risk of metabolic syndrome compared to Caucasians.

Furthermore, Dhawan et al., (1994) compared the risk factors of 83 British Asians, 87 white men and 30 Asians living in India with age matched controls. It was observed that, upper body obesity or central obesity was more strongly associated with insulin and triglycerides- risks factors for metabolic syndrome. In British Asians and Indian Asians, waist-to-hip ratio was the strongest predictor of coronary heart disease.

In a national Indian survey, consisting of 11216 sample (5288 men and 5928 women) aged 20y or more and representative of all socioeconomic strata, Ramachandran et al., (2001) showed that obesity defined by WHR and BMI was 50.3% and 30.8% respectively. Prevalence of diabetes and impaired glucose tolerance showed increasing trend with age. Diabetes showed a positive and independent association with age, BMI, WHR, family history of diabetes, monthly income and sedentary lifestyle. Impaired glucose tolerance showed association with age, BMI and family history of diabetes. The study showed that there was a large sample of population with impaired glucose tolerance on the way towards conversion to diabetes.

Mohan et al., (2001) compared 1262 individuals aged ≥ 20y from two economically distinct groups in urban Chennai, and showed that, high socioeconomic status also predicts risk in increased prevalence of diabetes, impaired glucose tolerance, central obesity, serum triglycerides and serum insulin. Also, symptoms of metabolic syndrome-central obesity and hyperinsulinemia were significantly higher in the higher income group.

The epidemic of diabetes is not limited to urban settings but has also affected the rural Deo et al., (2006). (ibid) observed a significantly high prevalence rate of diabetes (9.3%) in rural areas in I ndia compared to the prevalence rate of 2.4% reported fourteen years ago by Ramachandran et al., (1992).

In India, evidence of the problem has accumulated over the last two decades. In urban south Ramachandran et al., (1992) conducted a survey in urban area of south India from 1988-89, comprising of 900 individuals (457 men and 443 women), mean age 38y. They reported 8.2% prevalence of diabetes in this population. Five years later in 1994-95 Ramachandran et al., (1997) conducted a survey on 2183 individuals (1081 men and 1102 women), from the same urban area and similar socioeconomic status. They did not report any increase in anthropometric variables between the two surveys, but the prevalence of diabetes increased from 8.2 to 11.6% and prevalence of impaired glucose tolerance increased from 8.7% in 1988-89 to 9.1% in 1994-95. Prevalence of impaired glucose tolerance was dependent on age, BMI and waist-to-hip ratio. Parameters influencing diabetes were age, WHR, BMI and female sex in the enlisted order. Positive family history had a greater influence on diabetes in lean subjects compared to overweight subjects. As in the second survey there was no change in central adiposity, the researchers of the study attributed to prevalence of diabetes to an influential environmental factor compounded with a strong genetic predisposition driving the epidemic of diabetes in this population.

The epidemic of obesity is not restricted to adults in India, but has also affected children Ramachandran et al., (2002), observed 17% prevalence of overweight in boys and 18% in girls aged 13 to 18 years in urban south India. The prevalence was inversely related to physical activity scores and directly related to the parental occupation and socioeconomic status (i.e higher socioeconomic status more obesity).

Comparing British South Asian with Caucasian children aged 8 to 11years (y), Whincup et al., (2002), showed that children of south Asian origin weighed less ($0.01 \leq p \leq 0.05$), had a lower ponderal index ($p < 0.005$). Nevertheless, children of south Asian origin had higher fasting and post glucose load insulin ($p < 0.05$, for both), they also had higher fasting triglyceride concentration ($0.01 \leq p \leq 0.05$) and non-significantly lower HDL cholesterol concentrations compared with their Caucasian counterparts. Establishing correlation of fasting glucose and post glucose load insulin with ponderal index, waist circumference and waist-to-hip ratio using regression slopes it was observed that, the slopes of all the three measures were markedly steep in south Asian children. Thus, the above results with respect to increased insulin and triglyceride concentrations in South Asian children suggest that the features of insulin resistance are present at a young age in Indian children proposing the possibility of genetic predisposition. Changes in environment due to migration and low physical activity could have exacerbated insulin resistance by increasing central adiposity or intra-abdominal fat.

Indians are reported to be less physically active compared to European population (Fischbacher et al., 2004). In a national representative sample in the UK comprising of 840 Caucasian and Indian (from India, Pakistan and Bangladesh) males and females, Hayes et al., (2002) compared the physical activity levels based on the physical activity patterns in these two diverse populations. The physical activity patterns were classified as usual day time activity, physical activity, walking, cycling, sports and recreational activity. An index of physical activity with a range of 0-8 was created by awarding points for the responses to the question on patterns of physical activity. A score of two or more was considered equivalent to five episodes of moderate activity of thirty minutes duration per week.

On all measures, Europeans were reported to be more active compared to Indians (p<0.05). More European men scored at least two or more on the physical activity index compared to Indians (48% vs 18%, p <0.001). Similar patterns were found in women (36% vs 17%, p <0.001). A great proportion of the Indian sample did not participate in any structured recreational or sport activity (90% vs 57% in men and 88% vs 72% in women). The physical activity index in European men correlated negatively with waist-to-hip ratio in European men (r = -0.18, p = 0.001) and with 2h post glucose load insulin in Indian men (r = -0.15, p = 0.013). Amongst women, physical activity negatively correlated with waist- to-hip ratio, systolic blood pressure, 2h glucose and 2h insulin (p <0.005, for all) in European women. In south Asian men it negatively correlated with waist-to-hip ratio, systolic blood pressure and 2h glucose (p <0.005, for all). Thus, physical activity had positive effects on risk factors for diabetes in Indian population.

Bettiol et al., (1999) examined the cardio respiratory fitness of 9y old children (711 boys and 711 girls) in UK, belonging to different ethnic groups (white, African/Afro-Caribbean and those originating from Indian subcontinent). Physical fitness was assessed using power output against a load of 85% of maximum heart rate by cycle ergometer test. In all, 14.3% boys and 16.2% of the girls were unable to complete the test. The factors that significantly affected the ability of the children to perform were height, systolic blood pressure and Indian origin. Taller children performed significantly better (p<0.001, for both the sexes). Systolic blood pressure (p= 0.006 and p = 0.07, for boys and girls respectively) and sum of skinfold thickness (p <0.001 for both sexes) were negatively associated with ability to perform. The odds ratios for inability to perform in children from Indian subcontinent were OR = 0.34, 95% CI: 0.16-0.68 in boys and OR= 0.22 95% CI: 0.12-0.40 in girls. Thus, fitness was associated to obesity and Indian children had poor levels of physical fitness. The plausible explanation for the etiology of prevalence of diabetes and insulin resistance in Indians is lifestyle patterns, physical activity and genetic predisposition.

1.1.2 Genetic factors and associated metabolic risks

The Indian population could be predisposed to increased insulin and central adiposity since birth. Indian babies are the lightest babies in the world (Yajnik et al., 2002). Yajnik et al., (2002) compared Indian neonates from Pune (an urban city in India) with Caucasian babies born in the UK and reported that, Indian babies had a significantly higher cord plasma glucose (p<0.001) and insulin (p<0.001) concentrations. In Indian babies, cord plasma insulin concentration was directly correlated with subscapular skinfold thickness (r = 0.26, p<0.05) but not any other anthropometric variable. Thus, there is evidence to show that Indians are predisposed to central adiposity and its metabolic risk factors before birth which are carried forward through different stages of life.

Further, studies by Yajnik et al., (2003) have determined that, Indian babies showed a reduced abdominal circumference -reflecting visceral size and reduced mid arm circumference-reflecting reduced muscle bulk. On the contrary they had well preserved subscapular fat which is a surrogate of central fat. Thus, a thin Indian baby is relatively fat (Yajnik et al., 2003). As explained in section 1.1.1 the Indian population shows an increased tendency to accumulate central fat, which is associated with insulin resistance syndrome and risk factors for cardiovascular diseases. These metabolic risks could be programmed in early life (*in-utero*).

The intra-uterine environment is one of the important factors responsible for the development of the foetus. Adequate maternal nutrition is important to maintain a favourable intra-uterine environment. It is the most important not only in relation to energy intake by the mother but also requires the inclusion and balance of micronutrients and macronutrients in the diet. Lack of any essential nutrient could have a profound effect on the development of the foetus. The effects of maternal nutrition on the foetus have been studied in detail in India by Dr Yajnik and colleagues as referred to by James (2006). To understand the relationship between increased insulin resistance, central adiposity and maternal nutrition in this population, it is important to have knowledge regarding their present nutritional status and dietary patterns.

1.1.3 Nutritional status of Indians

India is at an early stage of epidemiologic nutritional transition. This is defined as the shift from a pattern of high prevalence of infectious disease associated with malnutrition, periodic famine, and poor environmental sanitation to one of high prevalence of chronic and degenerative disease associated with urban–industrial lifestyles (Popkin et al., 2004).

There have been only two nationally representative health surveys in India; National Family Health Survey-1, NFHS-1 (1992-93) by International Institute of Population Sciences (1995) and the National Family Health Survey, NFHS-2, (1998-99) by International Institute of Population Sciences and ORC Macro (2000).
NFHS-1 included 89,777 married women aged 13- 49y and 88,562 households. However, the data was presented only for women and their children. The 2nd National family Health Survey NFHS-2 included a representative sample of more than 90,000 eligible women aged 15-49y and children up to the age of four months. Both the health surveys did not present any information on men.

NFHS-1, in India, compared the data on children with international reference populations from the National Centre for Health Statistics (NCHS) and World Health Organization (WHO). It was reported that, more than half (53%) of all NFHS-1 children under the age of four were underweight (low weight for their age) and a similar proportion (52%) were stunted (that had a low height for their age). Twenty one to twenty nine percent of children were severely undernourished according to weight for age and height for age measures and 17% were wasted (low weight for their height). The report identified that, the period of active growth failure in children in every state was between the six and twenty four months of age period during which mean underweight quadrupled. Stunted children often are exposed to inappropriate weaning practices, repeated infections and poor diet in infancy due to poverty. They also have a reduced lean body mass resulting in lower metabolic rate and energy expenditure in physical activity (Popkin et al., 1996).

The second National Family Health Survey, NFHS-2, (1998-99) reported a 6% reduction in stunting (low height for age) but prevalence of wasting was similar at 16% in children. In women, 36% had a BMI <18.5 kg/ m^2 suggesting chronic energy deficiency, 11% were classified as overweight (BMI 25.0-30.0) and 2% with obesity (BMI>30.0). There were significant demographic differences in the prevalence of chronic energy deficiency, overweight and obesity. Chronic energy deficiency was 41% in rural women vs. 23% in urban women. Overweight and obesity were 6% in rural women vs. 24% in urban women. Thus, obesity was more prevalent in the urban population and energy deficiency was prevalent in the rural population.

In India, large shifts have occurred in dietary and physical activity patterns (Popkin, 2002). Popkin et al., (2001) reported that in India there was a large shift from consumption of high fibre coarse grain such as barley, rye, maize and millet to consumption of low fibre rice and wheat among all income groups. Indians consume a high amounts of clarified butter, which is associated with increased prevalence of coronary artery disease in rural and urban India (Singh et al., 1996). Popkin et al., (2001), further reported carbohydrate (particularly high in sugar) as being the highest source of energy (71%) followed by fat (18%) and protein (11%) being the least in urban India and a similar pattern observed in rural India. Rao et al., (2001), in a study on pregnant women and their babies in rural India, reported carbohydrate as the major source of energy followed by fat and protein.

The above information regarding the dietary patterns and body size information is in agreement with the suggestion by Uauy and Solomons (2006) that, malnutrition in the population does not comprise of under nutrition alone. Both under and over nutrition can be exhibited at all levels within a population.

When these exist in children within the same nation it creates complications in relation to the future health of the child. This is the problem encountered in India. Uauy and Solomons (2006) identified four different forms of malnutrition to categorise an individual's nutritional status:

- Underweight- defined by low weight-for-age.
- Wasting – low weight-for-height.
- Stunting – low height-for-age.
- Overweight- excess weight for one length and stature, measured as weight for height or BMI centile for age.

Popkin et al., (1996) compared data from national health surveys of Russia, China, South Africa and Brazil for examining the relationship between stunting and overweight status in children aged 3-6y and 7-9y. The prevalence of overweight in stunted children was 3.5% in children from Brazil to 45.1% in children from Russia. On examining the effect of increasing income, it was observed that, the relative risk ratio ranged from 1.7 in Brazil to 7.8 in Russia. The increase in risk ratio was attributed to the nutrition transition in these countries and the changes in activity and dietary patterns.

Obesity developed in childhood can track into adulthood. The prepubertal or preadolescent growth period is associated with adiposity rebound (Dietz, 1994). BMI rises during infancy and reaches the lowest point at approximately 6-8y of age and then starts rising again (Rolland-Cachera et al., 1987). Early adiposity rebound is associated with early maturation, which is in turn associated with increased adiposity in adulthood (Van Lenthe et al., 1996).Alternate explanation for stunting-overweight relationship focuses on hormonal changes occurring *in-utero*, programming and nutrition in early life which may promote adiposity but not linear growth (Popkin et al., 1996). The difference between linear growth and adipose tissue becomes enhanced in the presence of adequate energy intake but limited essential nutrients which favours deposition of adipose tissue but not linear growth (Shetty, 2002). The Dutch famine study by G. P. Ravelli et al., (1976) have determined that prenatal and early post natal nutrition direct subsequent obesity. Nutritional deprivation affects the differentiation of hypothalamic centres regulating food intake and growth. Subsequent increase in food availability results in an accumulation of excess fat in an organism growing to its predetermined maximum size (G. P. Ravelli et al., 1976).

1.1.4 Dietary patterns in India

In India, the family meal pattern is dictated by geographical region, religion, community and family practices that have developed over several generations (Jayanthi, 2001). It is also regulated by the socio-economic status. India is a country with diverse religious practices. Meat consuming habits are predominated by religion. People belonging to the Hindu religion, form a large part of the Indian population. Hindus consume chicken, lamb and pork but, do not consume beef, Muslims do not consume pork; and Christians/Catholics consume all types of meat, but they form a minor part of the Indian population. Meat consuming habits are also dependent on the geographical part of the country they originate from, e.g. meat-eaters in India originating from eastern zone and coastal areas of the western and the southern part of the country consume more fish than meat. In the northern and some southern parts of the country, meat-eaters consume more red meat and white meat compared to fish or sea foods. Consumption of meat is higher in higher socio-economic class.

The NFHS-2 (1998-99) reported some interesting findings in relation to meat consuming habits amongst women in India. It was reported that, one third of women participating in the survey consumed eggs, chicken, meat and fish at least once a week. One in three never consumed chicken, meat or fish. Among married women, aged 15-49y the total daily consumption of chicken + fish + meat was 5.8% and total daily consumption of eggs was only 2.8%. Women aged 15-24y consumed the least amount of eggs, meat, chicken and fish. Consumption was higher in urban compared to rural women.

NFHS-2 further reported that, in relation to other food sources rich in protein and vitamin B_{12}, for example milk and curds. One in ten women never consumed milk or curd and the low consumption of these above foods could have resulted in low protein quality diet, decreased vitamin B_{12} concentrations and nutritional anaemia following vitamin B_{12} deficiency. Further, it was reported that, the total daily consumption of green leafy vegetables and pulses and beans was greater than milk and milk products. As expected, Hindus consumed the least amount of eggs, chicken, meat and fish followed by Muslims and Christians who had the highest consumption. However, amongst the three groups, Hindus had the highest consumption of green leafy vegetables, pulses, beans and milk.

The low consumption of meat and meat products in Indians also reflects the vitamin B_{12} status of an individual. Khanduri et al., (2005), observed five times greater vitamin B_{12} deficiency compared to folate in vegetarians and non vegetarians in India. The meat consumption pattern of the non vegetarians in their study was similar to that reported in NFHS-2 (1998-99).

Further, absence of meat, meat products and eggs can also affect status of other micronutrients in the diet for example, iron. The NFHS-2 (1998-99) reported 52% prevalence of anaemia in women aged 15-40y and 74% in children aged 0-35 months. Anaemia was measured in children and women by direct measurement of haemoglobin only.

Gomber et al., (1998) reported 41.1% prevalence of iron deficiency anaemia in an urban slum in Delhi. Anaemia was exclusively due to lack of iron in 159 children aged three months to three years. Further, they also reported anaemia due to nutritional deficiency of B_{12} and folic acid as 14.4% and 2% respectively. Combined deficiency anaemia defined as deficiency anemia due to lack of all the three micronutrients; iron, folic acid and B_{12} or lack of any two was the second most common cause of anemia (25.5%) of which anaemia due to iron and B_{12} deficiency was seen in 87% of cases. The nutritional anaemias observed by (ibid) were due to malnutrition in relation to poor food intake, decreased stores, vegetarianism and prolonged breast feeding by anaemic mothers.

1.1.5 Vegetarian diets

Vegetarianism has been practiced for thousands of year in India, therefore there is an increased possibility that high number of this population is at risk of having low vitamin B_{12} status throughout life (Antony, 2003). The vegetarian diet evolved in India with advent of Jainism in 500 B.C. Before the advent of Jainism there was apparently no restriction in acceptance of food by Indian people. The popularity of Jainism bought along with it the concept of abstinence from flesh foods. In India abstinence from flesh foods represents a way of life that has cultural and spiritual implications. Vegetarianism is evident in most religious practices and represents a conscious choice with respect to diet (Jayanthi, 2001).

Vegetarian diets confer some advantages on health as a result of lower intakes of saturated fat, cholesterol and high intakes of complex carbohydrates, dietary fiber, magnesium, folic acid, vitamin C and E, carotenoids and other phytochemicals (Leitzmann, 2005). Simultaneously, there is serious health risk associated with this dietary pattern. Most of the health risks associated with vegetarian diets are found in strict vegetarians and to a lesser extent in lactoovovegetarians and lactovegetarians. Excluding animal products and ingesting only plant proteins affects the status of certain B-vitamins especially vitamin B_{12} (Huang et al., 2003). Vitamin B_{12} is only provided in animal derived proteins. All naturally occurring forms of vitamin B_{12} arise in microorganisms which are incorporated into animals, principally herbivorous animals. These animals are the food sources for other animals, thus vitamin B_{12} enters the food chain. There is complete lack of the enzymes for their biosynthesis in all plants and animals (Scott, 1999).

Vitamin B_{12} is essential for DNA synthesis, erythropoiesis, development and maintenance of the myelin sheath of nerves. Deficiency of vitamin B_{12} leads to pernicious anemia, gastrointestinal disorders, and neurological changes which include symptoms like, numbness and/or tingling of outer extremities, decreased vibration sense and/or position sense, decreased visual acuity, unsteadiness, poor muscular coordination with ataxia, moodiness, mental slowness, poor memory, confusion, agitation, depression, delusions, hallucinations, and even overt psychosis (Donaldson, 2000). The classical pathophysiological manifestations of B_{12} deficiency include megaloblastic anemia and neurological complications that run a gamut from peripheral neuropathy to depression, cognitive disturbances and dementia (Carmel et al., 2003).

Vitamin B_{12} along with folate is actively involved in maintaining the serum homocysteine concentration through the methylation process. Homocysteine- a thiol containing amino acid is derived from methionine which is an essential amino acid. Raised homocysteine has been identified as an independent risk factor for cardiovascular diseases and neurogenerative diseases (Geisel et al., 2003).Vitamin B_{12} and folate are synergistically and actively involved in cell growth and proliferation and are important determinants of fetal growth (Yajnik et al., 2005).

Yajnik et al., (2005), showed that a small maternal size and poor maternal nutritional intake of vitamin B_{12} and folate can affect the birth size of the foetus and also increase the risk for CVD in later life. In a population based study (Pune Maternal Nutrition Study) in 631 full term babies and their mothers across six villages in Pune, India, researchers investigated the relationship between maternal plasma total homocysteine, vitamin B_{12} folate concentrations and neonatal size.f

Nearly half of the women involved in the study never consumed meat, fish and eggs and one third never drank milk. Seventy percent of the women had low vitamin B_{12} status (plasma vitamin B_{12} <150pmol/L). None of the participants had folate deficiency. The mothers of small for gestation age (SGA) babies were themselves shorter and lighter and had higher total plasma homocysteine concentration (p <0.01) at 28 weeks of gestation.

They reported that increased plasma total homocysteine correlated negatively with birth weight (r = -0.28, $p<0.05$). Further in depth statistical analysis showed that, adjustment for red cell folate reduced the significance. This effect of red cell folate on birth weight despite the fact that none of them were folate deficient, emphasized the metabolic folate deficiency (folate trap) developed as a result of vitamin B_{12} deficiency. Also the vitamin B_{12} levels in the study group were too low to establish a correlation with neonatal weight. The researchers concluded that, the vitamin B_{12} deficiency in Indian mothers an associated functional folate deficiency could be the cause of increased risk of cardiovascular diseases in later life. Indians could be born with low vitamin B_{12} levels due to low maternal levels. The deficiency is carried forward due to breast feeding by nutrient deficient mothers and vegetarian diet (Yajnik et al., 2005).

Thus, vitamin B_{12} and folate play an important role in maintaining the homocysteine levels. A detailed explanation regarding their function in other metabolic reactions is discussed in the following section.

1.1.6 Vitamin B$_{12}$ and metabolic risk factors

Dr Yajnik has suggested a challenging role of low levels of vitamin B$_{12}$ in manifesting metabolic risk factors like fat accumulation, poor skeletal muscle and increasing insulin resistance in addition to the above mentioned disorders (Figure 1.1).

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Figure 1.1: Suggested metabolic mechanism for adiposity, insulin resistance and altered gene expression in non-meat-eating diets
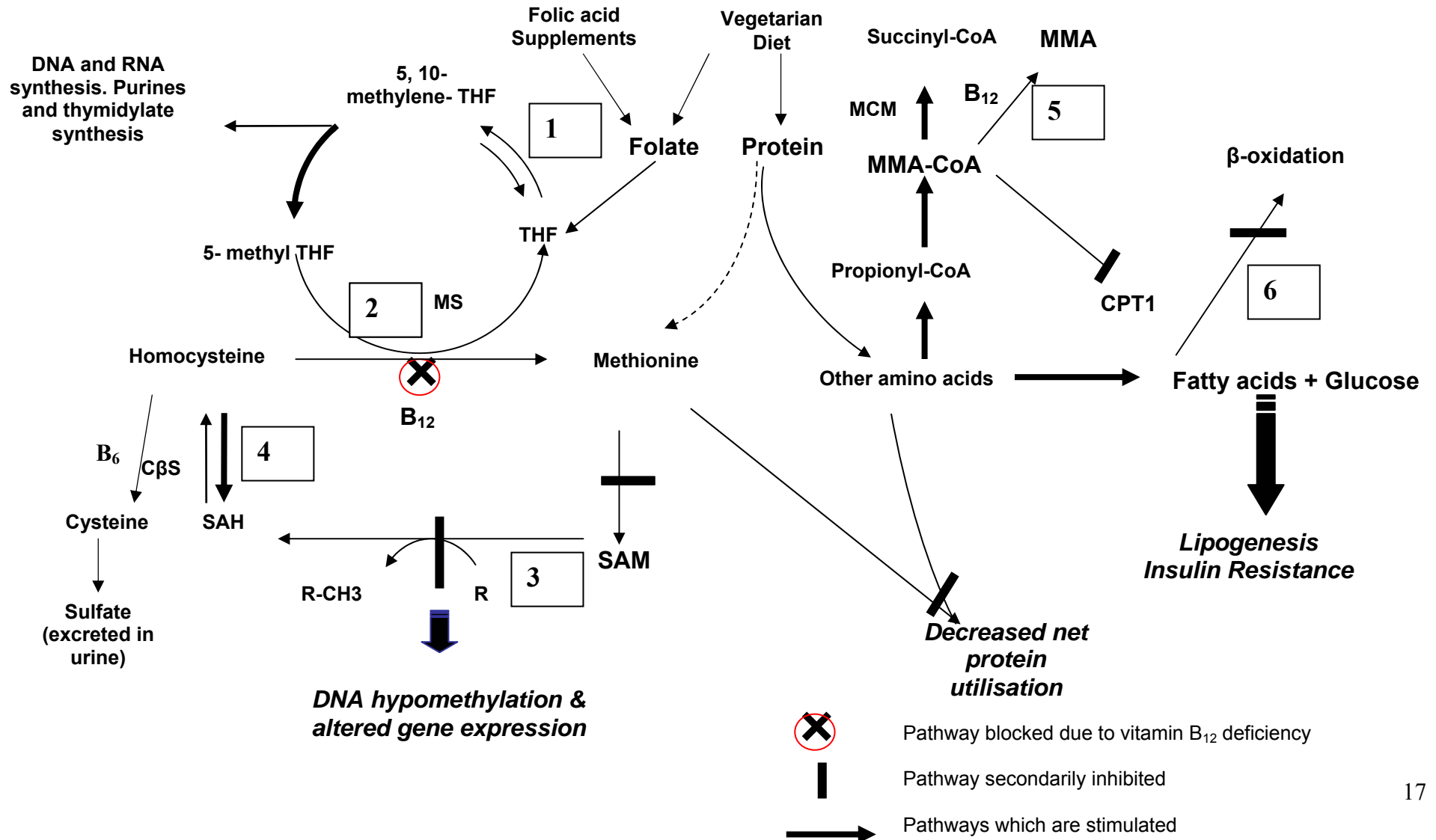
Vitamin B_{12} and folate are actively involved in "one-carbon metabolism". One-carbon metabolism is a network of interrelated and interdependent biochemical reactions involving the transfer of one carbon groups from one compound to another involved in methylation reactions. Figure 1.1 is a schematic representation of these reactions and suggested metabolic mechanisms for obesity and insulin resistance due to vegetarianism and low consumption of meat observed in the Indian population. Important co-enzymes required for these reactions include vitamins B_{12}, B_6 and folate. While the insufficiency of any of these essential nutrients may not cause overt classical deficiency syndromes, reduced levels can still contribute to important diseases like cardiovascular risks and cancers through one-carbon metabolic imbalance (Mason, 2003).

One-carbon metabolism involves methylation pathway in which nutrients play an important role by affecting DNA methylation. Methylation modifies the genomic DNA without altering its sequence. This process is also called 'Epigenetic modification'. Methylation also plays a role in functionally relevant modification of RNA, phospholipids, proteins, and the synthesis of neurotransmitters. The important nutrients that affect methylation are folic acid, zinc, betaine and vitamin B_{12} (Van den Veyver, 2002). As the present thesis is designed to explore the significance of vitamin B_{12} and protein with respect to the Indian dietary pattern, a systematic explanation of the processes involved in one-carbon metabolism is explained below in step 1 to 6.

<u>Step-1</u>

Folate enters this system in the form of tetrahydrofolate (THF) which is converted to 5, 10-Methyltetrahydrofolate (5,10- MTHF). Then, 5, 10 MTHF receives one carbon group from serine and glycine (non-essential amino acids and main donors of one-carbon groups in methylation process) and gets reduced to 5, methyltetrahydrofolate (5, MTHF) which is the active form of folate in the body. During this reduction reaction, 5, 10 MTHF releases methyl groups that are used in DNA, RNA synthesis, thymidylate and purine biosynthesis. Hence, folic acid is not only important for the methylation of genomic DNA but more importantly it also plays a role in its synthesis (Van den Veyver, 2002).

Step -2

5, MTHF, is further reduced to THF with the help of enzyme methionine synthase which requires vitamin B_{12} as a co-factor, during this process it transfers a methyl group to homocysteine (Van den Veyver, 2002). The amount of regenerated THF is a very small and replenishment through dietary intake is required (Scott, 1999).

Simultaneous to reduction of 5, MTHF, Homocysteine which receives a methyl group from 5-MTHF gets converted to methionine due to the synergistic effect of vitamin B_{12} and enzyme methionine synthase (MS, Figure 1.1). Partly it also converted to cysteine via transulfuration pathway with the help of co-enzymes cystathionine β synthase and vitamin B_6 (Carmel et al., 2003).

Step -3

Methionine (obtained from homocysteine) is an essential amino acid also obtained from the diet (found in abundance in meat products). It is further converted to S-adenosyl homocysteine (SAH), via conversion to S-adenosyl methionine (SAM), which is a major methyl donor of methyl group for DNA, RNA biosynthesis, proteins and neurotransmitters in the process of conversion to S-adenosyl homocysteine. The methyl group donated by SAM is a significant part of the cycle especially during the prenatal and post natal period during which may induce phenotypic changes in the embryo as shown in agouti mice by Waterland and Jirtle (2003). Thus, early nutrition can influence the establishment of epigenetic marks in the early embryo thereby affecting all the tissues including the germ line. Thus, vitamin B_{12} is an essential nutrient since it plays a significant role in the above methylation reactions. Methionine is not only required for methylation process, but is also required for protein synthesis (Van den Veyver, 2002).

<u>Step-4</u>

S-adenosyl homocysteine (obtained from methionine) is hydrolysed to homocysteine, which either enters the trans-sulfuration pathway and gets converted to cysteine or is converted back to methionine as explained in step 2. Hence vitamin B_{12} plays a significant role in the methylation pathway. Any discrepancy in the methylation pathway due to folate or vitamin B_{12} deficiency leads to

- DNA hypomethylation and altered gene expression as a result of adaptations to absence of methyl groups thereby resulting in decreased DNA biosynthesis and affects the cell division, primarily rapidly dividing cells like the erythrocytes thereby resulting in anemia (Van den Veyver, 2002).
- Increased level of serum homocysteine, a recognized as risk factor for cardiovascular and neurogenerative diseases (Geisel et al., 2003).
- Functional folate deficiency.

<u>Functional folate deficiency</u>

In the case of vitamin B_{12} deficiency, the two reactions are affected; conversion of 5-Methyl tetrahydrofolate to tetrahydrofolate (step 2) and conversion of homocysteine to methionine (step-2). The requirement of a methyl group by homocysteine to get converted to methionine stimulates enzyme methyl tetrahydrofolate reductase to convert 5, MTHF to free folate or tetrahyderofolate towards methionine synthesis. However, due to inadequate vitamin B_{12} status, the conversion of 5-MTHF to THF is disabled resulting in accumulation of free folate, while the intracellular folate pool available for methylation reaction decreases. Thus, folate cannot be utilized and gets trapped resulting in a methyl folate trap (Van den Veyver, 2002).

Step-5: Biochemical abnormalities exclusively due to B12 deficiency (Step 6)

Vitamin B_{12} is essential for the conversion of propionyl-CoA to succinyl CoA through formation of methylmalonyl CoA, by enzyme methylmalonyl CoA mutase and vitamin B_{12} as co-factor as shown in the step 5 in Figure 1.1 and also illustrated in Figure 1.2.

Figure 1.2: Role of vitamin B_{12} in formation of succinyl CoA

Methylmalonic acid

D-methylmalonyl
CoA hydrolase

Propionyl-CoA ⟶ D-methylmalonyl CoA

D-methylmalonyl CoA
Racemase

Valine, isoleucine, methionine,
threonine and odd chain fatty acids

L-methylmalonyl CoA

B_{12} L-methylmalonyl CoA
mutase
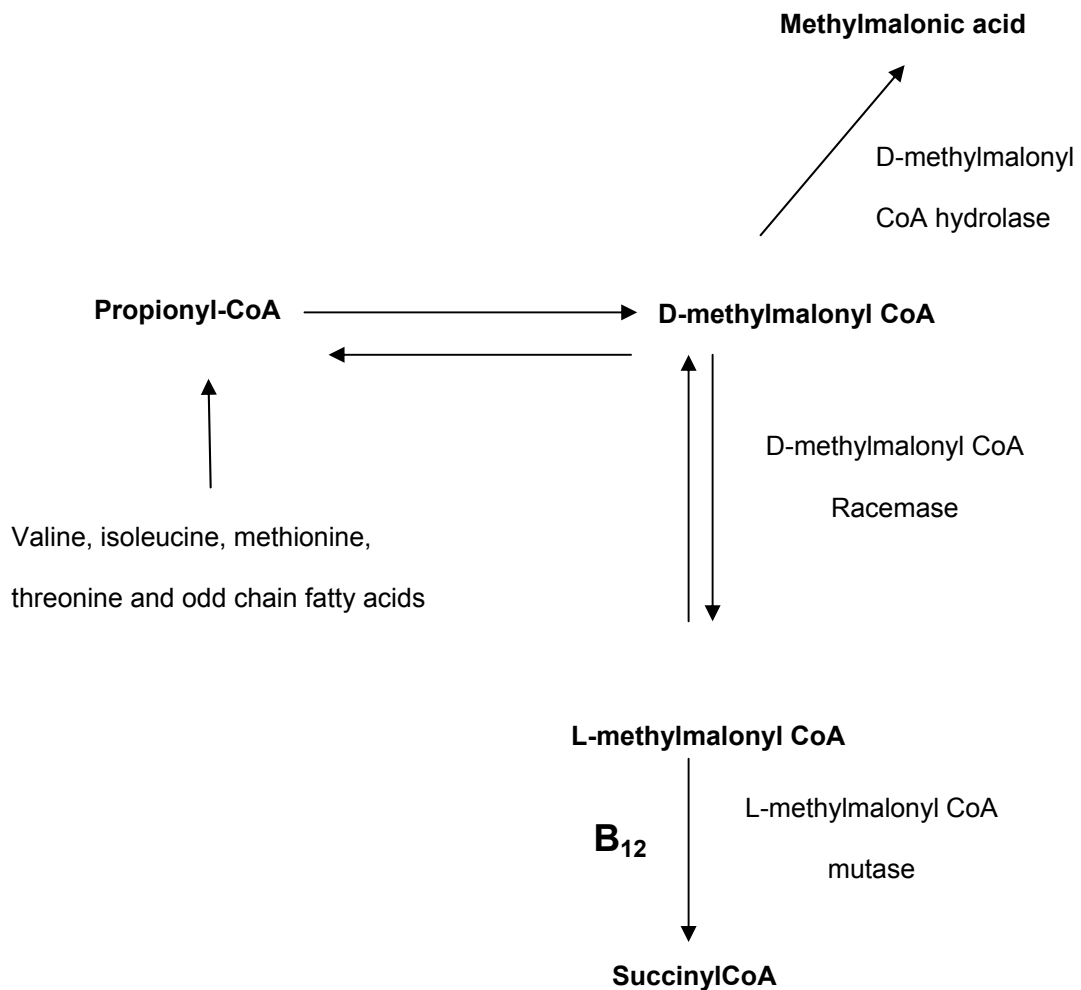
SuccinylCoA

Adapted from Allen, Stabler, and Lindenbaum (1998)

Under normal circumstances, propionyl –CoA is converted to succinyl-CoA through
methylmalonyl-CoA. Methylmalonyl-CoA utilizes vitamin B_{12} as a co-enzyme and gets
converted to succinyl-CoA. Due to vitamin B_{12} deficiency, methylmalonyl CoA
(MMA- CoA) is hydrolysed to methylmalonic acid (MMA). The increased serum level
of methylmalonic acid is a classical marker of vitamin B_{12} deficiency as this pathway is
independent of all forms of folate. Vitamin B_{12} deficiency can also result in increased
propionyl- CoA concentrations, as shown in Figure 1.2.

Increased propionyl-CoA can substitute acetyl-CoA in fatty acid biosynthesis and lead to formation of fatty acids with odd number of carbon atoms (Allen et al., 1998). The accumulated methylmalonyl- CoA due to vitamin B_{12} deficiency can substitute malonyl CoA in fatty acid synthesis and lead to formation of branch chain fatty acids (Allen et al., 1998).

Effect of increased malonyl-CoA concentrations on fatty acid oxidation.

In the liver, increased concentrations of malonyl CoA further inhibit the activity of carnitine palmitoyltransferase-1(CPT1) the enzyme that controls the rate of long chain fatty acyl-CoA transfer into the mitochondria (Ruderman et al., 1999), (Step-6, Figure 1.1). Inhibition of CPT1 results is accumulation of fatty acids in the cytosol of the nucleus and increased deposition of fatty acids into glycerolipids, thereby resulting in increased levels of triglycerides, diacylglycerol and fatty acids observed in insulin resistant muscle resulting in lipogenesis and insulin resistance (Ruderman et al., 1999).

Pan et al., (1997) showed that the mechanisms underlying the relationship between muscle triglyceride and insulin action on skeletal muscle glycogen synthesis may be central in understanding the etiology of insulin resistance. They performed biopsies of fasting skeletal muscles in 38 non-diabetic Pima Indian males aged 28±1y, and showed that, muscle triglycerides are the source of lipids that impair insulin mediated glucose metabolism. A direct correlation between fasting serum triglyceride and fasting plasma insulin was reported (r = 0.44, p<0.006). A negative association was reported between muscle triglyceride and insulin action at supraphysiological levels (r = -0.44, p<0.006). Measures of adiposity like percent body fat, BMI and waist to thigh ratio were also significantly negatively correlated with insulin action (p<0.005 for all).

They further reported that, in a multiple regression model, measures of insulin resistance were significantly related to muscle triglyceride independent of all measures of obesity. In turn, all measures of adiposity were related to insulin resistance independent of muscle triglycerides. All the measures of obesity and muscle triglyceride accounted for similar variance (44%) in insulin resistance. Thus, this study could be suggestive that, skeletal muscle insulin sensitivity is strongly regulated by supply of triglycerides. The supply of triglyceride depends on the rate of fatty oxidation which is regulated by dietary factors and muscle activity.

Further, malonyl CoA plays a vital role in fuel sensing and signaling mechanism as explained by Ruderman et al., (1999) while performing experiments on soleus muscle (broad flat muscle in the calf) of rats. The concentrations of malonyl- CoA increases two to six fold within 20 minutes in the after incubation of a rat soleus muscle with glucose and insulin and increased within six hours when the soleus muscle is made inactive. Conversely, its concentration decrease in a medium devoid of glucose and insulin and with increase in muscle activity. The effect glucose, insulin and physical activity on Malonyl-CoA levels are shown in Figure 1.3.

Figure 1.3: Regulatory factors of Malonyl CoA

| Glucose and Insulin | (+) → | Malonyl CoA | ← (-) | Decreased Fuel |
| | | | | |

(+)↗ from Decreased physical activity

(-)↖ from Increased physical activity
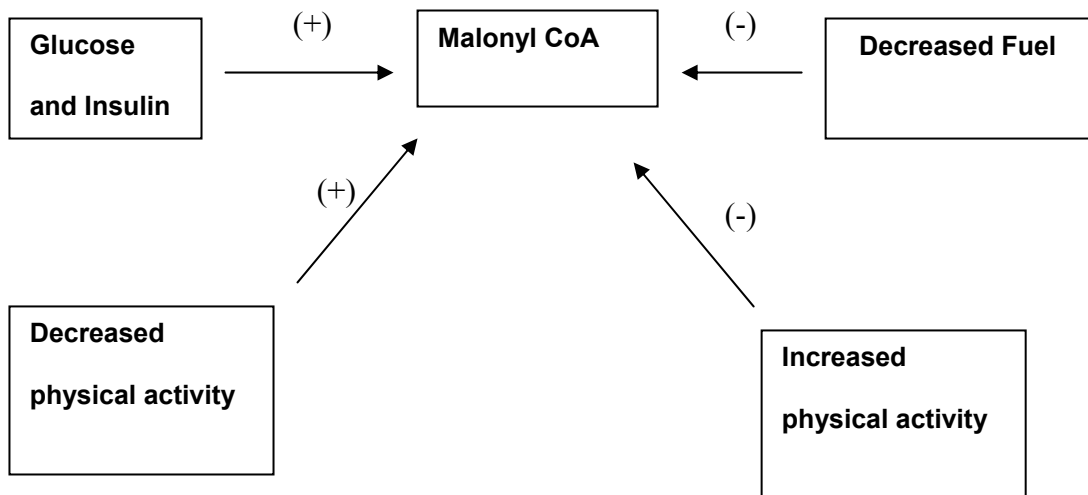
Adapted from Ruderman *et al.* (1999)

In vivo studies by Chien et al., (2000), in rats starved for 48 hours followed by 1, 3, 12, 18, 24h of refeeding, it was observed that, malonyl- CoA levels increase by 100% in soleus muscle (broad flat muscle in the calf) and by 60% in the gastrocnemius muscle (a muscle that forms greatest part of the rat leg). The increased malonyl-CoA affected the fatty acid metabolism which was apparent in rapid decrease in the long chain fatty acyl carnitine (LCFA- carnitine). The LCFA-carnitine helps in the oxidation of fatty acid. LCFA-carnitine concentrations in the soleus and gastrocnemius muscle of rats decreased by ~50% at one hour of refeeding.

Subsequently Chien et al., (2000) reported that, plasma free fatty acid concentrations decreased from 289µmol/L at 48h starving to 121µmol/L after 24h of refeeding suggesting inhibition of CPT1. Decreased plasma free fatty acids in the rats correlated positively with the increase in respiratory quotient ($r = 0.9$, $p<0.05$). There was a positive correlation between malonyl-CoA and respiratory quotient ($r = 0.98$, $p<0.01$). Both the variables increased with increase in duration of refeeding. The respiratory quotient increased from 0.82 at 48h of starvation to 1.08 after 24h of refeeding. Thus, the above findings are suggestive of link between changes in malonyl-CoA concentrations in the muscle post feeding and its effect on the rate of fatty acid oxidation.

Additionally, conditions characterized by high insulin glucagon concentration ratios in the portal circulation result in increased hepatic activity of Acetyl CoA carboxylase (ACC: A biotin dependent enzyme that catalyses carboxylation of acetyl CoA to produce malonyl CoA) accompanied by elevated concentrations of malonyl- CoA (Zammit, 1996).

Muscle activity also regulates malonyl- CoA concentrations. Dean et al., (2000) have shown the effect of exercise in human skeletal muscles in thirteen male volunteers aged $24 \pm 1y$. Subjects exercised on one leg dynamic knee extensor for 45 minutes at 60% Vo_{2max}, 10 minutes at 85% Vo_{2max} and until exhaustion (100% Vo_{2max}) following an overnight fast. Muscle biopsies were taken immediately after exercising. Acetyl CoA carboxylase (ACC) and malonyl CoA concentrations were determined from the post exercise muscle. It was determined that ACC activity decreased 50-75% during exercise. Malonyl CoA concentration decreased significantly by (-12%, $p<0.05$) after

exercise at 85% Vo_{2max} and by (-17%, p <0.05) at 100% Vo_{2max}. There was a reported increase in energy expenditure, carbohydrate and fat oxidation post exercise. Thus, exercise exerts positive effects on the biochemical regulators of fatty acid oxidation and also influences energy balance. Thus, lack of physical activity compounded with vegetarianism and infrequent meat consumption in the Indian population could have exposed them to a gamut of metabolic disturbances.

1.1.7 Protein status in relation to non-meat-eating and meat-eating habits

Protein quantity and quality are the major determinants of human growth. Human proteins are made up of 20 different amino acids. The amino acid profile of protein demonstrates the quality of protein. The amino acids form two groups. First are those that are indispensable (essential) and must be supplied in the diet, either because they cannot be synthesized in the body or cannot be synthesized at a sufficient rate to meet the body requirements. The essential amino acids include: histidine, isoleucine, leucine, valine, tryptophan, threonine, methionine, phenylalanine and lysine. The second group are those that can be made within the body and are termed as non-essential amino acids. The non-essential amino acids include: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, praline, serine and tyrosine (Read, 1997).

The World Health Organisation (2003) recommends that, 10-15% of daily energy intake should comprise of dietary proteins. Availability of ingested proteins to the tissues is highly influenced by the digestibility and the biological value of the ingested protein (Millward et al., 2003). Eggs, milk, meat, wheat gluten, wheat flour and soy isolates have a high protein digestibility (>95%). Polished rice, whole grain cereals, peas and pea protein isolates have a lower protein digestibility of 80-90%. Breakfast cereals, millets, beans and mixed diets in developing countries have the lowest protein digestibility of 50-80% (Millward et al., 2003).

The biological value of various food protein sources are largely determined by concentrations and availability of individual essential amino acids. Animal proteins contain a full and sufficient complement of essential amino acids, making them good sources of high biological value proteins. If an essential amino acid is present in low concentration in a particular food, it is known as the limiting amino acid. Proteins with limiting amino acids are recognized as low biological value proteins. Most vegetable proteins are lacking in at least one amino acid. Plant based foods like legumes, cereals, nuts, seeds and fruits have lower concentrations of lysine, threonine and tryptophan (all of which are essential amino acids) and sulfur amino acids (cysteine, methionine and homocysteine). Nuts and seeds have higher concentration of tryptophan compared to animal foods (Young et al., 1994).

When a plant food has a limiting amino acid, its nutritional value with regards to protein quality can be improved by using 'protein or amino acid complementation.' It involves, combining two food sources with two different limiting amino acids such that the amino acid of one protein may compensate for the limitation of the other resulting in higher biological value. Also, two food sources containing the same limiting amino acid in quantitatively different concentrations (one of them much higher than the other) can be supplemented. For example, soyabean is low in sulfur containing amino acids (cysteine and methionine), whereas cotton seeds, peanuts, sesame flour and cereal grains are deficient in lysine, combining soy protein which is high in lysine with a cereal that contains appreciable concentrations of sulfur-amino acids results in nutritional complementation or mutual supplementation of proteins. Also, corn and cotton seed have the same limiting amino acid; lysine, but in varying concentrations, cottonseed is relatively less inadequate than corn. However, combining these two different sources enhances the biological value of the protein. Issues related to amino acid complementation include the timing of ingestion of complementary proteins. It is not necessary to consume complementary proteins at the same time, separation of proteins among meals through the day permits nutritional benefits of complementation (Young et al., 1994).

Millward and Jackson (2003) have determined the nutritional value of dietary proteins using the protein-digestibility-corrected amino acid score (PDCAAS) suggested by Food and Agriculture Organisation and World Health organisation (1991). It is a product of digestibility and amino acid score. It is equivalent to net protein utilization. Further, they have also calculated the reference protein energy: total energy ratio (P/E) for some foods and diets. They have reported the P/E ratio, Protein digestibility score and PDCAAS-adjusted P/E ratio for some foods which revealed that, beef had the highest P/E ratio of 0.66 and protein digestibility and PDCAAS-adjusted P/E ratio 0.66 for both, soy had P/E ratio of 0.38, protein digestibility score and PDCAAS-adjusted P/E ratio 0.34, egg had a P/E ratio of 0.34, protein digestibility and PDCAAS-adjusted P/E ratio 0.34. Cow milk had a P/E ratio of 0.19 and protein digestibility and PDCAAS-adjusted P/E ratio 0.19. Contrary to the above foods, wheat had a P/E ratio of 0.19 and protein digestibility and PDCAAS-adjusted P/E ratio 0.95 and 0.08 respectively. The lowest values were reported for rice as P/E ratio 0.07, protein digestibility 0.82 and PDCAAS-adjusted P/E ratio 0.04.

They further reported that food intake of an average Indian had the lowest PDCAAS-adjusted P/E ratio 0.07 compared to food intake of omnivores and vegetarians in the UK. The lowest values are reported for the west Bengal region (eastern region of India) and Tamil Nadu in the south were 0.05 and 0.07 respectively. Rice is the staple and the major constituent of diet in both the regions.

Thus, people following a predominantly lactovegetarian diet ingest foods with decreased net protein utilization. Further, eliminating meat products from the diet results in decreased protein digestibility. This could result in decreased protein utilization. Albeit, including eggs and milk can improve the protein availability and utilization.

Ingestion of dietary protein intake has a direct effect on muscle protein (Wolfe, 2006). Muscle contains the major portion of protein in the body. One of the important functions of dietary protein is skeletal muscle synthesis. A diet low in protein also results in reduced muscle protein turnover due to decreased net protein utilization in the body (Figure 1.1).When exogenous sources (dietary sources) of amino acids are provided, only essential amino acids are required for the stimulation of muscle protein synthesis. Physical activity involving several muscles of the legs stimulates protein synthesis. It also elevates protein breakdown so that the net protein balance (protein synthesis- protein breakdown) does not become positive, thus a continuous nutritional intake of amino acids is elicited (Wolfe, 2002).

Poor muscle mass has negative physiologic outcomes on insulin sensitivity. Due to reduced muscle mass the insulin is unable to stimulate it to clear the glucose from the blood; as a result the insulin secretion is augmented in the initial phase of insulin resistance to enable muscle to clear glucose from plasma adequately to maintain normal glucose concentrations. The increased insulin secretion is unable to effectively counterbalance its inability to stimulate the glucose uptake resulting in the occurrence of glucose intolerance and progresses towards the development of diabetes and insulin resistance (Wolfe, 2006).

Consequently, increased body fat also confers a disadvantage on insulin sensitivity. With an increase in percentage body fat, plasma free fatty acid increases simultaneously resulting in increased deposition of triglycerides in the muscles which is also associated with insulin resistance. There is increased delivery of free fatty acids to muscle in obesity. However, triglyceride deposition is not elevated in obese subjects who are not insulin resistant. Therefore, impaired disposal via oxidation is the principal basis for accumulation of triglyceride in muscle compared to increased delivery of free fatty acid to muscle. The exact mechanisms by which disturbances in intramuscular accumulation of fatty acid in muscle are linked to impaired insulin signaling mechanism are currently under investigation. Complex alterations in metabolic functions of muscle play a pivotal role in development of insulin resistance (Wolfe, 2006). Thus, adequate intake of protein to maintain muscle mass and related metabolic functions is essential.

1.1.8 Iron status of non-meat-eating and meat-eating population

Iron is another important micronutrient which is compromised due to vegetarianism and irregular meat eating habits in Indians (Gomber et al., 1998). Iron occurs in a plant origin diet in the non-heme form, which is less bioavailable than the heme form in animal products. Decreased brain iron stores may impair the activity of iron dependent enzymes necessary for the synthesis, function and degradation of neurotransmitters such as dopamine, serotonin and noradrenaline (Bruner et al., 1996).

The effect on an individual's diet on iron homeostasis is highly variable and depends on the concomitant availability of other nutrients. A vegetarian diet is therefore common grounds for etiologies of Vitamin B_{12} and iron deficiencies (Obeid et al., 2002). In many developing countries iron deficiency anemia is highly prevalent. This may be attributed to high iron requirements during gestation. Studies have shown higher rates of low birth weight infants born to women with hemoglobin values<90 mg/dl compared to women with higher hemoglobin values (Preziosi et al., 1997).

Iron from a plant based diet has considerably less availability for absorption due to the differences in the chemical form of iron and accompanying constituents that enhance or inhibit iron absorption. Vegetarian diet or plant based diet consists of non-haem iron which is absorbed less efficiently compared to haem-iron from meat sources (Hunt, 2002). In vegetarian or plant based diet, ascorbic acid is an enhancer of iron absorption. Predominant inhibitor of iron absorption in vegetarian diet is phytic acid- present in abundance in legumes, whole grains, lentils and nuts. Other inhibitors present in vegetarian diet are polyphenols, such as tannic and chlorogenic acid in tea, red wine, coffee and variety of other cereals, vegetables and spices (Hunt, 2002).

Forty percent of the iron in meat, poultry and fish is in the form of haem (Monsen et al., 1978). In non-vegetarian diet, haem iron is reduced when poultry or fish are substituted for red meat, as they contain less total iron as compared to red meat, although the iron content present in haem form is similar (Hunt, 2002). Roughead and Hunt (2000) have explained that, iron absorption also depends on the iron stores of the body. They demonstrated in an omnivorous beef based meal, the absorption of haem and non-haem iron was high for individuals with low serum ferritin levels- exhibiting poor iron stores. But as the serum ferritin levels improved, the absorption of non-haem iron decreased by 10-15 fold. Although the absorption of haem iron did not change much, there was a decrease of 2-3 folds only. Hence, non-haem iron absorption can be limited in individuals with high iron stores. The discernible advantage of non-haem iron was that, it is as well absorbed as haem-iron in the case of low iron stores.

Hunt and Roughead (1999), in a controlled study for eight weeks, compared lactoovovegetarian and non-vegetarian diet to measure the non-haem iron absorption from a lactoovovegetarian diet and check for the presence of anaemia. It was observed that non-haem iron absorption from lactoovovegetarian diet was 70% less than from non-vegetarian diet. The amount of non-haem iron absorbed from lactoovo and nonvegetarian diet was 0.14mg/d and 0.48mg/d respectively. The total iron absorption from lactoovo and nonvegetarian diet was only 0.14 and 0.89mg/d respectively, because haem iron absorption contributed to the total iron in nonvegetarian diet only. There was a significant difference in the total iron absorption between the two groups. Surprisingly no signs of anaemia were observed from serum ferritin, iron, hemoglobin, erythrocyte, protoporphyrin and transferrin saturation. Serum ferritin was logarithmically and inversely associated with non-heam iron absorption. $R^2 = 0.60$, $p<0.01$ for nonvegetarian diets and $R^2 = 0.59$, $p <0.01$ for lactoovovegetarian diets.

The above study further determined the fecal ferritin concentrations and observed that it was significantly less in lactoovovegetarian diet. It was logarithmically correlated with serum ferritin. $R^2 = 0.43$, $p<0.01$ for nonvegetarian diet and $R^2 = 0.32$, $p<0.01$ for lactoovovegetarians. The only plausible explanation for this correlation provided by the researchers was the function of mucosal ferritin in blocking the absorption of excess iron by preventing serosal transfer, thereby retaining the iron in the mucosal cell until cell death. Muscosal ferritin is directly related to serum ferritin and inversely associated

to haem and non-haem iron absorption. The decrease in fecal ferritin was attributed to partial physiological adaptation to increase efficiency of iron absorption.

Hunt and Roughead (1999), explained that, vegetarians do not adapt to inhibitors of iron absorption such as phytate in wheat-bran but instead there is an overall increase in efficiency of iron absorption in response to lower iron stores, as apparent from the serum ferritin levels which were similar to non vegetarians. The vegetarian diet did contain enhancers of non-haem iron absorption like; vitamin A and ascorbic acid, but due to the lack of meat and increased consumption of phytates there was low iron absorption. Thus, the vegetarians had lower iron stores but were not iron deficient.

The above information regarding nutritional status and dietary patterns gives an insight to the possible etiology of accumulated metabolic risks observed in Indian population. Such a dietary pattern when followed by pregnant women can have deleterious effects on the developing foetus since it is lacking or has poor quantities of essential nutrients like vitamin B_{12}, protein and iron. Thus maternal nutrition plays a pivotal role in development of foetus.

1.1.9 Maternal nutrition and epigenetics

Maternal or *in-utero* environment plays a significant role in determining the phenotype of the resulting foetus (Gluckman et al., 2004). Models have been developed to explain the relation between pre-natal environment and the phenotype of resulting foetus. The 'thrifty genes' concept put forth by Neel (1962), explains that populations have been genetically selected for alleles favouring insulin resistance. This results in decreased glucose uptake and limits body growth. Such an adaptation is useful in case of poor food availability associated with increased energy expenditure which helped our ancestors to survive in case of scarcity of food e.g. hunter-gatherers. However, the descendants of such population are at a risk due to increased availability of food and increased longevity of life. Such a selection of genotype can result in low birth weight if insulin is selected as 'thrifty gene'. Although no specific gene markers have yet been described (Yajnik, 2000), and purely genetic models are unable to explain the effects of poor maternal nutrition on the developing foetus as observed in the famous Dutch Famine study (Gluckman et al., 2004).

The second model proposed by Hales and Barker (1992) explains that, the foetus become growth retarded in response to adverse uterine conditions. As a result, the phenotype suited to a deprived post food/energy environment is developed. Also poor fetal nutrition triggers an adaptive response in the foetus which optimizes the growth of key body organs but compromises other organs leading to altered post-natal metabolism. These adaptations may become detrimental when the energy intake in the post natal period is more abundant than it was in the prenatal environment. The phenotype model is unable to explain the effects of disease risk seen in normal birth weight. Thus, a combination of genetic and environmental factors can explain the etiology of disease risk observed in some populations (Gluckman et al., 2004).

Maternal constraints influence the development of disease risk in foetus (Gluckman et al., 2004). Experimental evidence of pre-natal and perinatal environment on adult physiology is available in mammalian species (Wolff et al., 1998). In a dietary experiment in pregnant mice Wolff et al., (1998), have shown the effects of methylated diets on epigenetic inheritance. The mice selected for the study exhibited epigenetic mosaics ranging from yellow phenotype with maximum ectopic agouti overexpression, through a continuum of mottled agouti/yellow phenotype with partial agouti expression, to a pseudoagouti phenotype with minimal agouti overexpression. Viable yellow (A^{vy}) "agouti" mice are large, obese, hyperinsulinemic, more susceptible to cancer, and are short lived compared to their non-yellow counterparts. Pseudoagouti mice are lean, normoinsulinemic, healthy and long lived compared to their yellow siblings.

The methylated diets given to the mice consisted of choline, betaine, vitamin B_{12} and folic acid. Three types of this diet were prepared. Methyl supplemented diet (containing all the above nutrients), HS diet (containing half of the supplement level in MS diet) and 3SZM diet (contains thrice as much methyl supplements in MS diet, additionally it also contained zinc and methionine). It was reported that pregnant mice fed on 3SZM diet gave birth to a new phenotype offspring 'pseudoagouti' they had only few thin yellow lines or tiny spots. (ibid) explain that, the expression of variable yellow allele (A^{vy}) can be down regulated by methylation of DNA and thus can be epigenetically regulated. Expression of A^{vy} allele in the off spring is modulated by maternal epigenetic inheritance and maternal diet. The epigenetic inheritance confers potential for multigenerational inheritance of epigenetically determined characteristics. Also,

paternal phenotype in not as influential as the maternal genotype and phenotype in exerting an effect on the phenotype of the off spring (Wolff et al., 1998).

Methyl supplements influence epigenetic phenotype by inducing changes in methyl metabolism that extend to the embryo. Mammalian development is dependent on DNA methyltransferase, which requires S-adenosylmethionine- chief methyl donor (SAM). Increased ingestion of methyl supplemented diets results in increased level of DNA methylation in early embryos (Wolff et al., 1998).

Additional to methyl supplements, protein is also shown to have profound effects on the foetal growth. Low protein diets given to dams throughout gestation have shown to decrease islet cell proliferation, islet size and pancreatic insulin content at birth (Snoeck et al., 1990). Merezak et al., (2001), have investigated the effects of maternal dietary proteins on off springs in relation to sensitivity of fetal β-cells against nitric oxide and interleukin - 1β (IL-1β). The experimental dams were fed a low protein diet (8%) as compared to a control diet providing 20% protein It was reported that apoptosis of islets was greater in off springs of dams fed a low protein diet compared to the control group. Further, exposure to IL-1β and nitric oxide (NO) augmented β-cell death. However, exposure to a medium containing taurine showed protective effects against increased β cell death caused by IL-1β and NO.

Thus, Merezak et al., (2001) demonstrated that, protein restriction during gestation increases the susceptibility of fetal β- cells to the damage caused by IL-1β and NO. Given the incidence of increased subclinical inflammation in Indians, characterized by elevated cytokines like C-reactive protein- high sensitivity (CRP-hs) (Misra, 2004), low protein diet could further exacerbate the pathogenesis of metabolic risk factors in this population.

In Pune Maternal Nutrition Study in India, Rao et al., (2001), collected dietary information from 633 pregnant women before and after pregnancy and correlated it to neonatal anthropometry. It was reported that, dietary intakes of protein and energy were low in rural Indian women. Most of the protein in the diet was derived from cereals and pulses. Only 38% of them consumed animal protein which contributed to 15% of daily intake. When the dietary intake was associated with neonatal anthropometry, it was observed that, higher fat intake by the mother at 18 weeks of gestation was associated

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with greater length (p<0.01) and triceps skinfold thickness (p<0.001). At 18 weeks of gestation, milk consumption was related to birth measurements like, weight, length, mid upper arm circumference (MUAC), head circumference and placental weight. These relationships were stronger at 18 weeks compared to 28 weeks. Milk consumption was not related to neonatal fat or abdominal circumference. Later at 28 weeks of gestation, the consumption of green leafy vegetables was associated birth measurements like birth weight, length, head circumference, MUAC, abdominal circumference, triceps and subscapular skinfold thickness and placental weight. Energy and protein intake were not related to birth size.

Milk is the best source of vitamin B_{12} and protein for lactovegetarians. In lactoovovegetarians it is preceded by eggs. It could be possible that B_{12} had a contribution in maintaining the length, MUAC (marker of increasing muscle mass) and weight. Although energy and protein were not related to birth size, the association of milk consumption with MUAC is suggestive of a role for protein. Since green leafy vegetables and not milk consumption was associated with subscapular and triceps skinfold thickness. Green leafy vegetables are good sources of iron and folate and beans are good sources of folate. Consumption of green leafy vegetables and beans is higher than milk and milk products in Indian women.

Thus, the phenomenon of thin-fat Indian baby with reduced MUAC and visceral size but well preserved subscapular fat determined by Yajnik et al., (2003) could be due to the increased intake of green leafy vegetables and comparatively less quantities of milk and milk products by the mothers, leading to foetal undernutrition. Additionally, foetal under nutrition favours the growth of key body organs at the cost of other organs leading to altered post-natal metabolism (Hales et al., 1992).

The genetic predisposition of Indians to insulin resistance can be explained using the hypothesis suggested by Hales and Barker (1992), which states that, nutrient supply to the foetus is determined by the mother's own foetal and childhood growth, her nutrition before and during pregnancy and transfer across placenta (A. C. Ravelli et al., 1998). Thus, nutrition, particularly of a girl from conception to child bearing is important for the health of future generations as the ova for her children are formed in the first three months of her foetal life. The important stages of human life which require

consideration with respect to nutritional and lifestyle factors can be explained using the 'Life course model' suggested by World Health Organisation, (2004b) in Figure 1.4.

Figure 1.4: Life course approach to health

Foetal life

- *SES
- Mother's nutrition
- Growth
- Birth weight

Infancy & childhood

Breast feeding

*SES

Infection

Growth rate

Physical activity

Food behaviour

TV viewing

Adolescence

Obesity

Sedentarism

Inactivity

Smoking

Physical activity

Food behaviour

TV Viewing

Adult life

Established adult risk

Diet/Physical activity

Alcohol

Biological risks

Socio economic status

Accumulated risk

Genetic susceptibility to malnutrition

Elderly

Behaviours

Tobacco

Age

Source World Health Organisation (2004)

*SES socio economic status

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1.1.10 The life course approach to good health

The various stages of life can be broadly divided into foetal life; infancy and childhood; adolescence; adult life and old age (Figure 1.4). This model is applicable to the present thesis and the study of the accumulated chronic disease risk as the factors that operate within each particular stage of life influence the health in relation to nutritional status of the individual as he/she passes that phase of life (Uauy et al., 2006). Many of the factors relate to nutritional status. The factors at each stage in relation to manifestation and accumulation of risk to the next stage deserve more attention for the development of prevention strategies. These associations show that, results of exposure to a particular factor at a particular stage not only manifest in that phase of life but are also carried forward to later stages of life. The manifestation of the factors is also affected by their interactions with the genetic constitution of the individual (Uauy et al., 2006).

Hence, the life course model serves as a template for recognising the lifestyle factors involved in molding individuals. Further, Dietz (1994), explained that the three distinct periods of growth and development during which risks for obesity markedly increases are; early infancy, prepubertal growth (associated with adiposity rebound) and the adolescent growth phase. Obesity occurring at these periods increases the risk of persistent obesity and its complications.

It is therefore important to monitor female nutrition and health since birth as the development of her foetus depends not only on the nutritional intake during pregnancy, but depends on her health and nutrition throughout her life (A. C. Ravelli et al., 1998). The preadolescent ages are of particular interest because, the eating and activity pattern of children may be changing as children become established in school and other routines of middle childhood (Ball et al., 2001). Although parents play a significant role in moulding their children's dietary and activity patterns. The dietary patterns also depend on the demographic and socioeconomic status of the individual and the country they belong to.

The aims and hypotheses of the study were established based upon the evidence and knowledge regarding the dietary patterns, nutritional status and accumulated risk factors in the Indian population.

1.2 Aims

The present thesis proposed to study Indian preadolescent girls aged 9-10 years. The aims of the thesis were to

1. Examine the dietary differences between meat and non-meat-eating girls particularly in relation to vitamin B_{12} status, protein and fibre.
2. Examine differences between biomarkers of nutrient absorption and inflammation in meat and non-meat-eating girls. This includes B_{12}, folate, iron absorption markers including blood count, C-reactive protein and blood lipids.
3. Examine the differences in body composition between the two groups.
4. Evaluate the time spent in static and moving physical activity by these groups.

1.3 Hypotheses

1. Non-meat-eaters have more body fat and are centrally obese compared to meat-eaters.
2. Non-meat-eaters have a higher respiratory exchange ratio than meat-eaters.
3. Non-meat-eaters have lower serum vitamin B_{12} and higher folate concentrations than meat eaters.
4. Non-meat-eaters have higher fasting glucose and less favourable lipids than meat-eaters.
5. Non-meat eaters consume a higher carbohydrate and fibre-enriched but a lower protein diet compared to meat-eaters.
6. Non-meat-eaters are physically less active than meat eaters.

1.4 Significance

Findings from the present thesis carried out in the Indian preadolescent girls will have future implications for

1. Metabolic and cardiovascular disease risk associated with non-meat-eating diets.
2. The importance of physical activity, not only for this age group but for the larger Indian population
3. The effect of non-meat-eating diet and physical activity on body composition.
4. The importance of maternal nutrition in this population.

In order to meet the aims and the hypotheses of the present study, specific measurements were used to measure the body composition, resting energy expenditure, biomarkers (for one-carbon metabolism and risks for diabetes and cardiovascular diseases), nutrient intake (quantitative and qualitative) and physical activity levels. The rationale for using these measurements will be discussed in the rest of the chapter.

1.5 Measurements used for assessing body composition and obesity

1.5.1 Body mass index (BMI)

Obesity and overweight are commonly defined by measurements of BMI. It has been used by most of the studies as an index of body fatness to report increase in adiposity in children and has been recommended as the best measurement for monitoring overweight in individuals in pediatric populations (Prentice, 1998). Body weight not only correlates with body fat, but also with height. Therefore, BMI (weight adjusted for height squared) is a useful index to assess overweight and is a consistent surrogate for obesity. It correlates with measures of body fatness in children and adolescents (Bhave et al., 2004) and can be used as a reliable tool to assess fatness in children and adolescents (Dietz et al., 1999). BMI cut off points are used clinically to identify high risk individuals for screening and identify individuals for absolute risk assessment (World Health Organisation, 2004a). Thus, BMI was used as a useful tool for the initial screening of overweight, obesity and associated risk factors.

<u>**Limitations of BMI**</u>

Although BMI is the most commonly used surrogate for measuring overweight and obesity, there are certain limitations to its application. BMI does not differentiate between obese and muscular individual since it is defined as normalized weight for height (Rush et al., 1997), i.e. it does not distinguish between fat mass and fat free mass. Wells (2000) reported in 64 prepubertal children aged 8-12y that BMI embraced a wide range of body fat percentage with wide ranges for the confidence limits. It also does not account for variation in body composition among different ethnicities Tyrrell et al., (2001). This is particularly applicable to Asians, who have a high BF% compared to a Caucasian with the same BMI (Deurenberg et al., 2002). They also reported that, the Asian population in their study (Singaporean Chinese, Malay, Indians and Hong Kong Chinese) for similar BMI exhibited 3-5% higher BF% and for the same BF% their BMI was 3-4 kg/m^2 lower than Caucasians. There are differences in body composition amongst the Asians as well. For the same age, gender and BMI, Indians have the

highest BF% compared to Malay and Chinese (ibid). Therefore, in the present thesis, BMI was used for the initial screening of overweight and obesity.

1.5.2 Bioelectrical impedance analysis (BIA).

Due to the limitation of BMI is measuring body composition, a more accurate method is required which can measure body fat percentage (BF %), fat mass (FM) and fat free mass (FFM). Bioelectrical impedance was the choice of measurement since it is used extensively in pediatric populations (Rush et al., 2003; Tyrrell et al., 2001). Also it is inexpensive and less time consuming compared to other techniques like Magnetic resonance Imaging (MRI) and computed Tomography (CT) scan. The use of CT scan also confers a danger of children being exposed to the radiations (Goran & Gower, 1999).

BIA is based on the theoretical relationship between the volume of a conductor and its impedance. In biological systems, electrical conduction is related to water and ionic distribution in the conductor. Fat free mass (FFM) comprises of a protein matrix of adipose tissue, contains virtually all the water and conducting electrolytes in the body, hence conductivity is greater in fat free mass compared to fat mass (FM) (Lukaski et al., 1985). The impedance value is combined with anthropometric data like weight and height in prediction equations validated against a standard (explained further in the methods chapter) to give body compartment measures. It has been shown to be accurate at predicting FFM and total body water in children (Rush et al., 2003).

In New Zealand, Tyrell et al., (2001) have shown strong correlation between FM, FFM and BF% measured using DEXA and BIA in children aged 5-10 years of age from three different ethnicities (Maori, Pacific Island, and NZ European). Mean differences between DEXA and BIA derived FM, FFM and BF% were not very large with correlation for FM, FFM and BF% of 0.98, 0.98 and 0.94 respectively. However, the two methods could not be interchanged because BIA had poor limits of agreement for percentage body fat, where it could vary from percentage body fat derived from DEXA by -4.29 to 9.36%. However, they argue that the limits of agreement for FM and FFM were not very wide and therefore BIA was a reliable tool for measuring FM and FFM. Bandini, Vu, Must, and Dietz (1997), showed that BF% percentage fat determined by BIA highly correlated with BF% determined by deuterium dilution or total body water

(r = 0.84), followed by tricep skinfold (r = 0.83), followed by BMI (r = 0.62) in 132 non obese premenarcheal girls in the age group 8-12 years. In the present thesis, BF% of 30% is used as a reference value to determine increased body fat in preadolescent females as suggested by Williams et al., (1992), and recommended by the Indian Academy of Pediatrics (Bhave et al., 2004).

1.5.3 Waist circumference

As observed above, BIA provides an estimate of total body fat, but not body-fat distribution. An assessment of body fat distribution, particularly central obesity, can possibly identify subjects with risk of adverse lipid profile and hypertension (Yudkin et al., 2000). Intra abdominal fat is clinically the most relevant type of fat in humans. Preadolescent growth is associated with an expansion of intra-abdominal adipose tissue depot (Goran et al., 1995). Adverse effects of increased intra abdominal adipose tissue include high blood pressure, hyperinsulinemia, type 2 diabetes and dyslipidemia (Maffeis et al., 2001).

Intra abdominal adipose tissue can be measured accurately using techniques like DEXA, MRI and CT scan. It is not feasible to use CT scanning in children due to the radiation exposure. Whereas, MRI and DEXA could be costly and the technical protocol demands cooperation from children, which could be difficult. Therefore to estimate the body-fat distribution in children body circumferences are the preferred measurements which also provide a sufficient degree of accuracy (Maffeis et al., 2001).

In 818 Italian children aged 3 to 11 years Maffeis et al., (2001) showed that waist circumference correlated positively with systolic blood pressure (r = 0.40, p<0.001) and diastolic blood pressure (r = 0.29, p<0.001). In a multivariate linear model analysis waist circumference was significantly associated with HDL cholesterol, Total cholesterol:HDL cholesterol ratio, systolic and diastolic blood pressure (p<0.01). Approximately, 19% of children with waist circumference more than 90th percentile had two or more cardiovascular risk factors compared with 9% of children with waist circumference less than 90th percentile.

Also, children with a waist circumference more than 90th percentile had high risk of having lower HDL cholesterol (odds ratio 0.97, 95% confidence interval 0.96-0.99, p<0.01) and higher blood pressure (odds ratio 2.3, 95% confidence interval 1.41-3.72, p<0.001). Waist circumference strongly correlated with subscapular (r = 0.66, p<0.001) and triceps (r = 0.71, p <0.001) skinfold thickness, which warranted the use of waist circumference in determining body-fat distribution.

Goran and Malina (1999), showed that in 101 African American and white prepubertal children the strongest correlate of intra abdominal adipose tissue (IAAT) was skinfold thickness (r = 0.85) followed by waist circumference (r = 0.84). They also observed that waist circumference correlated most strongly with subcutaneous abdominal adipose tissue (r = 0.93) followed by triceps (r = 0.93) and abdominal skinfold thickness (r = 0.91). Further in Asians, central adiposity is an important predictor of risk factors of obesity compared to generalized obesity (Shelgikar et al., 1991).

As previously observed in section 1.1.1, the relatively easy measure of central adiposity, WHR, has proved to be a more important determinant of hyperglycemia compared to BMI (Shelgikar et al., 1991) and therefore measuring waist circumference in this population can be very helpful in identifying risk factors of obesity. In the present thesis a cut off value of < 71cm for waist circumference is used. It is recommended by the Indian Academy of pediatrics (Bhave et al., 2004). Also Higgins et al., (2001) showed the highest likelihood ratio for cardiovascular disease risk in prepubertal children with waist circumference between 71-72.9cm.

1.5.4 Skinfold measurement

The use of skinfold measurements depends on the assumption that, the subcutaneous fat constitutes a constant or at least predictable proportion of body fat (Womersley, 1977), and the sites selected for measurement represent the average thickness of subcutaneous adipose tissue (Lukaski, 1987). Skinfold thickness can be used to predict percentage body fat in children and adolescents (Deurenberg et al., 1990).

Goran et al., (1995), examined the correlation between intra abdominal adipose tissue (IAAT, determined using CT scan) and body fat distribution estimated from individual eight skinfold thicknesses, ratio of three trunk skinfold (chest, axilla and abdomen) to three extremity skinfold (triceps, thigh and calf) thickness in 16 children aged $6.4 \pm 1.2y$ (12 Mohawk Indians, 4 Caucasians, 12 girls and 4 boys). IAAT significantly correlated with all skinfold measurements ($r = 0.60$ to 0.78, $p = 0.02$ to 0.003) except at the calf. IAAT highly correlated with trunk to extremity skinfold ratio ($r = 0.78$, $p = 0.0003$) and suprailiac ($r = 0.78$, $p = 0.0003$) skinfold thickness (skinfold measured at the waist). The correlation between trunk: extremity skinfold ratio and IAAT, warrants the use of the use of trunk: extremity skinfold ratio as a reliable measure of central obesity. Increased IAAT or central obesity in children is associated with greater risk of hyperlipidemia, cardiovascular diseases, hyperinsulinemia and hypertension.

In post pubertal Indian females, Misra et al., (2004), have shown that subscapular skinfold thickness and triceps skinfold thickness correlated significantly with fasting insulin, $p<0.05$ and $p<0.01$ respectively. Maffeis et al., (2001), (section 1.5.3) have also shown significant correlation between skinfold thickness and other reliable anthropometric measurements like waist circumference, used for measuring body-fat distribution. Thus, using skinfold measurement not only helps in understanding body fat distribution but also helps in assessing the exposure to metabolic disease risk factors.

1.5.5 Arm circumference

Mid upper arm circumference (MUAC) is a standard technique used for assessment of body fatness to provide an estimate of nutritional status of an individual or population (Lukaski, 1987). Due to the impracticality of using laboratory methods in field studies upper arm circumference and triceps are used to assess nutritional status (Lukaski, 1987).The triceps skinfold thickness which is measured at the level of MUAC allows an index to derive subcutaneous adipose tissue in the arm. MUAC and triceps skinfold thickness together and independently are indicators of protein energy malnutrition (Jelliffe et al., 1969).

Rolland-Cachera et al., (1997) used arm circumference along with triceps skinfold thickness to calculate total upper arm area (TAA), upper arm muscle area (AMA) and upper arm fat area (AFA). They proposed that, the unrolled fat rim around the arm is a rectangle with length 'C' (arm circumference) and width of half the triceps skinfold (TS) thickness (TS/2). They also used approximations that, mid arm muscle compartment is circular and bone included in anthropometric arm muscle area atrophies proportionally with the muscle. This proposition appears to be more sound compared to the assumption of Gurney and Jelliffe (1973) and E. F. Jelliffe and Jelliffe (1969) which assumed that upper arm muscle area (AMA) is constituted of a circular limb, a muscle compartment and a symmetrically distributed fat rim. Based on their assumption (Rolland-Cachera et al., 1997) proposed the following formulas which are used in the present study

Total Arm Area (TAA) = C2/4π.

Upper arm Fat Area (AFA) = C$\times$ (TS/2).

Upper arm Muscle Area (AMA) = TAA-AFA. The fat area calculated using these formulae had closer association with fat area calculated using MRI scan (Rolland-Cachera et al., 1997).

Basit et al., (2005) determined that arm fat percentage had a significant effect on LDL cholesterol ($p = 0.037$) and insulin ($p = 0.044$) in ninety-two, 8-10 years preadolescent Pakistani children. (ibid) calculated AFA using the above formulas and then calculated arm fat percentage as (AFA/TAA) $\times$100

Currently there are no cut-off values available for arm fat percentage in children which could help in identifying children at risk of obesity. However, its correlation with metabolic factors like insulin and LDL cholesterol warrants its use in identifying risks factors of obesity and nutritional status of children. Hence, these measurements and calculations are used in the present thesis.

1.6 Choice of measures in assessing metabolism.

1.6.1 Resting energy expenditure (REE) and substrate metabolism

Total energy expenditure is the sum of resting energy expenditure, thermic effect of food, and the energy expenditure related to activity of which resting energy expenditure is the largest component. The resting metabolic requirements of splanchnic tissues, brain, and skin vary slightly under normal conditions due to relatively constant tissue mass and protein turnover rates. On the contrary, the energy expenditure related to muscle metabolism varies considerably due to synthesis and break down of muscle protein (Wolfe, 2006).

As fat mass increases due to obesity, muscle mass increases simultaneously and therefore resting energy of individuals with the same body weight but different levels of muscle and physical activity can vary widely. Stimulation of muscle protein turnover and metabolic restoration in the presence of increased muscle mass can have a significant effect on resting energy expenditure and thus, energy balance.
The increased amino acid from the diet can help in muscle mass and increased muscle protein turnover. The energy required in the form of adenosine triphosphate (ATP) for the above process is derived by oxidation of fat since it is preferred energy substrate of resting muscle. The above process facilitates a loss in fat mass and can be capitalized on to promote fat loss (Wolfe, 2006).

There is a strong positive relationship between resting metabolic rate and body weight. Fat free mass is the best predictor of energy expenditure. REE increases more slowly as body weight increases, REE per kg body weight falls as body weight increases and fat free mass decreases (Weinsier et al., 1992). They also observed a lower REE in obese preadolescents compared to non-obese aged 10 years, after adjusting for body weight, suggesting prepubertal age as a critical period for exacerbation of overweight characterized by reduced REE and increased body weight. In the older age groups (11 and 12 years), obese children had a higher REE. Hence, the prepubertal age is a critical period for determining the REE.

Respiratory exchange ratio (RER) measured along with REE is a measure of the fuel mix in the diet. Rueda-Maza et al., (1996) investigated the effect of a mixed diet comprising of carbohydrate enriched with ^{13}C isotope on resting energy expenditure in ten obese and eight normal weight prepubertal children. Following the meal continuous measurements were made for 570 minutes of total oxygen uptake and carbon dioxide output. Total (exogeneous+endogeneous) carbohydrate oxidation was calculated using indirect calorimetry. Exogenous carbohydrate oxidation was estimated from carbon dioxide production (V_{CO2}), the isotopic enrichment of breath $^{13}Co_2$ and the abundance of $[^{13}C]$ carbohydrate in the meal ingested. The endogenous carbohydrate oxidation is primarily glycogen breakdown in muscle and liver.

A positive correlation was observed between the percentage body fat of children and the ratio between exogenous carbohydrate oxidation and total carbohydrate oxidation for both obese and control group. The exogenous oxidation of carbohydrate was significantly greater ($p<0.03$) and endogenous oxidation significantly less ($p<0.05$) in the obese children. The total oxidation of carbohydrate was greater in the normal weight group. This suggested that normal weight children utilized the glycogen stores for resting energy expenditure but the obese children did not. Glycogen is an energy substrate in postprandial phase. Decreased utilization of glycogen is indicative of increased availability of carbohydrate in the diet.

Isotopic enrichment is not feasible in all experimental set up. In such cases, the respiratory exchange ratio measured with resting energy expenditure using indirect calorimetry provides a measure of the fuel (carbohydrate, protein or fat) metabolized. The numerical values for carbohydrate, protein and fat metabolism are 1, 0.8 and 0.7 respectively (Weir, 1949).

In the present thesis, REE was measured to calculate energy requirements for the normal body functions and the respiratory exchange ratio (RER) determined the macronutrient mix in the diet. The respiratory exchange ratio at rest is dependent on glycogen and fat stores (Weir, 1949). Therefore, RER is a combined measure of recent diet history and body composition.

REE improves the ability of diet diaries in detecting invalid reports and avoids misclassification of individuals (under-reporters and over-reporters) which could distort analysis (Black, 2000).

1.6.2 Biochemical markers of obesity

A very large number of biomarkers can be measured in the blood but the selection of those to be measured is determined both by relevance to the main hypotheses, amount of sample needed and cost. The main hypotheses were related to one-carbon metabolism, and risk factors for diabetes and cardiovascular disease.

1.6.2.1 One carbon metabolism biomarkers (vitamin B_{12} and folate)

One –carbon metabolism as described above is a network of interrelated biochemical reactions that involve transfer of one-carbon from one compound to another involved in the methylation processes. Some of the important nutrients involved in the mechanism are vitamin B_{12} and folate were measured in the serum samples. As explained in section 1.1.6, deficiency of vitamin B_{12} and folate can lead to abnormalities in DNA, RNA synthesis, DNA methylation, gene expression and red blood cell formation. Due to the role of vitamin B_{12} in metabolic mechanisms for adiposity and insulin resistance (Figure 1.1), measuring these biomarkers in a young population could provide an indication of the preventive measures to be adopted to reduce the risk of metabolic diseases in the Indian population.

1.6.2.2 Risk for diabetes and cardiovascular disease (Glucose, insulin and lipids)

Glucose, insulin and lipids are classical markers of impaired glucose tolerance, diabetes and cardiovascular risks. Fasting insulin is elevated prior to increase in glucose in insulin resistant subjects. Increased triglycerides and decreased HDL cholesterol are important risk factors of insulin resistance syndrome. Having known the prevalence of insulin resistance in this population, it was important to measure these parameters in the present thesis.

1.6.2.3 Inflammatory markers associated with obesity (C-reactive protein and Ferritin)

C - reactive protein high sensitivity (CRP-hs) is an acute phase reactant i.e. produced in response to cell damage or imbalance resulting in the inflammatory response. Synthesis of CRP-hs occurs in the hepatocytes of the liver and is regulated by pro-inflammatory cytokines. Proinflammatory cytokines are closely related to insulin-glucose metabolism and insulin resistance. CRP- hs levels increase non-specifically in a wide variety of infections, immunoinflammatory diseases, trauma and malignancies. It has a direct role in the pathogenesis of atherosclerosis and vascular inflammations (Misra, 2004). As discussed earlier Indians are at a greater risk of developing insulin resistance, type 2 diabetes and coronary heart diseases compared to their Caucasian counterparts with higher prevalence and earlier appearance of risks factors like insulin resistance, abdominal obesity, artherogenic dyslipidemia, increased pro-coagulant factors and endothelial dysfunction (Forouhi et al., 2001).

Misra (2004), reported a high (25%) prevalence of subclinical inflammation in Asian children and adults. Subclinical inflammation is characterized by persistent elevation of CRP-hs concentration not associated with increased infections and appears asymptomatic. Asian Indians living in India are exposed to infection and parasite infestations largely driven by the fact that 40% live below the poverty line which may lead to persistently increased CRP-hs levels. Furthermore, elevation of pro-inflammatory cytokines due to recurrent and persistent infection may induce or exacerbate insulin resistance (Misra, 2004). In the same paper, (Misra, 2004) also recognized that protein deficiency in India and developing countries may modify CRP levels, since the inflammatory response in such people is subdued. Overweight and obesity which can also occur with protein deficiency in childhood are conversely associated with elevated CRP levels.

Forouhi et al., (2001), compared 113 healthy men and women of South Asian and European origin (aged 40-55y with BMI 17-34 kg.m^{-2}) to test the association between circulating CRP-hs concentrations and a detailed panel of body fat measures, to establish a correlation between CRP-hs levels and other measures of insulin resistance syndrome (insulin and lipid levels) and to compare the circulating levels of CRP-hs. Percentage body fat was measured using dual energy X-ray absorptiometry (DEXA) and visceral fat was measured by CT scan technique. It was observed that South Asian women had significantly higher percentage fat ($p<0.007$) and visceral fat area ($p<0.027$) compared to European women. All south Asians had significantly higher levels of fasting insulin ($p<0.075$) and 2h insulin ($p<0.001$). Median CRP-hs levels in south Asians [1.35mg/dl (0.72-3.04)] was nearly double that in Europeans [0.70mg/dl (0.41-1.7); $p=0.05$). The association of CRP-hs with visceral fat area persisted after correction for BMI and fat percentage (all, $p<0.05$) in south Asians but not in Europeans.

In the above study, in the entire study group visceral fat area was the only independent correlate of CRP-hs ($\beta=0.87$, $p=0.005$) amongst predictors tested i.e. age, sex, ethnic group, smoking status or percentage fat. In age, sex and smoking adjusted regression analyses, there was an independent association between CRP-hs and insulin and lipid levels in both the ethnic groups. Independent associations were observed between CRP-hs and fasting and 2h insulin, fasting and 8h triglycerides (post glucose load) and negative association with fasting HDL cholesterol after adjusting for age, sex, ethnic group and smoking. Further adjustment for total percentage body fat reduced the significance of the association between insulin CRP-hs and lipids in most cases to $0.05<p<0.10$ but abolished completely for HDL cholesterol. When statistically adjusted for visceral fat area the significance of the associations was abolished in all cases ($p>0.15$). Therefore low grade chronic inflammation was associated with central obesity.

Further, Chandalia et al., (2003), observed that 82 Asian Indian men had significantly higher CRP-hs levels compared to 52 Caucasians ($p<0.03$) of similar age, body fat and both the groups were non-diabetic. After adjustment for waist circumference Asian Indian had significantly higher CRP-hs levels ($p<0.0003$). CRP-hs differences between the two ethnic groups paralleled changes in insulin sensitivity. The parallel relationship between insulin sensitivity and hs-CRP-HS concentrations in the two groups could be attributed to obesity which results in high production of cytokines by the excess adipose

tissue. This in turn could induce higher CRP-hs production by liver (Forouhi et al., 2001).

Cook et al., (2000) examined the relationship of CRP to potential determinants and cardiovascular risk factors in 628 Caucasian, 56 south Asian and 15 children of Afro-Caribbean or other ethnic origin aged 10-11y. They reported that CRP was 104% higher in south Asian children. Further, in all the three groups, girls had 47% higher CRP levels compared to boys. After adjustments for age, sex, town and ethnicity, they observed a significant negative correlation between CRP and HDL cholesterol (r = -0.13, p = 0.0006) and positive correlation between CRP and heart rate (r = 0.12, p = 0.002). As heart rate is an indicator of physical fitness, the positive correlation between CRP and heart rate was an indicator of increasing inflammation with decreasing physical fitness.

<u>Ferritin as an inflammatory marker</u>

Biochemical markers like CRP-hs and lipids are markers of cardiovascular disease although they are not related (Mainous et al., 2004). (ibid) also reported in a representative sample from National Health and Nutrition Examination Survey Ш, that patients with increased ferritin and LDL cholesterol were more likely to have elevated CRP (OR 1.68; 95% CI 1.06 to 2.68). Also patients with elevated ferritin and low HDL cholesterol were more likely to have elevated CRP (OR 1.71; 95% CI 1.28 to 2.27). The above findings indicate a significant role of iron and lipids in inflammation.

1.7 Dietary assessment

For individuals in energy balance, habitual energy intake must equal energy expenditure. Assuming that an individual is in energy balance, dietary assessment provides a valid measure of habitual energy intake (Black et al., 1997).

A seven day diet diary is a representative tool of usual energy intake and dietary pattern (Livingstone et al., 1992). The ability to detect an invalid report is improved by using seven day diet diaries as it provides a narrower 95% confidence limit range compared to a 24h recall (Black, 2000). She has shown, wide range of 95% confidence limits (0.87-2.75) for 24h recall compared to narrower range 95% confidence limits for seven day diet diary (1.05-2.28). A seven day diet diary gives additional information about the timing and frequency of eating occasions, foods and food combinations eaten and fluid intake, energy density, and sources of nutrients like protein and vitamin B_{12}.

1.8 Physical activity assessment.

Physical activity assessed using diaries is highly cost-effective and does not involve complicated procedures. They can be used in individuals over a wide age range of age groups 10-50y (Bouchard et al., 1983). Physical activity diary developed by Bratteby et al., (1997) requires the subject to record the physical activity for every 15 minutes period. The activities are categorised into nine levels according to their average energy costs representing multiples of BMR. The dominant activity of every quarter of an hour is recorded. Thus, physical activity diary presents a habitual energy expenditure pattern of an individual (Bouchard et al., 1983). Human energy expenditure varies everyday, especially between weekdays and weekends and therefore one weekend should be included in the diary (Bouchard et al., 1983). Although seven day physical activity diary does not present the same precision in measurements of energy expenditure as REE but using it in conjunction with a seven day diet diary, presents an approximation of habitual energy intake and energy expenditure of an individual. It is a helpful tool next to doubly labeled water in validating the diet diary (Rothenberg et al., 1998).

CHAPTER 2: DESIGN AND METHODS

2.1 Participants

The study population was self-selected from group of migrant Indian preadolescent girls 9-11 years of age, in Tanner stage 1 of development, following a non-meat-eating or a meat eating diet and living in Auckland. One child was born in Dubai, the others were all born in India. The number of years in Auckland ranged between 1 and 6. Ethical approval was gained from the Auckland University of Technology on April 07, 2006 (Appendix 1). Assent and consent forms were obtained from the girls and parents respectively.

2.1.1 Determination of number of subjects

Twelve girls were included (six non-meat-eating and six meat-eating) in this pilot study to determine the feasibility of the measurement techniques and the sample size necessary for a larger study. Vitamin B_{12} levels in this population were not known and therefore the effect size of any difference between non-meat-eating and meat-eating was also unknown. For a pilot study, the practical use of a power calculation is not feasible as there were so many assumptions that had to be made. In this population the feasibility of the recruitment technique and the compliance of the families was also not known. What was known was that meat-eating and non-meat-eating are common practices within Indian families.

An extensive literature search did not provide information about the percentage of the population that does eat meat. What is known is that even when meat is eaten it is in relatively small quantities <6% energy as determined in NFHS-2 in India by (International Institute of population sciences and ORC Macro, 2000). While milk is consumed daily and eggs are eaten 4-5 times a week, intake of B_{12} is likely to be much lower than children of other ethnicities e.g. New Zealand European children. In particular beef has ten times more B_{12} than milk, while milk is the main source of B_{12} for "lactovegetarian"

Examination of the New Zealand National Children Nutrition Survey (NZCNS) data (Ministry of Health, 2003) showed that those girls who did not eat any meat (adjusted for age) weighed less (36 kg vs. 41 kg) than those who did. By selecting these two "extreme" dietary groups, it was hoped that differences will be detected. The initial sample size was limited to twelve subjects due to the high cost involved for the extensive blood biochemical analysis and the cost of doubly labeled water.

2.1.2 Participant recruitment

Participants were recruited through personal contacts with the mothers, by word of mouth and establishing contacts with some Indian families. More references and contacts with families were obtained from colleagues who helped in recruiting subjects. As the sample size was limited to 12 participants recruitment could be accomplished using the above contacts. Once the subjects were recognized, a follow up telephone contact was made with each mother of the prospective participant to explain the study. An appointment was made to explain the participant information sheet (Appendix 2) to the mother and child. A figure depicting Tanner stage 1 was also shown to the mother this helped the mothers in recognizing and confirming the Tanner stage of the participant (Appendix 3).

Beunen (2001) explained that Tanner stages are a biological system used to assess biological maturity status for breast, pubic hair and genital development. There are five described Tanner stages. Tanner stage 1 is the preadolescent stage in which there is no breast development and no pubic hair. The pictorial depiction (Appendix 3) also included pictures of resting energy expenditure being measured in a child and a child drinking the doubly labeled water. Once the participant and the mother agreed, a consent and assent form (Appendix 4) was signed by the mother and the child respectively.

2.1.3 Inclusion and exclusion criteria for the study

It was essential that girls were at preadolescent stage (Tanner stage 1) and they were of Indian origin. Participants were excluded, if they were male, were not between 9 and 11 years, not in Tanner stage 1, had type 1 diabetes, congenital cardiac anomalies, chronic diseases or were ingesting any drugs that could influence body composition or energy metabolism. It was explained that if they could not or did not want to have any of the measurements conducted on them then they were not eligible for this study.

2.2 Design

The study "Indian preadolescent girls: Lifestyle patterns and accumulated risks" was a pilot study designed to look at developmental factors that influence future health in non-meat-eating and meat-eating Indian preadolescents girls and differences between them in relation to body composition, energy consumption and energy expenditure. Anthropometric measurements, bioelectrical Impedance Analysis (BIA) and resting energy expenditure measurements were made. Seven days dietary recall and physical activity information was collected from the diaries completed daily by the girls with assistance from their mothers. Additionally, total energy expenditure was measured using the doubly labeled water technique (water labeled with the stable isotopes deuterium, ^{2}H and oxygen 18, ^{18}O) to measure total energy expenditure. Data from the doubly labeled water technique procedure will not be included in this thesis as it forms part of a separate study on the same girls performed concurrently with the thesis and the analysis of the samples is not yet available. The data were collected over a period of two weeks with a minimum of four field visits (Figure 2.1). Visits 1 and 2 were at the start of the study. Visit 3 and visit 4 were each a week apart following visit 2. The girls visited Diagnostic Medlab Ltd, Auckland with their parent in the second week of the study.

Figure 2.1: Data collection protocol
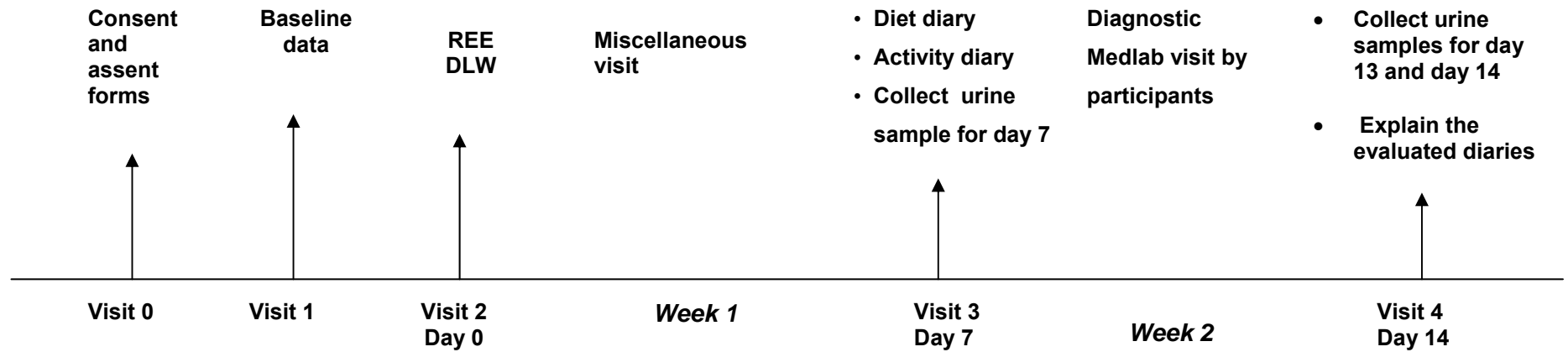
Baseline measurements included anthropometry, bio electrical impedance analysis and providing training on completion of seven days diet and physical activity diary; REE Resting energy expenditure, DLW dosing with doubly labelled water.
Miscellaneous visits involved collecting 5 hr post dose, day 1 and day 2 urine samples and explaining the evaluated diaries and blood reports.

Visit 1

This visit was arranged with parent and child both present and informed consent was obtained. During the first visit, anthropometric measurements of height, weight, sitting height, waist circumference, hip circumference, Mid Upper Arm Circumference (MUAC), skinfold thickness (triceps and subscapular), and head circumference were made. Bioelectrical impedance analysis was used for estimating FFM and FM and BF%. Functional measurements included right and left hand grip strength, blood pressure and resting pulse rate. Personal history including length of stay of the child and family in New Zealand, their dietary pattern (meat-eating or non-meat-eating), foods they liked and foods not eaten were recorded. Birth record, history and child health record if available or known was obtained from the mother. Information was obtained from the mothers as to whether the child was taking vitamin supplements. The mother's dietary pattern and diet history during pregnancy was noted and any existing family history of diabetes or cardiovascular diseases was also noted. The food and the activity diaries were explained to the participant and were given at least two to three days to practice recording foods eaten and physical activity diaries prior to the study period. The mothers were instructed about collecting a fasting urine sample from the child (preferably the morning sample) prior to dosing with doubly labeled water. The resting energy expenditure was to be measured in visit 2. Therefore as a requirement of the procedure for resting energy expenditure measurement, the girls were familiarized with the mask that they would breathe into using the best size of mask determined and the procedure explained and a picture shown (Appendix 3).

Visit 2

The day of visit 2 was marked as day 0 of the study. The second visit involved measuring the resting energy expenditure, dosing the child with doubly labeled water, and revising the procedure for diet and physical activity diary completion. Weight was checked before measuring the resting energy expenditure. They were given the "Very Important Pee diaries (VIP)" for recording the timings of urine collection after the dosing. They were instructed to collect 5hr, day 1 and day 2 post dosing urine sample. The mothers were given instruction sheets for blood collection, physical address and operating hours of the closest Diagnostic Medlab in their vicinity, along with pamphlet for children provided by Diagnostic Medlab Ltd, Auckland. Girls filled the diet and the physical activity diaries from day 0 onwards for the following six days.

Visit 3

The third visit was on day 7 of the study. The diet diary was cross checked and evaluated by comparing their household measures with a standard measuring cup, which included standardized measurements of milliliters (ml) and ounce (Oz). It was ensured that all the information written in the diet diary was understood. Any missing or unclear information in the diet and the physical activity diary was obtained by probing the mother and the participant. The urine samples for day 7 were also collected from the participants.

Blood analysis

The girls accompanied by their parents visited Diagnostic Medlab Ltd, for blood collection in the second week of the study. Negotiations were made with Diagnostic Medlab for collecting fasting venous blood samples. Biochemical examination of the serum samples included fasting plasma glucose, Total Cholesterol, High Density Lipoproteins (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol, Total/HDL cholesterol ratio, triglycerides, insulin, folate, vitamin B_{12} and methylmalonic acid (MMA). Hematological examination included Complete blood count, serum iron, ferritin and C-reactive protein high sensitivity. The venous blood samples were drawn by an experienced phlebotomist at Diagnostic Medlab Ltd. Approximately 15 ml of blood sample was drawn from the participants. The samples were collected in EDTA coated sterile tubes. Fresh blood was analysed for fasting glucose, lipids and complete blood count at Diagnostic Medlab. Following the analysis, the remaining blood samples were centrifuged, aliquoted and stored in small vials to be delivered A plus Laboratories (Lab + Auckland) for temporary storage before they were stored at -85ºC for further analysis. Negotiations were made with the help of Professor Elaine Rush with Dr Lindsay Plank at the body composition unit at the University of Auckland to grant the permission for storing the samples in the freezers. Further analysis included serum insulin, ferritin, iron, vitamin B_{12}, folate, C reactive proteins high sensitivity and MMA.

Methods used for blood analysis

Glucose and serum lipids were measured by Diagnostic Medlab Ltd, Auckland using Roche-Hitachi automated clinical chemistry analysers. Complete blood count was measured using the Sysmex-XE-2100 hematology analyzer. At a later date the plasma samples were analysed for serum iron, ferritin, folate, C-reactive protein high sensitivity, insulin, vitamin B_{12} and folate at Health Waikato laboratories. Insulin was measured using the Immulite 2000 Analyser, serum iron was measured using the Roche Hitachi analyzer, serum ferritin, vitamin B_{12} and folate were measured using the Roche Elecsys 2010 and MODULAR ANALYTICS E170 inmmunoassay analyzer. C-reactive protein high sensitivity was measured using Beckmann Image Immunochemistry systems kit. Methylmalonic acid (MMA) was analysed by Canterbury District Health Board.

The method for plasma MMA analysis involved solvent extraction of acidified plasma with added internal standard (deuterated methylmalonic acid i.e. the methyl group hydrogens have been replaced with deuterium) into ethylacetate. The solvent was then dried and then derivatised with N-methyl-tertbutyldimethylsilyltrifluoracetamide (MTBSFTA). Samples were then injected into a Shimadzu Gas Chromatography Mass Spectrometer (GCMS) and the ratio of peak area MMA/peak area D3MMA plotted as a standard curve versus concentration in µmol/L (personal communication, Christine Leaver). Homeostasis model assessment (HOMA) was used to measure β- cell function (HOMA B %) and insulin sensitivity (HOMA S%). This required the input of fasting insulin and glucose into an algorithm kindly provided by Jonathan Levy (Hermans et al., 1999; Levy et al., 1998).

The blood reports were checked by Dr Janet Rowan for a medical opinion with particular attention to any signs of anaemia and vitamin B_{12} deficiency. The final results and the medical advice if necessary was given to the parents in the form of a letter which enclosed serum vitamin B_{12}, ferritin, C- reactive protein and folate.

Visit 4

The fourth visit was marked on day 14 of the study. On the fourth visit, copies of the blood reports for lipids, fasting glucose and blood counts were given to the parents of the participants. Evaluated 7 day diet analysis was discussed with the parents, who were given booklets on New Zealand Ministry of Health dietary guidelines for preadolescent and adolescent children with an additional booklet for vegetarians. The urine samples for day 13 and day 14 were collected from the participants. As a token of appreciation for participating in the study the parents of the girls were given $20 petrol vouchers as commuting charges to Diagnostic Medlab collection room.

Miscellaneous visits

Miscellaneous visits involved the collection of 5 hour, day 1 day 2 urine samples. The girls were revisited if there were gaps recognized in the information in the diet or physical activity diaries which required further clarifications.

2.3 Measurements

All the measurements were made in the participant's house. Most of the anthropometric measurements were repeated three times to ensure precision was known for the measurements.

2.3.1 Height

Height was measured using a movable stadiometer (in-house constructed using a steel carpenters' measure as used in the pilot for the child nutrition survey, 2000), consisting of a flat horizontal board at the base, a movable measuring rod and a horizontal headboard that slides to contact the vertex of the head. The horizontal headboard had a spirit level to ensure the horizontal placement of the headboard. It was ensured that the horizontal board at the base of the stadiometer was placed on a hard flat surface. The girls were asked to stand straight on the horizontal board at the base with weight evenly distributed between the two feet, heels together and arms hanging by the sides with palms facing the thighs. It was ensured that the head, scapula and buttocks touched the measuring rod. The rod was kept straight through out the procedure and not bent or curved. The girls were asked to keep their head erect with eyes focused straight ahead. As the girls inhaled deeply and stretched tall, the horizontal board of the stadiometer was lowered to the top of the head firmly without exerting extreme pressure and ensuring the horizontal plane of the board using the carpenter's level. The standing height was measured thrice to the nearest $\pm$ 0.1cm.

2.3.2 Sitting height

The sitting height was measured using the same stadiometer (used for measuring height). The girls were asked to sit on the flat surface of the stadiometer with their legs stretched straight in front and a straight back. It was ensured that their head was erect with eyes focused straight ahead. As the girls inhaled deeply, the horizontal board of the stadiometer was lowered to the vertex firmly without exerting extreme pressure. The sitting height was measured thrice to the nearest ± 0.1 cm.

2.3.3 Weight

The weight was measured using electronic digital scale (Tanita/model 1609, Wedderburn scales Ltd, Napier). The accuracy of the weighing scale was confirmed by cross checking its readings at a number of measurement points with a standard weighing scale in the anthropometry laboratory at Auckland University of Technology, on the day or day before visit 1. Before weighing the girls, they were asked to empty their bladder and to remove any extra clothing e.g. sweat shirts and shoes. They were requested to wear bear minimum clothing. The girls were asked to stand in the centre of the scale platform with weight evenly distributed between the feet. If their clothes were thick enough due to the winter season a remark was made and later the weight of those clothing was on subtracted from the recorded weight. At least two measurements were recorded and weight was measured to the nearest ± 0.1kg. Measuring the body weight immediately after a meal was avoided.

2.3.4 Mid upper arm circumference (MUAC)

The mid upper arm circumference was measured at the midpoint of the distance between the lateral projection of the acromial process and the inferior margin of olecranon process on the lateral aspect of the arm using a measuring tape with the forearm kept straight in supinated position. The midpoint was marked on the lateral side of the arm. The MUAC was measured thrice to the nearest ± 0.1 cm. All measurements were made on the right side of the body.

2.3.5 Skinfold thickness

Harpenden calipers (John Bull, British indicators Ltd, UK) were used for the measuring the skinfold thickness with a precision of ± 0.2 mm. During the measurements the girls were asked to stand erect but in a relaxed position from shoulders and arms. The sites were carefully identified and marked with a washable felt pen. The subscapular skinfold thickness was measured by obliquely raising twice the thickness of subcutaneous fat below the inferior angle of the scapula at approximately 45º to the horizontal plane following the natural cleavage line of the skin. The triceps skinfold thickness was measured by vertically raising the skinfold on the posterior aspect of mid triceps exactly halfway between the olecranon process of the ulna and acromion process of the scapula (MUAC). The skinfolds were firmly grasped between the thumb and the index finger of the right hand at a distance of 1cm above the marked site. The fold was lifted by raising a double fold of skin and subcutaneous adipose tissue leaving the underlying muscle undisturbed. The jaws of the caliper were placed perpendicular to the fold 1cm below the thumb and index finger and halfway between the crest and base of the fold.

The jaw pressure was released gradually and the measurement was taken a few seconds after releasing the pressure. The skinfold thickness measurements were measured at least twice with an interval to allow the tissue to restore its uncompressed form. The measurements were made to the nearest ± 0.2 mm. The MUAC measurement and the triceps skinfold were used to calculate the Total upper arm area (TAA), upper arm muscle area (AMA) and Upper arm fat area (AFA) using the formulas proposed by Rolland-Cachera et al., (1997) as described previously (Section 1.5.5)

2.3.6 Girth measurements

An anthropometric non stretchable tape (Figure finder tape measure, Novel products Inc, Rockton, IL) with tension gauge was used for measuring the girths.

2.3.7 Waist circumference

The waist was measured using the midpoint between the inferior lateral margin of the ribs and top of the iliac crest using horizontal position. The girls were asked to stand erect, abdomen relaxed, arms at sides and feet together. Measurement was taken at the end of normal expiration to the nearest ± 0.1cm.

2.3.8 Hip circumference

The girls were asked to stand erect, buttocks relaxed, feet together. The measurement was taken by placing the tape around the hips at horizontal level of the greatest gluteal protuberance and anteriorly at the level of symphysis pubis. Measurements were made to the nearest ± 0.1cm at least thrice.

2.3.9 Head circumference

The girls were asked to stand straight looking straight ahead. The tape was comfortably wrapped around the head; measurements were taken at the midpoint between the eyebrows. Measurements were made to the nearest ± 0.1cm at least thrice.

2.3.10 Grip strength

The girls were asked to hold the hand dynamometer (Smedley's dynamometer, TTM, Tokyo) in their pronated hand and compress it to the best of their ability. Three measurements were taken for right and left hand alternately and repeated at least twice. The highest measurement to the nearest kilogram (± 1kg) for each hand was used for analysis.

2.3.11 Bioelectrical impedance analysis (BIA)

A tetrapolar single frequency 50 kHz analyzer (Imp DF50, Impedimed Ltd, Town needed, Australia) was used to perform the measurement. The bioimpedance analyser was checked weekly against a standard resistance block and the checked resistance measurement was recorded 409±8Ω. The bioimpedance measures were taken on the right hand side of the body of the girls while they were lying in a supine position with legs slightly apart and thighs not touching each other. It was ensured that there was no contact between the thighs or the arm and the trunk as this would create a short circuit in the electrical path thereby dramatically affecting the impedance value. The room temperature was kept warm (20-25°C). As it was the winter season, it was ensured that an electric heater was used to keep the room warm.

The skin was cleaned at the electrode sites with alcohol wipes, four electrodes with electro conducting gel were attached at the following places:
a) Just proximal to the dorsal surface of the third metacarpal-phalangeal joint on the right hand (red coloured connection).
b) On the dorsal surface of the right wrist adjacent to the head of ulna (yellow coloured connection).
c) On the dorsal surface of the right foot just proximal to the second metatarsal-phalangeal joint (blue coloured connection).
d) On the anterior surface of the right ankle between medial and lateral malleoli (black coloured connection).
It was assured that the distance between the centre of the proximal and distal electrodes was at least 5 cm. The girls were asked to lie quietly while the analyzer was turned on and off. The readings were obtained for impedance (Z ohm), resistance (R ohm), reactance (Xc ohm) and phase angle (P°). The results for FM, FFM and BF% were obtained by substituting the above resistance measurement in the equation derived by Professor Elaine Rush (Rush et al., 2003).
$FFM = 0.622*(height^2/resistance) +0.234*weight+1.166$.
Further, FM (kg) = weight- FFM (kg).
Body fat percentage = FM(kg)/weight (kg) x 100.

2.3.12 Blood pressure

The arterial blood pressure was measured using digital sphygmomanometer (Omron T8, Omron Healthcare, Australia). The cuff size was selected depending on the mid upper arm circumference, for circumferences less than 16cm, the child cuff size was used. Blood pressure was measured on the left arm. The girls were requested to sit in a relaxed position with their left arm stretched out, arm and hand relaxed and fist closed comfortably. Three readings were recorded.

2.3.13 Resting energy expenditure (REE)

The REE was measured in the participant's house early in the morning, after overnight fast so the measured REE could also be considered equivalent to basal metabolic rate, BMR. It was measured to determine energy requirement along with the physical activity diary, validate the diet diary and observe the macronutrient mix in the diet from the ratio of carbon dioxide to oxygen in the expired air as respiratory exchange ratio (RER). The measurement was made using the indirect calorimeter Vacumed CPX metabolic cart (Vista mini CPX, Vacuumed, CA, USA). Before each measurement, calibration of this device was performed against a reference O_2–CO_2 mixture and fresh air. Barometric pressure, ambient humidity and temperature measured by the device were checked against reference laboratory instruments. The turbine was checked for accurate volume measurement using a standard one litre syringe.

It was ensured that the environment was noise-free. The procedure was explained to the child before start up and they were assured that it was a comfortable procedure and that they remain calm and quiet without moving during the period of measurements. The participants were also asked to indicate by a hand signal if they became uncomfortable. The participant lay on the couch in relaxed supine position ensuring that they were warm enough; they were kept comfortable by covering them with a rug or duvet or keeping a heater near the couch. After five minutes, a mask with the turbine and sampling tube attached (Appendix 3) was placed on the face comfortably covering the nose and the mouth; it was fastened around the head.

They were asked to breathe in to the mask in a relaxed manner without being distracted. Any kind of action that would cause anxiety was avoided. The child was permitted to watch television if she preferred to. Some girls were told stories to keep them calm and settled throughout the twenty minutes period as measurements were recorded. Efforts were made to prevent extra body movements or hyperventilation by the child, since the former increases the resting energy expenditure and the latter results in an increase in respiratory exchange ratio. Oxygen and carbon dioxide analyzer contained within the metabolic cart sampled the expired air from the subjects and analyzed the rates of oxygen consumption and carbon dioxide production. The data was displayed and stored in a computer breath by breath. The readings were recorded at the end of 60 seconds. Heart rate was measured using a digital pulse oximeter (Nonin Onyx, Nonin Medical Inc, MN, USA), the heart rate was updated every 5 seconds and noted manually every minute while REE was measured. This measurement also served as an early warning of discomfort in the children and also was a good measure of resting heart rate.

2.3.14 Diet diary analysis

The seven day diet diary format as used in previous study by Rush et al., (2004) was provided by Professor Elaine Rush (Appendix 5). Information on the timings of the eating or drinking occasion, the meal and foods consumed and the quantity were listed. Standard and household measurements were given as references for recording dietary intake. Household measures included a teaspoon, a cup and a portion of a plate. A ruler and a series of circles to estimate for example the size of an apple were printed on the back cover of the diary. The diary was kept by the participant for seven days starting from the day of drinking the doubly labeled water. They had completed a practice day previously and this was reviewed with them to ensure the instructions were clear and the foods noted in enough detail to enable analysis. For foods like bread, the type of bread (wholemeal, white and mixed grains), thickness; toast or sandwich was reported. For ethnic foods, like chapatti the size and recipe was noted. For the purpose of dietary analysis, eggs were included as dairy products. For maximising compliance, girls were telephoned twice during the week of record keeping to offer help and a reminder. To control for observer effects and bias, all the interviews and dietary analyses were conducted by the researcher (PC).

All the information obtained from the diaries was translated into gram equivalents and then entered into the FoodWorks $^{®}$ program (FoodWorks professional 2005 XyrisT Australia,Queensland). For some Indian foods which were not available in the program, standardized recipes were developed using ingredients within the FoodWorks program. While examining the dietary analysis for micronutrient intakes like vitamin B_{12} the two most commonly consumed breakfast cereals, Sanitarium^{TM} and Kellogs^{TM} were identified as a major source of vitamin B_{12} as they were fortified with 1.95 μg and 3.95 μg per 100g respectively. The non-meat eaters with a high intake of breakfast cereals, showed a high intake of vitamin B_{12} but their fasting serum analysis showed the converse. This triggered an investigation into the fortification of breakfast cereals. The nutritional information on the packet did not bear any information on fortification of cereals with vitamin B_{12}. Personal communication with Sanitarium and then Julian Lee at the Food and Crop organization confirmed that cereals in New Zealand were not fortified with vitamin B_{12} and that there was an error in the FoodFiles 2005 data entry (and also previous years) for vitamin B_{12} in cereals (Appendix 6) as data external to New Zealand had been used. The dietary analysis was then adjusted for this error.

2.3.15 Physical activity diary

The physical activity diary was adapted from Bratteby et al., (1997) with the help of Professor Elaine Rush (Appendix 7). Some modifications were made with respect to the description of physical activities for some categories so that the girls could comprehend it easily in terms of their daily life (Table 2.1). The dairy recorded physical activity for every quarter of an hour with each day divided into 96 periods of 15 minutes each. One hour was represented by four squares in each row (Appendix 7). The activities were categorized into nine levels, with level 9 representing the maximum intensity of physical activity and level 1 representing minimum intensity of physical activity, for example sitting, resting in bed etc (Table 2.1). For each 15 minute period the girls were asked to enter the activity level into the appropriate square. They were strictly instructed not to record two levels of activity in the same square. In such a case the dominant activity of that period was recorded. If an activity was not listed, the girls were asked to record the closest activity of comparable intensity or make a note of it in the space provided on the page.

For each day the number of 15 minute periods for each activity was summed and it was ensured that the total was 96. These values were entered for each day into an Excel spreadsheet along with the measured resting energy expenditure for each child. By multiplying each time period for each activity by the physical activity ratio (Table 2.1) and averaging over the seven days total energy expenditure was determined. The total sum of time in each level of activity was also determined.

Table 2.1: Physical activity ratios and examples of physical activities.

Category	Activities	Physical Activity Ratio
1	Sleeping, resting in bed	0.95
2	Sitting, eating, writing, listening, sitting in a car or bus, watching television etc.	1.5
3	Standing, washing etc.	2
4	Walking indoors, light home activities	2.8
5	Walking outdoors, light work, carrying a small bag	3.3
6	Leisure activities, sports and relaxed movement, running i.e light intensity	4.4
7	Leisure activities, sports and manual work of moderate intensity	6.5
8	Leisure activities, sports and manual work of high intensity- sweating and breathing hard	10.0
9	Sports activities and work of very high to maximal intensity.	15.0

2.4 Statistical analysis

Descriptive statistics of data are presented as mean, standard deviation and maximum and minimum values as ranges. Due to the small sample size, group data were compared using unequal variance unpaired t test. The 95% confidence intervals of the effect size of the difference was also determined as Cohen's d (Cohen, 1988). Confidence limits gives value of magnitude of the standardized difference in the means. A significant test statistic does not indicate that the effect it measures is meaningful or important. But, using an effect size, which is an objective and standardized measure of the magnitude of the observed effect is a meaningful way of comparing variables (Field, 2005b). Due to the small sample size, only the effect sizes of 0.5 and above are used to explain the qualitative difference between the two groups. According to Cohen (ibid) an effect size (d) of 0.2 is considered small, 0.5 medium and $0.8 \geq$ large. In the present thesis effect size is abbreviated as (ES).

Assuming that there are equal number of non-meat eaters and meat-eaters in a similar but a larger population. Bivariate correlations were used to investigate relationships among anthropometric, fasting blood biochemical, functional, dietary intake and energy expenditure parameters using the Pearson's product-moment correlation coefficient (r). Pearson's product-moment correlation coefficient is a standardized measure of the strength of relationship between two variables. The value for r lies between -1 to +1. A coefficient of +1 indicates that the two variables are perfectly positively correlated conversely a coefficient of -1 indicates that the two variables are perfectly negatively correlated. Since it is a standardized measure of an observed effect, it is commonly used measure (Field, 2005a).

According to Cohen's classification for the correlation coefficient if:

r= 0.10 this is a small effect: in this case effect explains 1% of total variance

r=0.30 this is a medium effect: in this case effect explains 9% of total variance and

r=>0.50 this is a large effect and the effect accounts for 25% of total variance

"Although the above values look small for r, they represent stronger degrees of association than they may seem" (Cohen, 1988).

Due to some non linear relationships the Spearman rank correlation was used to establish the relationship between some variables. Spearman's test first ranks the data and then applies Pearson's equation to those ranks (Field, 2005a).

All data were analysed using the statistical program SPSS 14.0™ provided by SPSS Inc, Chicago and Excel program™ and the confidence limits for the effect size calculated using an Excel spreadsheet kindly provided by Professor Will Hopkins. The significance level was set at $p < 0.05$.

CHAPTER 3: RESULTS

Data gathered from the twelve Indian preadolescent girls involved in the study "Indian preadolescent girls: Lifestyle patterns and accumulated risk" are presented in the following chapter. Results are divided into the following sections:

- Anthropometry
- Physiological measurements
- Fasting blood measurements of biomarkers
- Reported seven day dietary intake of foods and nutrient analysis
- Reported seven day physical activity diary analysis

3.1 Anthropometry

All measurements were completed for twelve preadolescent girls (10.0 ±0.8y). Six non-meat eating (9.8±0.9y) and six meat eating (10.0±0.6y) girls were selected for the study. Mothers confirmed that each girl was at Tanner stage 1. All belonged to the socio economically middle class society. Nine of the twelve girls were from decile 5.0 school, one was from decile 7.0 and the remaining two were from decile 3.0 (Education Review Office, 2005).

Reported mean birth weight was not different for the two groups, 2.8 (±0.6) kg and 3.0 (±0.4) kg for non-meat-eating and meat eating respectively. Mothers and daughters in each group followed the same dietary pattern and all mothers had followed a non-meat-eating or a meat eating diet through out their life including pregnancy. The mean gestation period was 39 weeks and 40 weeks for non meat eating and meat eating respectively. Five girls (three non-meat-eating and two meat eating) had their birth weight below 3 kg and could be considered small. Weight, height, BMI was compared with the recently published New Zealand and Australian reference values obtained from the New Zealand Ministry of Health (2005).

3.1.1 Physical characteristics

3.1.1.1 Weight and Height

Mean weight, height and BMI of all the girls were close to the reference values (Table 3.1). Two non-meat eaters and two meat-eaters were (~3-15kg) more than the reference value. For height measurement, two non-meat eaters and four meat-eaters were ~3-8 cm taller than the reference value. Reference value for BMI is not given in the NZ standard. But, when derived from reference value for height and weight, it was 17.3 kgm^{-2}. In the present study, average BMI was 16.9 kgm^{-2}. It ranged form 13.3 to 22.5 kgm^{-2}. This means that there was a wide range of BMI 9.2 kgm^{-2} in a relatively small sample.

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Table 3.1: Physical characteristics of six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y

Physical characteristics	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size
Age		10.0 ± 0.8 (9.0-11.3)	9.8 ± 0.9 (9.0-11.3)	10.0 ± 0.6 (9.1-10.7)		
Height (cm)	138.0[†]	138.7 ± 5.9 (129.6-146.8)	138.0 ± 6.3 (129.6-146.8)	140.0 ± 6.0 (130.7-146.6)	0.65	-0.27
Weight (kg)	32.9[†]	32.2 ± 7.6 (22.8-48.3)	31.2 ± 5.5 (25.4-39.0)	33.3 ± 9.6 (22.8-48.3)	0.65	-0.27
BMI	17.3[‡]	16.6 ± 2.8 (13.3-22.5)	16.3 ± 1.9 (14.5-19.2)	16.9 ± 3.7 (13.3-22.5)	0.75	-0.19
Sitting Height (cm)		68.2 ± 3.1 (64.2-72.2)	67.8 ± 3.1 (64.2-72.1)	68.6 ± 3.4 (64.2-72.2)	0.70	-0.23
Leg length (cm)		70.6 ± 4.3 (60.4-76.8)	70.1 ± 5.5 (60.4-76.8)	71.0 ± 3.1 (66.6-76.0)	0.74	-0.12

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; effect size calculated using 95%CI; §95% CI not reported for effect size less than 0.5; [†] Reference from Ministry of Health (2005); [‡] reference value derived using the reference value for weight[†] and height[†]; BMI, Body mass index.

3.1.1.2 Girths

There was no difference between the two groups for waist circumference (Table 3.2). Two non-meat eaters and two meat-eaters had waist circumferences 7-15 cm above the mean value for the group. The waist circumference was within the reference value for both the groups. The reference value used for waist circumference (<71 cm) in the present study is referred from Higgins et al., (2001) for prepubertal children. They have shown the highest value for positive likelihood ratio for cardiovascular diseases in prepubertal children with waist circumference 71-72.9cm. This value is also recommended by Indian academic of pediatrics (Bhave et al., 2004).

The hip circumference tended to be higher in the meat-eaters (p = 0.39, ES= 0.52). The waist-to-hip ratio tended to be higher in the non-meat eaters (p= 0.37, ES= 0.56) and was on the borderline of upper level of reference value recommended by Indian Academy of pediatrics (Bhave et al., 2004) for defining central obesity in Indian children.

Table 3.2: Girth measurements of six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y.

Girth measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat eaters N=6	*p value	§Effect size	95% confidence limits	
Waist circumference (cm)	<71 cm[†]	62.3 ± 8.7 (50.5-78.7)	61.7 ± 7.0 (54.3-70.6)	62.9 ± 10.8 (50.5-78.7)	0.83	-0.13		
Hip circumference (cm)		72.5 ± 8.9 (52.2-81.4)	70.1± 10.2 (52.2-81.4)	74.8 ± 7.4 (65.7-86.1)	0.39	-0.52	-1.83	0.79
WHR	<0.9[‡]	0.86 ± 0.09 (0.77-1.1)	0.89 ± 0.12 (079-1.12)	0.84 ± 0.06 (0.77-0.92)	0.37	+0.56	-0.81	1.92

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §effect size calculated using 95% CI; §95% CI for effect size less than 0.5 not reported; WHR, waist to hip ratio; †reference value obtained from Higgins *et al.* (2001); ‡ reference value obtained from Bhave *et al.* (2004) for Indian Academy of Pediatrics.

3.1.1.3 Arm anthropometry

Meat-eaters tended to have a larger MUAC (p =0.39, ES =0.53), larger TAA (p = 0.37, ES = 0.55) larger subscapular skinfold thickness (p = 0.35, ES= 0.59) and higher subscapular to triceps skinfold ratio (p= 0.41, ES= 0.53), Table 3.3. But there was no difference between the two groups for AFA and AMA. One of the meat-eaters had the largest MUAC, TAA and AFA but the AMA was not proportionately larger inferring proportionately less muscle mass and more fat in this individual.

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Table 3.3: Arm anthropometry of six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y

Arm Anthropometry	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
MUAC (cm)		21.7 ± 3.7 (17.9-30.7)	20.7 ± 2.6 (17.9-24.0)	22.7 ± 4.7 (18.1-30.7)	0.39	-0.53	-1.90	0.84
Triceps skinfold thickness(mm)		15.7 ± 8.1 (6.5-35.5)	14.3 ± 5.1 (9.1-23.9)	17.0 ± 10.7 (6.5-35.5)	0.59	-0.33		
Subscapular skinfold thickness (mm)		11.3 ± 7.7 (5.1-27.5)	9.1 ± 4.0 (5.8-15.1)	13.6 ± 10.1 (5.1-27.5)	0.35	-0.59	-2.0	0.83
Subscapular to triceps ratio		0.7 ± 0.3 (0.4-1.3)	0.7 ± 0.3 (0.5-1.2)	0.8 ± 0.3 (0.4-1.3)	0.41	-0.53	-1.83	0.78
TAA (cm^2)		38.8 ± 14.1 (25.0-75.0)	34.8 ± 8.8 (25.0-46.0)	42.7 ± 18.0 (26.0-75.0)	0.37	-0.55	-1.92	0.81
AFA (cm^2)		18.3 ± 13.4 (6.0-54.4)	15.2 ± 7.1 (8.5-28.6)	21.4 ± 17.9 (6.0-54.4)	0.46	-0.46		
AMA (cm^2)		20.3 ± 3.8 (13.0-28.9)	19.5 ± 5.2 (13.0-28.9)	21.1 ± 1.4 (19.2-23.4)	0.48	-0.43		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; effect size calculated using 95% CI; §95% CI for effect size less than 0.5 not reported; MUAC, mid upper arm circumference; TAA, total arm area; AFA, upper arm fat Area; AMA, upper arm muscle area.

3.1.1.4 Body fat percentage determined using bioelectrical impedance analysis

There was no difference in the body composition of the two groups in relation to fat free mass, fat mass and body fat percentage measured using bioelectrical impedance analysis (Table 3.4). The BF% of all the 12 girls (29.4%) was on the borderline of upper level of normality. Comparing the data with British reference range using One Sample T test, the Indian girls in the present study were 34 percentile points above the British median adjusted for age. Graphical representation of comparisons for each of the girls made with British reference ranges is shown in Figure 3.1.

Table 3.4: Body composition by bioimpedance analysis of six non-meat-eating and six meat-eating preadolescent Indian girls aged 9-10y

Body composition	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size
FM (kg)		9.8 ± 4.0 (5.2-18.0)	9.5 ± 3.5 (5.6-13.8)	10.0 ± 4.8 (5.2-18.0)	0.83	-0.13
FFM (kg)		22.5 ± 4.1 (17.6-30.3)	21.7 ± 2.7 (18.5-26.1)	23.3 ± 5.3 (17.6-30.3)	0.52	-0.38
BF%	30%[†]	29.4 ± 6.1 (20.8-38.1)	29.7 ± 6.6 (21.7-38.1)	29.0 ± 6.2 (20.8-37.3)	0.85	+0.11

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; effect size calculated using 95%CI; §95% CI for effect size less than 0.5 not reported; FM fat mass; FFM, Fat free mass; BF%, Body fat percentage; † reference value obtained from Williams *et al* .(1992).

Figure 3.1: Body fat percentages of six non-meat-eating and six meat eating preadolescent Indian girls 9-10 years

Adapted from (McCarthy et al., 2006)

According to McCarthy et al., (2006) BF% from 28.2 to 32.2% for girls aged 10y were categorized between 91st to 95th percentile. All except one were above the 50th percentile. Eight girls (4 non-meat-eaters and 4 meat-eaters) had BF% between 27.5- 32 %, one non-meat eater and one meat-eater had BF% 38.1 and 37.1% respectively, both above the 98th percentile cutoff. The Caucasian girls at the age of 10y showed a relatively flat 50th centile with BF% varying between 15-18% showing a peak at the age of 11y. The above cutoffs when used with BMI to clinically define cut offs used by the International Obesity Task force (IOTF). The 85th and 95th centile lines closely corresponded with overweight and obese boundaries of the IOTF curves respectively. Hence the subjects in both the groups were overfat compared to Caucasian girls despite of a normal BMI. They were overweight nearing obesity.

3.1.1.5 Measures of central adiposity.

Considering the three measures of central adiposity, waist circumference, waist-to-hip ratio (Table 3.2) and subscapular to triceps ratio (Table 3.3), there were no significant differences in the waist circumference. Waist to hip ratio appeared to be higher in non-meat eaters ($p = 0.37$, ES 0.56) but subscapular to triceps ratio appeared to be higher in meat-eaters ($p = 0.41$, ES= 0.53).

3.2 Physiological measurements

Criterion measurement of energy expenditure like resting energy expenditure (REE) was used. A major portion of energy expenditure at rest and during exercise is a due to muscle metabolic activity and therefore REE provides an estimation of body mass. Additional measurements included: Respiratory exchange ratio (a measure of the substrate mix in the diet), hand grip strength (right and left), blood pressure and pulse rate were compared between the two groups as shown in Tables 3.5 and 3.6.

The non-meat eaters appeared to have a higher REE ($p = 0.30$, ES= 0.63), RER ($p = 0.21$, ES = 0.78) and heart rate ($p= 0.36$, ES= 0.56). Grip strength was not different between groups; the right hand was the dominant hand for all participants and this was the stronger hand in both the groups (Table 3.5). All the 12 girls had blood pressure readings within the reference range. Meat-eaters had a higher systolic blood pressure than non-meat eaters ($p = 0.13$, ES 0.98) Table 3.6.

Table 3.5: Functional measurements in six non-meat-eating and six-meat-eating preadolescent Indian girls aged 9-10y

Functional Measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
REE (kcal/day)		1240 ± 244 (899-1774)	1317 ± 258 (980-1774)	1163 ± 224 (899-1560)	0.30	+0.63	-0.67	1.94
REE (MJ/day)		5.2 ± 1.0 (3.8- 7.4)	5.5 ± 1.1 (4.1- 7.4)	4.9 ± 0.9 (3.8- 6.5)	0.29	+0.63	-0.62	1.89
RER		0.89± 0.06 (0.78-0.98)	0.91± 0.05 (0.86-0.98)	0.87 ± 0.07 (0.78-0.98)	0.21	+0.78	-0.55	2.11
Heart rate (bpm)		85 ± 5 (76-94)	86 ± 6 (76-94)	83 ± 4 (80-90)	0.36	+0.56	-0.77	1.89
Grip strength left hand (kg)		16.1 ± 4.6 (11.0-24.0)	15.5 ± 3.5 (11.0-21.0)	16.7 ±5.8 (14.0-24.0)	0.64	-0.26		
Grip strength right hand (kg)		16.6 ± 3.4 (12.0-24.0)	17.8 ± 3.8 (12.0-21.0)	17.1 ± 3.5 (14.0-24.0)	0.54	-0.36		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported;

bpm, beats per minute; REE, resting energy expenditure; RER, respiratory exchange ratio.

Table 3.6: Blood pressure readings of six non-meat-eating and six meat eating preadolescent Indian girls aged 9-10y

Functional Measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Systolic blood pressure (mmHg)	120	110 ±10 (90-122)	106 ± 9 (90-117)	115 ± 8 (103-122)	0.13	-0.98	-2.29	0.32
Diastolic blood pressure (mmHg)	80	72 ± 5 (62-82)	71 ± 6 (62.0-78)	73 ± 5 (66-82)	0.41	-0.50	-1.80	0.81
Pulse		93 ± 9 (76-105)	94 ± 11 (76-105)	92 ± 8 (80-102)	0.64	+0.28		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported.

3.3 Fasting blood measurements

The blood biochemistry involved measuring fasting serum lipids, metabolic biochemical parameters (fasting glucose, serum insulin, homeostasis model assessment of insulin sensitivity and β- cell function), serum haematological measurements (complete blood count, iron and ferritin) and biomarkers of one-carbon metabolism and obesity (vitamin B_{12}, folate, MMA and CRP-hs) are reported in Tables 3.7-3.11.

3.3.1 Lipids

Fasting HDL cholesterol appeared to be higher on average by 0.3 mmol/L in meat-eaters (p = 0.31, ES= 0.62) compared to non-meat-eaters (Table 3.7). Two meat-eaters had a HDL cholesterol ≥2.0 mmol/L; the upper limit of the observed range. The mean value of LDL cholesterol for meat-eaters was greater than the reference value (<2.0 mmol/L) by 0.6 mmol/L and appeared to be greater than the non-meat eaters (p = 0.08, 1.16). Four of six meat-eaters had LDL cholesterol normal value (>2.0 mmol/L) and three of six non-meat eaters had LDL cholesterol normal value (> 2.0 mmol/L). Total cholesterol tended to be higher in meat-eaters (p = 0.07, ES= 1.18). Although, the mean values for total cholesterol were within the reference range for both the groups, three of six meat-eaters had total cholesterol >5.0 mmol/L (reference value, <5.0 mmol/L). The total/HDL cholesterol ratio was not different between the two groups and was within the reference value for both the groups. Triglyceride levels were not different between the two groups, only one meat-eater had triglycerides >2.0 mmol/L (reference value, < 2.0 mmol/L).

Table 3.7: Fasting lipids in six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y.

Serum lipids	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
HDL cholesterol (mmol/L)	>1.0[†]	1.5 ± 0.4 (0.9-2.2)	1.4 ± 0.4 (0.9-1.8)	1.7 ± 0.4 (1.2-2.2)	0.31	-0.62	-1.93	0.69
LDL cholesterol (mmol/L)	<2.0	2.3 ± 0.7 (1.1-3.4)	1.9 ± 0.5 (1.1-2.5)	2.6 ± 0.7 (1.7-3.4)	0.08	-1.16	-2.47	0.14
Total cholesterol (mmol/L)	<5.0[†]	4.2 ± 0.9 (2.7-5.9)	3.8 ± 0.6 (2.7-4.4)	4.7 ± 1.0 (3.3-5.9)	0.07	-1.18	-2.52	0.15
Total/HDL cholesterol ratio	<4.5[†]	2.9 ± 0.7 (1.9-4.2)	2.8 ± 0.8 (2.1-4.2)	3.0 ± 0.8 (1.9-3.9)	0.69	-0.24		
Triglyceride (mmol/L)	<2.0[†]	0.9 ± 0.5 (0.4-2.2)	0.9 ± 0.4 (0.5-1.6)	1.0± 0.7 (0.4-2.2)	0.88	-0.09		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; HDL, high density lipoprotein cholesterol; LDL; low density lipoprotein cholesterol, †reference values provided by Diagnostic Medlab Ltd.

3.3.2 Metabolic biochemical parameters

The fasting glucose was within the normal range for both the groups with no difference between the two groups but the serum insulin appeared to be higher in meat eaters (p = 0.35, ES = 0.57) with no difference in functional measurements of β-cell function and insulin sensitivity.

Table 3.8: Metabolic biochemical parameters in six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y

Metabolic biochemical parameters	Reference range	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Fasting glucose (mmol/L)	3.5-6.0 [†]	4.5 ± 0.3 (4.2-5.0)	4.5 ± 0.2 (4.2-4.8)	4.5 ± 0.3 (4.2-5.0)	0.92	-0.06		
Serum Insulin pmol/L	43-210 [‡]	53.6 ± 22.6 (24-88)	47.2 ± 18.9 (31-80)	60.0 ± 25.9 (24-88)	0.35	-0.57	-1.82	0.69
HOMA B%[¶]		117.1 ± 38.5 (74.9-181.2)	108.4 ± 32.4 (74.9-162.2)	125.9 ± 45.0 (76.0-181.2)	0.46	-0.44		
HOMA S%[¶]		117.1 ± 49.8 (61.1-220.8)	125.0 ± 39.2 (66.9-169.3)	109.3 ± 61.5 (61.1-220.8)	0.61	+0.31		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; HOMAB%, Homeostasis model assessment β cell function; HOMA S%, Homeostasis model assessment insulin sensitivity; †reference range provided by Diagnostic Medlab Ltd; ‡ reference range provided by Waikato Health Laboratories ¶calculated using algorithm provided by Hermans *et al.* (1999) and Levy *et al.* (1998).

3.3.3 Haematological measurements

Serum haematological measurements were done to check for anaemia, Table 3.9 and 3.10. No child had overt clinical anaemia but two non-meat-eaters had a low serum iron, below 10 30μmol/L. The reference range is 10-30μmol/L. The meat eaters appeared to have a higher serum iron (p = 0.39, ES = 0.52) and haemoglobin (p =0.35, ES= 0.58) Table 3.9. All the girls had haemoglobin values within the reference range. Although there was no difference in the ferritin levels between the two groups, two of six non-meat eaters had levels <15.0 ng/L; the reference range is 15-150ng/L.On observing the ranges, it becomes clear that only one non-meat eater had ferritin value 110ng/L and only one meat-eater had ferritin value 130ng/L.

The platelet counts were within the normal range for both the groups; meat-eaters appeared to have higher platelet counts (p = 0.24, ES = 0.73). One of the non-meat eaters had platelet count as low as 155 b/l and only one of them had a count of 339 b/l (reference value 150-500 b/l). Comparatively the meat-eaters had a higher range. The packed cell volume (PCV) appeared to be higher in non-meat eaters (p = 0.2, ES= 0.78). The mean cell volume (MCV) and mean corpuscular haemoglobin (MCH) were not different between the two groups. The monocyte count appeared to be higher in the meat-eaters (p = 0.37, ES = 0.54).The non-meat eaters had a higher basophil (p = 0.08, ES= 1.1). There were no differences between the two groups for lymphocytes, WBC, neutrophil and eosinophils.

Table 3.9: Hematological measurements in six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y.

Haematological measurements	Reference value	Total N=12	Non- meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Haemoglobin (g/l)	115-152[†]	131.0 ± 8.8 (120-149)	129.0 ± 10.4 (120-149)	133.7 ± 7.2 (124-143)	0.39	-0.52	-1.86	0.81
Platelet (b/l)	150-500[†]	276 ± 69 (155-434)	251 ± 63 (155-339)	300 ± 71 (227-434)	0.24	-0.73	-2.04	0.57
PCV (l/l)	0.33-0.47[†]	0.41 ± 0.03 (0.4)	0.41 ± 0.1 (0.4)	0.39 ± 0.03 (0.4)	0.2	-0.78	-2.12	0.55
MCV(fl)	74-90[†]	82.4 ± 4.1 (75-87)	81.8 ± 2.3 (79-86)	83.0 ± 5.5 (75-87)	0.65	-0.28		
MCH pg	23-31[†]	27.3 ± 1.2 (25-29)	27.3 ± 1.0 (26-29)	27.3 ± 1.5 (25-29)	1.00	0.00		
Serum iron µmol/L	10-30.0[‡]	16 ± 6 (7-25)	15 ± 7 (7-25)	19 ± 2 (12-22)	0.35	-0.58	-1.94	0.79
Ferritin ng/L	15-150[‡]	50 ± 38 (9-130)	45 ± 37 (9-110)	55 ± 42 (18-130)	0.68	-0.24		

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; PCV ,Packed cell volume; MCV, Mean cell volume; MCH ,Mean corpuscular hemoglobin; †reference values provided by Diagnostic Medlab Ltd; ‡ reference value provided by Health Waikato Laboratories.

Table 3.10: Hematological measurements count cont'd

Haematological measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Monocyte (b/l)	0-0.9[†]	0.5 ± 0.1 (0.3-0.6)	0.4 ± 0.1 (0.3-0.6)	0.5 ± 0.1 (0.3-0.6)	0.37	-0.54	-1.85	0.76
Lymphocyte (b/l)	1.5-7.3[†]	2.9 ± 0.7 (2.0-4.1)	2.9 ± 0.8 (2.0-4.1)	3.0 ± 0.7 (2.0-4.1)	0.96	-0.03		
WBC (b/l)	4.5-14.0[†]	5.9 ± 1.6 (3.9-8.5)	5.5 ± 1.6 (3.9-7.8)	6.3 ±1.7 (4.2-8.5)	0.45	-0.46		
Neutrophil (b/l)	1.2-8.3[†]	2.3 ± 1.3 (1.1-4.8)	2.0 ± 0.8 (1.3-3.3)	2.7 ± 1.7 (1.1-4.8)	-0.52	-1.88	0.85	
Basophil (b/l)	0-0.2[†]	0.02 ± 0.01 (0-0.1)	0.03 ± 0.02 (0-0.1)	0.02 ± 0.01 (0-0.1)	0.08	+1.1	-0.2	2.41
Eosinophil (b/l)	0-0.5[†]	0.2 ± 0.1 (0.1-0.4)	0.2 ± 0.1 (0.1-0.4)	0.2 ± 0.1 (0.1-0.4)	0.08			

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; WBC White blood cell count; †reference values provided by Diagnostic Medlab Ltd.

3.3.4 Biomarkers for one-carbon metabolism and obesity.

The serum vitamin B_{12} was significantly higher in meat-eaters (p = 0.01, ES=1.99) (Table 3.11). Two non-meat eaters were deficient in Vitamin B_{12} i.e. serum vitamin B_{12} concentration less than 170 pmol/L at 110 and 116 pmol/L. Ironically the highest value for serum vitamin B_{12} in the non-meat-eaters was 380 pmol/Land the lowest value for the meat-eaters was 340 pmol/L, this shows the difference or relative lack of overlap between the two groups (Figure 3.2). Methylmalonic acid (MMA), a classical marker for B_{12} deficiency was high in non-meat eaters (p=0.3, ES=0.63, Figure 3.2). One of the non-meat-eating girls had serum MMA concentrations above the reference range (0.40µmol/L) and one of them had high normal concentrations of MMA close to the reference value, she also had vitamin B_{12} concentrations below the reference value (110pmol/L). All the meat-eating girls had serum MMA concentrations within the reference range. Hence, the significantly lower levels of Vitamin B_{12} and higher levels of MMA in non-meat eaters were suggestive that they were insufficient in vitamin $B_{12,}$ Figure 3.2.

There was a distinct difference in the two groups in regards to the relationship between vitamin B_{12} and MMA concentrations (Figure 3.2). At 170 pmol/L (lower value of the reference range used in the present thesis), the MMA concentrations in the non-meat-eaters are as high as 0.60µmol/L. The serum MMA concentrations in the non-meat-eaters start decreasing (0.25-0.15µmol/L) when the serum vitamin B_{12} concentration increases to 250-300pmol/L. further, the reference values for serum vitamin B_{12} (170-800pmol/L) used in the present thesis, are based on studies in European population (personal communication with Roche Diagnostics, Germany) and (Kolhouse et al., 1978). The consumption of meat and meat products is lower in Indians compared to Caucasians. Therefore, a reference value higher than 170pmol/L should be set as the lower reference value for vitamin B_{12} in this population.

The non-meat eaters appeared to have higher serum folate concentrations (p= 0.1, ES=0.99). None of the girls in either group had folate levels lower than the reference range (9.0-45.0 pmol/L).Further, two markers of inflammation and cardiovascular risk; CRP-hs appeared to be higher in non-meat eaters (p = 0.21, ES = 0.79) Table 3.11, with no difference in the serum ferritin levels Table 3.9.

Table 3.11: Biomarkers for one-carbon metabolism and obesity in six non-meat-eating and meat-eating Indian preadolescent girls aged 9-10y.

Biomarkers	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Vitamin B12 pmol/L	170-800[†]	389 ± 221 (110-870)	232 ± 95 (110-380)	543 ± 212 (340-870)	0.01	-1.99	-3.35	0.62
Folate pmol/L	9.0-45.0[†]	27.0 ± 8.1 (16.5-45)	30.4 ± 9.2 (19-45)	23.1 ± 4.8 (16.5-31)	0.1	0.84	-0.41	2.10
MMAµmol/L	<0.4[‡]	0.2 ± 0.1 (0.1-0.6)	0.3 ± 0.2 (0.1-0.6)	0.2 ± 0.1 (0.1-0.2)	0.3	+0.63	-0.63	1.89
CRP-hs mg/L	0.00-8.00[†]	1.4 ± 1.1 (0-3)	1.9± 1.3 (0-3)	1.0 ± 0.8 (0-2)	0.21	0.79	-0.47	2.05

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; MMA, Methylmalonic acid; †reference values provided by Health Waikato Laboratories; ‡ reference value provided by Canterbury District Health Board.

Figure 3.2: Fasting serum concentrations of vitamin B12 and MMA of six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y
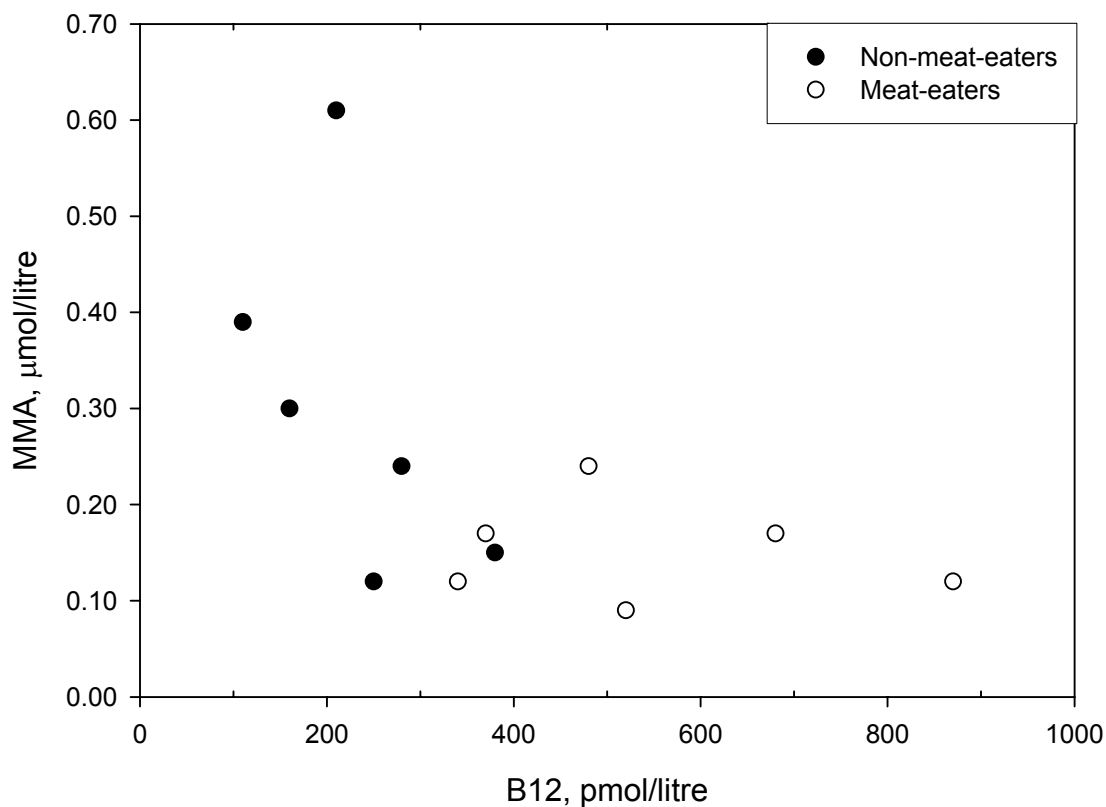
3.4 Dietary Analysis

Qualitative analysis

The dietary habits of meat-eating and non-meat-eating girls are summarized in Tables 3.12 and 3.13. Meat eating practices varied even within the six girls because of religious and cultural practices which have determined the eating pattern of Indians for centuries. Three of the six girls were Hindus and therefore did not consume beef. One of them was a Muslim and therefore did not consume pork. The remaining two were Catholics and ate all kinds of meat.

Based on geographical distribution, three meat eating girls originated from west coastal areas and therefore fish was included in the diet. One of the meat-eating girls was from the eastern zone of the country and therefore consumed fish.

From the remaining two girls, one was from the coastal southern zone of the country but did not consume fish, and the sixth participant was from southern part of the country that consumes more red meat. There were distinctive dietary patterns observed between the groups and within the meat-eating and non-meat-eating girls Table 3.12 and Table 3.13. As explained earlier, the information obtained from the mothers regarding the foods not consumed by their daughters was related to individual taste and cultural and religious practices.

Table 3.12: Qualitative dietary habits of six non-meat-eating Indian preadolescent girls

Dietary groups	Foods eaten/Dietary pattern	Foods not eaten	Serum vitamin B_{12} (pmol/L) [†]
NME1	Lactovegetarian diet	Eggs, meat and meat products	160 pmol/L
NME2	Lactoovovegetarian, Eggs only once a week	Meat and meat products	380 pmol/L
NME3	Lactoovovegetarian and Fish once a month	Meat and meat products	280 pmol/L
NME4	Lactoovovegetarian and eggs three times a week	Meat and meat products	250 pmol/L
NME5	Lactoovovegetarian and eggs consumed twice a week	Meat and meat products	210 pmol/L
NME6	Lactovegetarian diet, Yoghurt and dairy products not consumed often	Eggs, meat and meat products	110 pmol/L

NME, Non-meat-eaters; † reference values (170-800 pmol/L) provided by Diagnostic Medlab Ltd

Table 3.13: Qualitative dietary habits of six meat-eating Indian preadolescent girls

Dietary group	Foods eaten/Dietary pattern	Foods not eaten	Serum vitamin B_{12} (pmol/L)[†]
ME1	Chicken and lamb	Vegetables, fruits and Ham. Eggs consumed only twice a week	340 pmol/L
ME2	Chicken, fish and eggs only twice a week	Lamb, beef and ham	870 pmol/L
ME3	Chicken, prawns and lamb. Eggs consumed four times a week	Beef and ham	480 pmol/L
ME4	Beef, chicken, pork and tuna fish	Fruits, vegetables and eggs	370 pmol/L
ME5	Beef, chicken, pork and fish. Eggs consumed twice a week only.	Fruits, butter and cheese	520 pmol/L
ME6	Beef and chicken. Eggs four times a week only. Milk only twice a week	Pork	680 pmol/L

ME,Meat eaters; [†] reference values (170-800 pmol/L) provided by Diagnostic Medlab Ltd.

Quantitative analysis

Analysis of the diet diaries for studying the dietary patterns, foods and nutrients intake of the girls particularly in relation to protein, B_{12} intake and fibre are summarized in Table 3.14 and Table 3.15.

Table 3.14: Reported dietary intakes from seven days diet diaries in six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y

Measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Energy consumption (MJ/day) for 32.2kg	7.3[†]	9.7 ± 1.5 (6.2-14.4)	10.1 ±1.7 (6.2-14.4)	9.3 ± 1.4 (6.1-9.7)	0.37	+0.56	-0.74	1.87
%Energy Carbohydrate	55-75%[‡]	57.3 ± 8.1 (45.6-73.7)	57.9 ± 10.0 (45.6-73.7)	56.4 ±6.6 (49.4-65.8)	0.80	+0.15		
%Energy protein	10-15%[‡]	11.3 ± 1.5 (8.8-14.9)	10.5 ± 0.9 (8.8-11.3)	12.0 ± 1.7 (9.9-14.9)	0.09	-1.12	-2.38	0.14
%Energy fat	15-30%[‡]	31.9 ± 8.3 (15.8-46.1)	32.1 ± 10.5 (15.8-46.1)	31.7 ± 6.5 (23.1-39.0)	0.94	+0.04		
Protein (g/day)	24[†]	65 ± 11 (50-84)	64 ± 12 (51-80)	66 ± 11 (50-84)	0.70	-0.23		
Protein (g/day) meat sources				22 ± 3 (11-55)				
%Energy from sucrose [‡]	<10%[‡]	8 ±4 (4-19)	8±3 (4-12)	9±5 (4-19)	0.68	0.24	0.004	

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; ‡ reference values obtained from World Health Organisation (2003); †reference value obtained from Ministry of Health (2005)

Table 3.15: **Reported dietary intake cont'd**

Measurements	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	§Effect size	95% confidence limits	
Energy density kJ/g		1.76 ± 0.19 (1.5-2.0)	1.73 ± 0.16 (1.5-2.0)	1.79 ± 0.22 (1.5-2.0)	0.60	-0.31		
P:S ratio	0.7-1.0[¶]	0.6 ± 0.4 (0.4-1.4)	0.6 ± 0.4 (0.4-1.4)	0.6 ± 0.4 (0.4-1.3)	0.96	-0.32		
Saturated fat as % of total energy ‡	<10%	13.9 ±6.0 (5.0-25.0)	13.0± 6.0 (5.0-23.0)	15.9 ±6.3 (10.0-25.0)	0.60	0.15		
Dietary B12 µg/person/ day [†]	EAR 1.5µg/day[†] RDI 1.8µg/day[†]	2.2 ± 0.8 (1.2-3.8)	1.8 ± 0.6 (1.2-2.6)	2.5 ± 0.8 (1.8-3.8)	0.11	-1.0	-2.25	0.27
Dietary fibre (g/day) [†]	20 g /day[†]	25.2 ± 9.0 (10.4-44.6)	30.4 ± 8.0 (22.5-44.6)	19.9 ± 6.9 (10.4-29.5)	0.03	1.4	0.10	2.70
B_{12} milk (µg/day)		0.75 ± 0.36	1.00±0.23 (0.60-1.20)	0.45 ± 0.24 (0.07-0.70)	0.003	2.32	1.06	3.58

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95% CI for effect size less than 0.5 not reported; EAR Estimated average requirement; RDI Recommended dietary allowance; ‡ reference value obtained from World Health Organisation (2003); † reference value obtained from Ministry of Health (2005); ¶ reference value obtained from Harding *et al.*(2001)and Oliver (1997).

3.4.1 Energy

The non-meat eaters tended to have a higher absolute energy intake than meat-eaters (p=0.37, ES=0.56, Table 3.14). Girls in both the groups reported energy intake greater than the recommended values of 7.6-8.5 MJ/day by the Ministry of Health (2005) for girls weighing 32.2kg at the age of 10.0y. The percentage energy obtained from reported carbohydrate: protein: fat intake of all the 12 girls was 57:11:32.

3.4.2 Macronutrients

3.4.2.1 Carbohydrate

Carbohydrate intake of all the girls was within the reference range at 57% of energy. There was no difference between the two groups. The mean contribution of sucrose to the reported total energy intake was within the reference values (<10%) recommended by World Health Organisation (2003).

3.4.2.2 Protein

The protein intake of non-meat eaters was significantly lower than meat eaters (p= 0.09, ES=1.12). Amongst meat-eaters on average only 30% of the total daily protein was from meat products but meat was not eaten every day. For the purpose of dietary analysis, eggs were included in dairy products. Schematic representations of food consumed and their contribution to daily protein intake in six non-meat-eating and six meat-eating girls are presented in Figure 3.3 and 3.4.

<u>Figure 3.3: Average protein intakes in non-meat eaters 63.6g/day.</u>
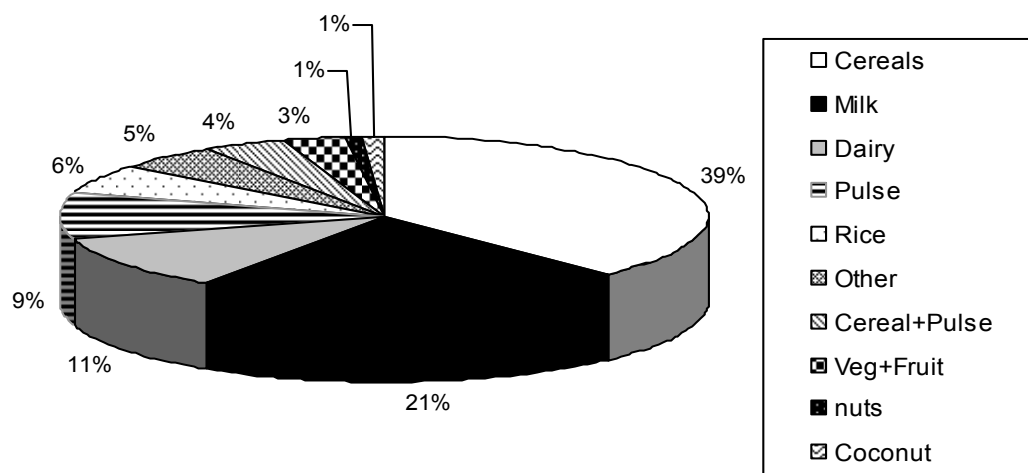
Breakfast cereals were the highest contributors to protein intake in the non-meat eaters (Figure 3.3); 43% of protein energy came from cereal being made up of whole wheat flour (11%), breakfast cereals (11%), bread (10%), rice (9%), pasta, noodles (1%) and Weetbix™ (1%). Milk contributed 21%, dairy products (9%) and pulses contributed only 9%. The remaining 29% contribution was from a mixture of foods containing rice and pulse; an indeterminate mixture (4%), the remaining contribution was from other sources like nuts, coconut, potato and other ready-to-eat foods.

In meat-eaters (Figure 3.4) the highest sources of protein were chicken, rice and cereal followed by milk, diary products, red meat and fish in the given order. Only 26% of the protein was contributed by meat, of which 17% was from chicken (white meat). Beef mutton and pork contributed only 6%, 2% and 1% respectively. Fish contributed only 4%.Cereals contributed 30% [rice (17%), bread (7%), whole-wheat flour (3%), pasta and noodles (2%) and breakfast cereals (1%)]. Milk contributed only 12% and other dairy products contributed 12% [eggs (5%), yoghurt (2%), cheese (3%) and ice cream (2%)]. The remaining 16% was from sources like vegetable and fruits, potato and ready-to –eat foods. The meat eating patterns of all the six meat-eaters was different depending on the religion and cultural practice as explained earlier. All the meat-eaters did not consume meat daily; the consumption of meat was not more than four times a week. In addition, the variety of meat consumed was limited. Only one participant consumed all types of meats.

Figure 3.4: Average protein intakes in meat-eaters 66.3g/day
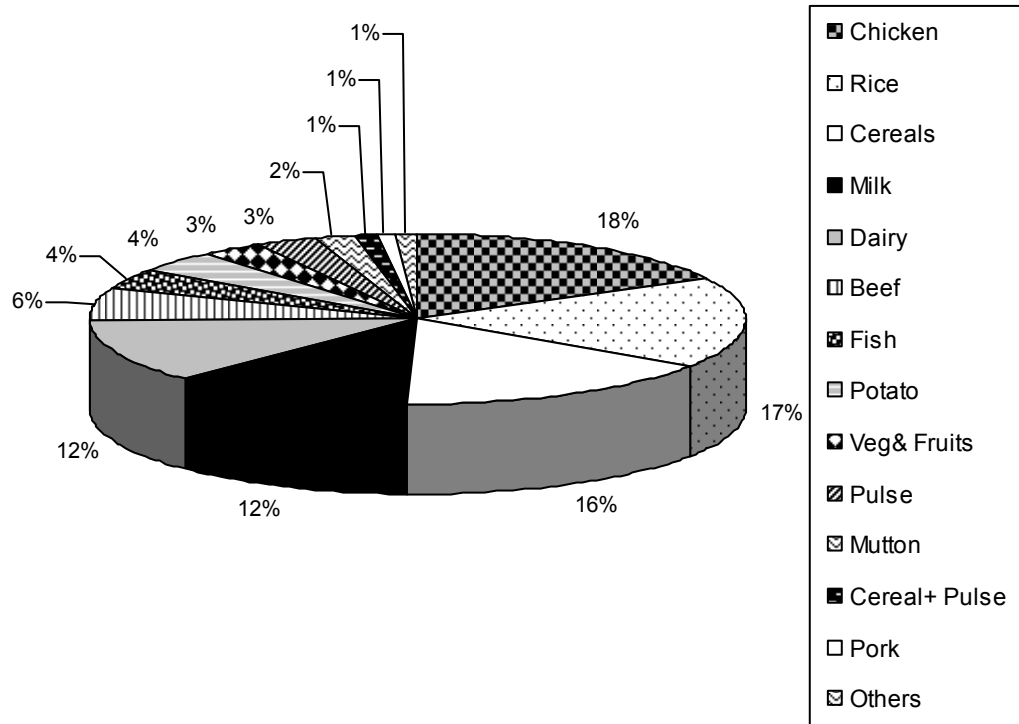
3.4.2.3 Fat

The mean fat intake of all the girls was slightly higher than the reference value. Four non-meat-eaters and three meat-eaters consumed diets with more than 30% of total energy from fats. It was observed that, 10 girls except two consumed standard milk. One serving of standard milk (100ml) contains 3.3 gm of total fat of which 2.4 gm is saturated fat i.e. almost three quarters of the total fat in milk is saturated fat. Saturated fat was more than 10% reference value provided by of the total energy intake. The polyunsaturated :saturated fat (P:S) ratio was lower than the reference value, implicating a risk for heart diseases and hyperglycemia (Harding et al., 2001; Oliver, 1997).

The twelve most commonly consumed ethnic foods were analysed separately for their macronutrient energy composition. Some of the commonly consumed foods are enlisted in Table 3.16. All the foods were high in carbohydrate and fat content, but were low in protein except for the meat and pulse preparations. Also, it is worth noting that, oil was the basic ingredient for all the recipes. Cooking oil was an important contributor to the high energy from fats.

Table 3.16: Energy composition of some commonly consumed foods by six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10y.

Food	% energy carbohydrate:protein:fat	Energy kJ/100g	Main ingredients
Bhindi (okra veg)	17:8:75	389	Okra, onion and oil
Butter Chicken	15:14:71	732	Chicken composite cuts, lean and fat, tomato, safflower oil and cream
Chicken curry	5:21:73	672	Chicken composite cuts lean and fat, tomato and oil
Potato curry	47:8:45	424	Potato, tomato, onion and oil
Paratha (Indian bread)	41:8:51	1352	Whole wheat flour and oil
Pooris (Indian bread, fried)	21:7:72	1693	Whole wheat flour and oil
Dal (pulse preparation)	39:24:36	180	Mung dal (pulse), water and oil
Stir fried mixed vegetable	40:12:48	446	Vegetables, onions and oil
Mixed vegetable khicdi (rice and pulse preparation)	69:28:3	424	Rice, mung dal and water.
Dosa filling	63:10:27	405	Potato, onion ad oil
Dosa (pancake made up of rice flour and milled pulse)	68:16:16	1463	Rice flour , pulse (milled)and oil
Veg cutlet	29:6:65	992	Mixed vegetables, deep fried in oil.

3.4.3 Micronutrients (vitamin B$_{12}$ and fibre)

3.4.3.1 Vitamin B$_{12}$ intake in non-meat eaters.

The reported dietary intake of vitamin B$_{12}$, by the meat-eaters and non-meat eaters is given in Table 3.15. Meat-eaters tended to report foods than contained more vitamin B$_{12}$ (p= 0.10, ES=1.02) .The mean intake of vitamin B$_{12}$ in non-meat eaters was 1.8µg/day. Which was in agreement with the values for Estimated Average Requirement (EAR) 1.5 µg/day, and Recommended Dietary intake (RDI) 1.5 µg/day of vitamin B$_{12}$ (Ministry of Health, 2005). The different sources of vitaminB$_{12}$ in non-meat eaters are shown in the figure below.

Figure 3.5: Average vitamin B$_{12}$ intake in non-meat eaters 1.8µg/day
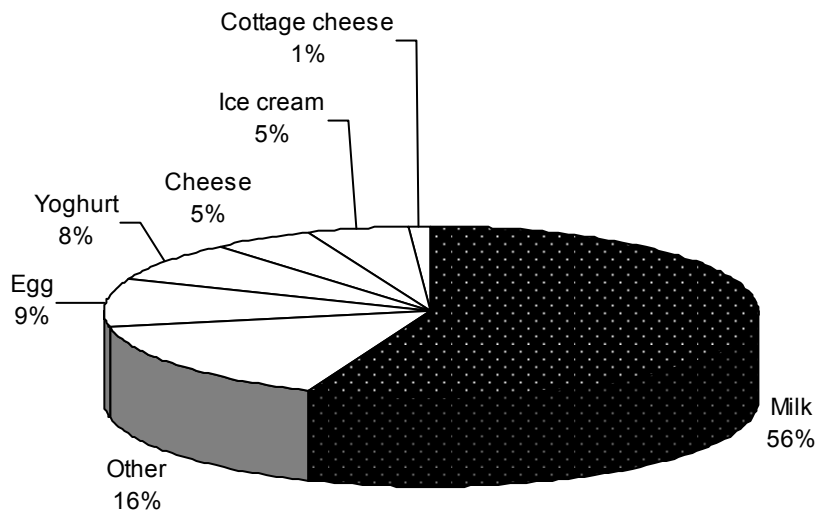
3.4.3.2 Vitamin B$_{12}$ intake in meat-eaters.

The mean intakes of vitamin B$_{12}$ of meat-eaters was 2.6μg/day which was above the EAR and RDI values provided by Ministry of Health (2005). The different food sources of vitamin B$_{12}$ in non-meat eaters and meat-eaters are shown below in figure below

Figure 3.6: Average vitamin B$_{12}$ intakes in meat-eaters 2.5μg/day
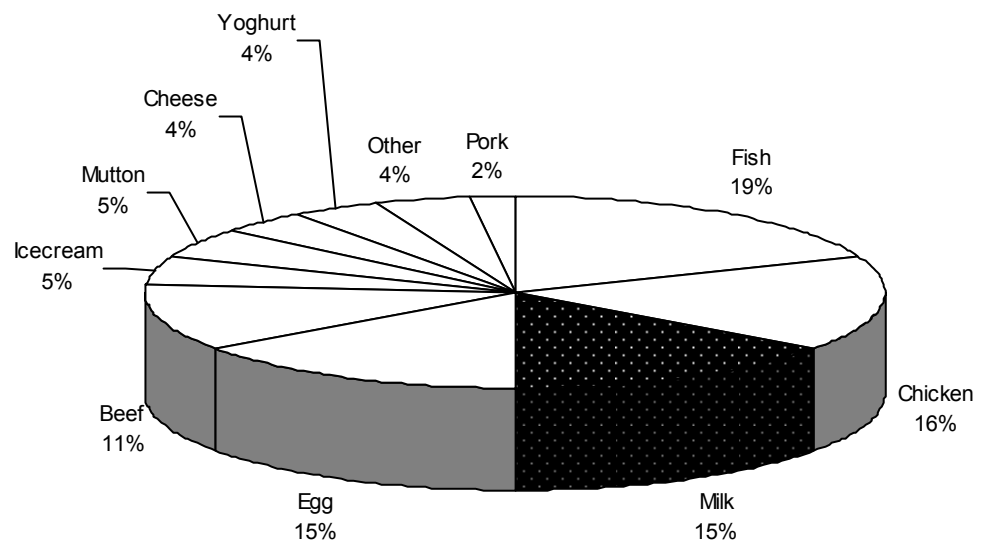
For the meat-eaters, (Figure 3.6), a third, 34%, of the seven day B$_{12}$ was obtained from meat, of which 16% was chicken (white meat). The contribution of red meat like beef, mutton and pork was 11%, 5% and 2% respectively. Fish contributed fairly up to 19%. Eggs contributed 15% followed by dairy products 14% [yoghurt (5%), ice cream (5%) and cheese (4%)]. Although, the non vegetarians consumed more B$_{12}$ than the vegetarians and also included meat in the diet the low consumption of red meat reduced the amount of B$_{12}$ since beef and lamb are better sources of B$_{12}$ compared to white meat (Narayanan et al., 1991).

3.4.4 Dietary fibre

Non-meat eaters had a significantly higher fibre intake than meat eaters. (30.4 vs 19.9 g/day, p=0.03, ES 1.40) Table 3.15. This was related to the high consumption of breakfast cereals, whole wheat flour, wholemeal and multigrain bread, fruits and vegetables in non-meat eaters. All six non-meat-eating girls consumed wholemeal or multigrain bread all the time. In the meat eaters, all but one girl consumed white bread and the consumption of fruits and vegetables was low compared to non-meat eaters. A diagrammatic representation of the difference between the non-meat eaters and meat and meat-eaters for fibre and protein intake is shown in Figure 3.7.

Figure 3.7 : Differences between meat-eaters and non-meat eaters for total fibre and protein intake
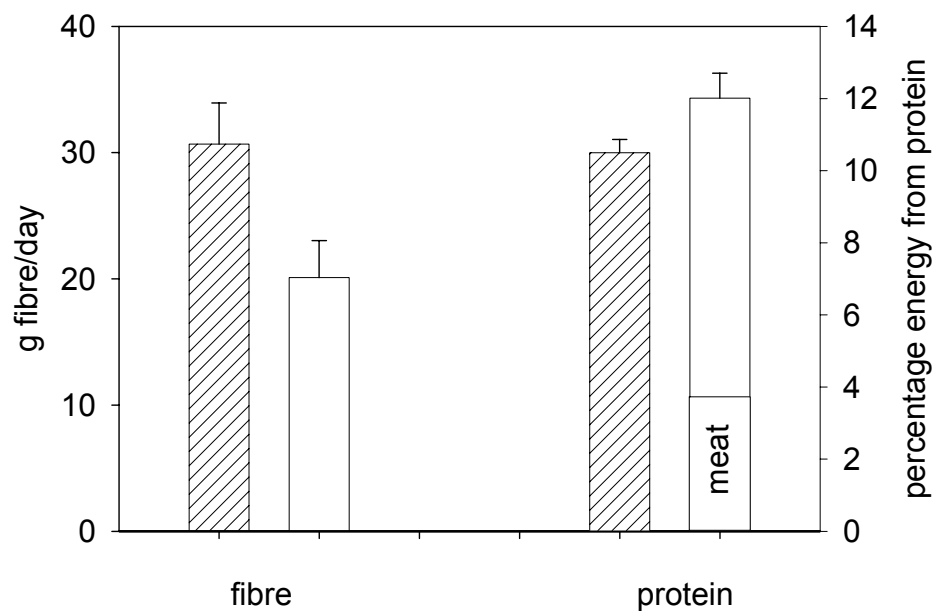
3.5 Reported physical activity

There was no difference between the two dietary groups in relation to energy expenditure measured in kilojoules per day derived from the seven day physical activity diary (Table 3.17). Non-meat-eaters reported higher energy expenditure (P=0.17, ES 0.89). Further, both the groups spent a large time in sedentary activities~21 hours/day (including sleep, sitting and standing) and 3 hours/day in moving activities (Figure 3.8) with no significant difference (P=0. 33) between the two groups but a large effect size 0.59. As reported by the participants, moving activities involved only more light intensity activities (categories 4,5 and 6. Table 2.1) than moderate activity, high or maximal intensity activities (categories 7,8 and 9. Table 2.1). On an average they spent approximately fifteen minutes per day in moderate intensity activity (category 7, Table 2.1). They reported nil participation in high and maximal intensity activities (category 8 and 9, Table 2.1).

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Table 3.17: Reported physical activity from seven days physical activity diaries in six non-meat-eating and six meat-eating Indian preadolescent girls aged 9-10 years

Energy expenditure	Reference value	Total N=12	Non-meat eaters N=6	Meat-eaters N=6	*p value	Effect size	95% confidence limits	
Energy expenditure (MJ/day)		8.7 ± 2.6 (6.1-14.4)	9.8 ± 3.2 (6.2-14.4)	7.5 ± 1.3 (6.1-9.7)	0.17	0.89	-0.37	2.15
Sedentary hours/day		21.1 ± 1.3 (19.1-23.1)	20.7 ± 1.2 (19.1-22.0)	21.4 ± 1.3 (19.4-23.1)	0.33	-0.59	-1.85	0.67
Moving hours per day		2.9 ± 1.3 (0.9-4.9)	3.3 ± 1.2 (2.0-4.9)	2.6 ± 1.3 (0.9-4.6)	0.33	0.59	-0.67	1.85

Mean ± SD; Range in parenthesis; *p value calculated using unequal variance t test; §95%CI for effect size less than 0.5 not reported.

Figure 3.8: Total time spent in static and physical activities per day by six non-meat-eating and six meat-eating Indian preadolescent girls.
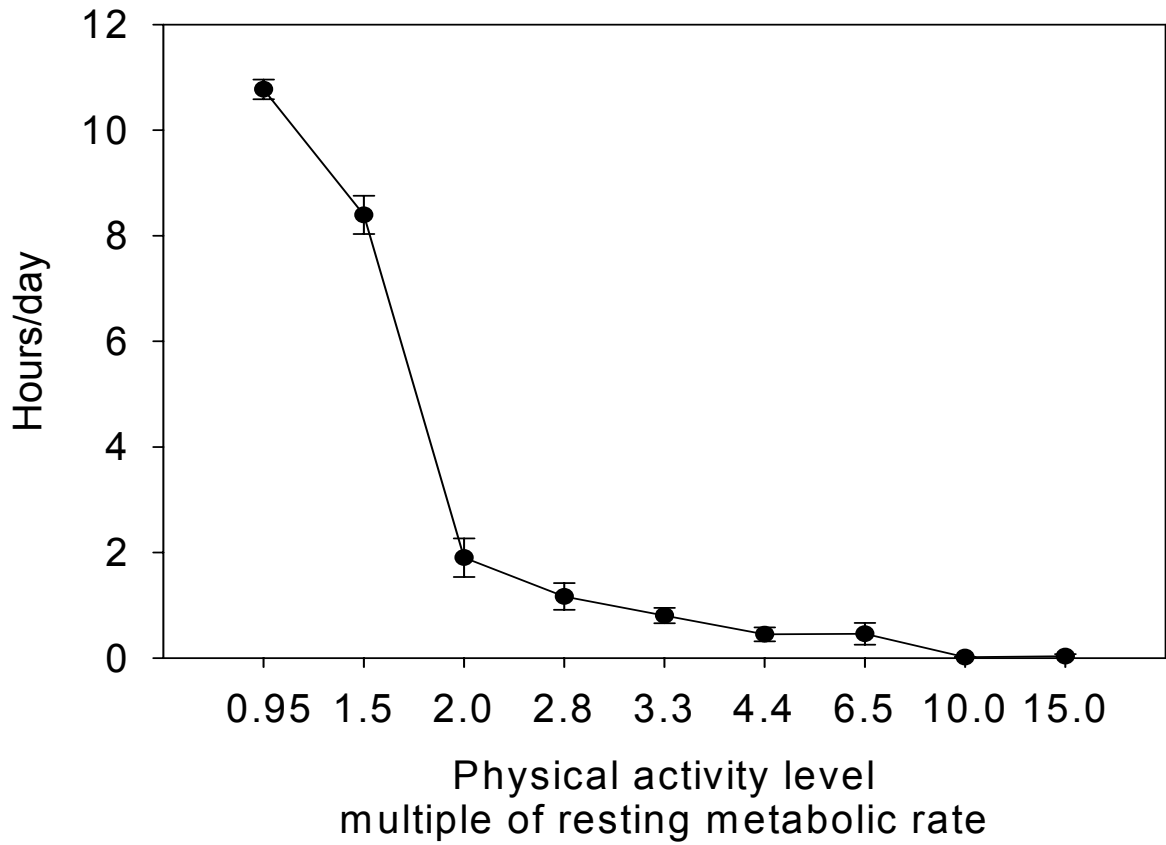
3.6 Associations

A large number of variables were measured in a relatively small number of subjects. Therefore it is possible that a Type I error may have occured because of the large number of comparisons that were made i.e. finding a difference where there is not one. Conversely a Type II error of not finding association because of the small number of subjects is also possible. Bivariate correlations explored associations between body composition, physiological measurements, biomarkers, diet and physical activity. Selected, significant associations are grouped and summarised in Tables 8.1-8.6 in Appendix 8. Within body composition measurements as expected there was good correlation, e.g. Subscapular skinfold was correlated with both FM and FFM, waist and BMI (all r>0.80, p=0.05,Appendix 8.1).

The measure of central fatness, the subscapular to tricep skinfold ratio was positively associated with insulin, serum triglycerides, grip strength and arm muscle area (Appendix 8, Table 8.3). Grip strength was also associated with WHR, the size and composition of the upper arm, LDL cholesterol and triglycerides (Appendix 8, Table 8.5). In the above associations, subscapular skinfold thickness was positively associated with left hand grip strength (r = 0.83, p =0.001) and right hand grip strength (r = 0.70, p= 0.01) which in turn positively correlated with the serum triglycerides (r = 0.87, p < 0.001) and (r = 0.94, p <0.001). The triceps skinfold thickness did not correlate with the grip strength, but tended to correlate with triglycerides (r = 0.51, p = 0.09). This could be suggestive of increasing muscle strength accompanied by increasing fat which is evident from the strong correlation between subscapular skinfold thickness and serum triglycerides, and the tendency of triceps skinfold thickness to correlate with triglycerides (r = 0.51, p = 0.09).

3.7 Summary of differences between non-meat-eating and meat groups

It was observed that, in relation to body composition there was no difference in the waist circumference between the two groups. However, the non-meat eaters had a higher WHR. Arm anthropometric measurements like MUAC, subscapular skinfold thickness, subscapular to triceps ratio, TAA was higher in meat eaters. But there was no difference in the AMA and AFA. The resting energy expenditure and respiratory exchange ratio tended to be higher in non-meat eaters. There was no difference in the fasting glucose, however, the all forms of cholesterol were higher in meat-eaters (LDL cholesterol, HDL cholesterol and total cholesterol). Insulin was higher in the meat-eaters which is reverse of the suggested hypothesis. Haemoglobin, serum iron and platelet were higher in meat-eaters. The CRP-hs was higher in non-meat eaters.

Further, non-meat eaters tended to report a higher energy intake. There was no difference in energy obtained from carbohydrate and fat, but meat-eaters had higher percentage of energy from protein and non-meat eaters had a high fibre intake as hypothesized. Vitamin B_{12} was higher in the meat-eaters compared to non-meat eaters and vice-a-versa for serum folate as hypothesized. Physical activity levels were not different between the two groups. Both the groups spent maximum amount of the time in sedentary activities. Over the week, the girls spent less approximately fifteen minutes per day on moderate physical activity (category 7, Table 2.1).

CHAPTER 4: DISCUSSION

The present thesis was designed to look at lifestyle risks in preadolescent Indian girls in relation to the meat-eating and non-meat-eating practices followed by themselves and their mothers i.e. from conception to preadolescence. This thesis was successful in showing that:

1. Diet in meat-eaters had marked differences to non-meat eaters. Non-meat eaters reported a significantly high intake of fibre (p = 0.03, ES 1.4) and folate as measured in the fasting serum samples (p = 0.1, ES=0.99) but tended to have a low energy from proteins (p = 0.09, ES = 1.12).

2. Biomarkers in the blood for one carbon metabolism were different between meat-eaters and non-meat- eaters. Non–meat eaters had a significantly low level of vitamin B_{12} (p = 0.01, ES = 1.99) and high levels of methylmalonic acid (MMA) compared to meat- eaters (p= 0.3, ES= 0.63).

3. Meat-eating and non-meat-eating girls had higher body fat percentage at a lower BMI when compared to a contemporary British reference range.

4. Both the groups spent approximately 21 hours of the day in sedentary activities (including sleeping) and only three hours a day in any form of movement.

The implications of each of these findings, diet, biomarkers, body composition and physical activity will each be discussed in turn and then interrelated. Limitations of the study and future recommendations will then be described and conclusions stated.

4.1 Diet

It is recognized that energy intake is often under reported and physical activity over reported. Diet is very hard to measure but seven day diet diaries are recognized as a reliable method of determining recent dietary intake. They are preferred over food frequency questionnaire when validated against urinary biomarkers like nitrogen, potassium and sodium (Day et al., 2001). (ibid) compared food frequency questionnaire and 7 day diet diaries using the urinary biomarkers and reported that diet diaries compared to food frequency questionnaires are more strongly associated with the true intake of sodium and potassium. As well as giving an estimate of usual seven day energy intake the dairies have the advantage of recording dietary pattern and timing of

the eating occasions of the children and the actual foods eaten. Particularly, a qualitative and quantitative evaluation of the intake of core foods like milk and milk products, dairy products, fruits, meat and cereals was possible. The present thesis cannot report any doubly labeled water findings, but these diet diaries have been used in earlier studies by Rush et al., (2004)

To answer the question: "Are meat-eaters and non-meat-eaters different in their diet?" the findings of the diet analysis was examined against criteria. The diaries were checked for reporting accuracy using the ratios of energy intake and basal metabolic rate (BMR) as suggested by Goldberg et al., (1991). *(ibid)* have suggested a ratio of 1.41 with confidence limit 95% as cut-off value for recognizing under-reporters. The cut-off is applicable for studies with a sample size, n= 10 and study period of 7 days. The present thesis reported a mean ratio of energy intake: REE as 1.9, thus there was no bias to under-reporting.

4.1.1 Dietary patterns

There was a definite difference in dietary pattern as defined by selection of meat-eating and non-meat-eating girls. Dietary practice was determined by religion and culture of the mothers. Non-meat-eating girls had poor consumption of eggs and abstained from meat and meat products and this contention was supported by lower levels of serum vitamin B_{12} levels in their blood. The meat-eaters did not consume meat more than four times a week but had higher serum vitamin B_{12} levels compared to non-meat eaters. Apart from the meat-eating characteristics, examination of the diet for macro and micronutrient analysis revealed that the diet did not follow the New Zealand dietary guidelines in a number of aspects and it was concluded that the diet quality of all the girls was "poor'. This is explained in the next part of the discussion.

4.1.2 Macronutrients

4.1.2.1 Carbohydrate

Meat eaters and non-meat eaters consumed 57% of their energy as carbohydrate. The mean intake of females aged 7-10y in the New Zealand National Children Nutrition Survey (NZCNS) (Ministry of Health, 2003) was 53% so this intake was slightly higher. The relatively high intake of carbohydrate was also reflected in the increased respiratory exchange ratio (RER) 0.89 for both the groups and slightly higher in the non-meat eaters. Popkin et al*.,* (2001), have reported a large shift from consumption of coarse grain to rice in all income groups in India. This is also shown in this group with the increased intake of carbohydrate due to a high intake of breakfast cereals, bread and rice which are mainly refined. Non-meat-eaters ate more breakfast cereal than meat eaters. The consumption of sucrose was within the recommended range of no more than 10% of energy (WHO, 2003), but the sucrose in the diet was contributed highly to by ready-to-eat foods like noodles, chips, biscuits, fruits juices and soft drinks and not fruit. Less than 3 servings of fruit a day were reported.

While mean fasting triglyceride was not high there is a risk that if high consumption of carbohydrate particularly sucrose and fructose continues triglyceride may increase in the high risk Indian population (Fried et al., 2003). There was no correlation between energy obtained from carbohydrate and serum triglycerides. But serum triglyceride positively correlated with total arm area ($r = 0.60$, $p = 0.04$), grip strength of right and left hand ($r = 0.76$, $p = 0.004$) ($r = 0.87$, $p<0.001$) respectively. Since grip strength is a measure of the muscle strength the positive correlation of serum triglyceride with grip strength could be an indication of increased intramuscular and adipose tissue triglyceride uptake. Also the correlation of serum triglyceride with FM ($r = 0.62$, $p = 0.03$) and FFM ($r = 0.72$, $p = 0.009$) is suggestive of increased triglyceride concentrations in the fat tissues (adipose tissues) and fat free mass (muscles). The strong correlation of serum triglycerides with total arm area and FM (Appendix 8.6) could be an indicator of high uptake of carbohydrate and conversion to fat by the muscles and the adipose tissue. However, this possibility needs to be explored further.

4.1.2.2 Protein

The mean value of energy obtained from protein for non - meat-eaters and meat eaters was 10.5% and 12.0 respectively which was on the borderline of lower level of recommended reference value. The reference value for protein intake is 10-15% (World Health Organisation, 2003). The recommended values by Food and Nutrition Board: Institute of Medicine, FNB:IOM (2002) for protein intake for children aged 4-18 y is 10-30% and therefore the girls in the present thesis were on the borderline of lower levels of the recommended amount. This protein intake for both the groups was below the 50th percentile value (13.9%) of females aged 7-10y as reported in NZCNS. In the present thesis, among non-meat eaters, milk was the highest source of protein followed by whole-wheat flour, breakfast cereals, bread, rice and dairy products. Also, the ratio of pulses to cereals and rice was not high 1:4.5. A mixture of cereals and pulses is important for the non-meat eaters to ensure complementation as explained by Young and Pellett (1994) but as they point out "there is no evidence that amino acid imbalances per se are important". Adequate protein in the diet is essential for ingestion of essential amino acids. But Krajcovicova-Kudlackova et al., (2000) further reported a significantly low (p<0.001) dietary methionine intake in vegans, lactovegetarians and lactoovovegetarians compared to omnivores. Omnivores in their study had adequate intake of methionine.

In the meat –eaters, chicken was the most commonly consumed meat although the quantity was not high, only 17% of daily protein intake and meat of any kind was not eaten more than 4 times a week. Red meat- beef, lamb and pork were not consumed more than once a week and had a poor contribution to daily protein intake. Similar findings of poor meat consumption are reported earlier by Rao et al., (2001) in pregnant rural Indian women. The consumption of red meat in this study was lower than the children in NZCNS. This was apparent from the contribution of red meat to the protein intake in the present thesis and NZCNS. The contribution of red meat like beef and veal in females aged 7-10y in NZCNS was 10% and in the present thesis, beef, lamb and pork together contributed only 9%. Red meat like beef has the highest protein digestibility as reported by Millward and Jackson (2003).

Cereals contributed more protein than meat 30% vs 26%. Rao et al., (2001) also observed a higher protein contribution from cereals and pulses in the Indian women. However, Young and Pellett (1994) reported that, in the United States only 30% of protein is from plant sources. In the present thesis, the contribution of pulses was only 9% and 4% in non-meat eaters and meat eaters respectively. Protein digestibility of cereals is lower than meat (Millward et al., 2003) and cereal proteins have a low biological value (Young et al., 1994). Further, all meat-eaters ate polished rice, which is not a good source of proteins since there are losses due the procedure involved in polishing it. Milk and dairy products did not contribute appreciably to the total protein intake of meat-eaters (12%).

The consumption of the eggs in both the groups was not more than four times a week. Eggs are the best and cheap source of proteins. The poor consumption of dairy products observed in the meat- eaters and non-meat eaters also reflects on their total calcium intake and the serum calcium levels in their body. The present thesis did not focus on the dietary or serum calcium levels, but this should be explored further in future studies.

4.1.2.3 Fat

The total fat intake of the girls was within the reference range: it coincided with the 50th percentile value of females aged 7-10y in the New Zealand Child Nutrition Survey. The saturated fat intake was 13.0% and 15.9% in non-meat eaters and meat-eaters respectively, which is higher than the recommended values of <10% of total energy WHO (2003). The dietary factors contributing to increased saturated fat intake were consumption of whole milk in both the groups, increased snacking habits, high consumption of ready-to-eat foods like noodles and cream biscuits. There was no difference between the two groups in the polyunsaturated: saturated fat ratio (P: S ratio), the P: S ratio was lower than the recommended values in the both the groups and this could lead to hyperglycemia and risks of ischemic heart diseases.

4.1.3 Micronutrients

4.1.3.1 Vitamin B_{12}

The main biomarker of interest in this thesis was vitamin B_{12}. Consumption was lower in the non-meat eaters (p= 0.11, ES = 1.0) and this observation was validated by significant (p=0.01,ES= 1.99) difference in serum vitamin B_{12} levels between the two groups. The mean serum B_{12} concentration for all the twelve participants was not appreciably high and the levels of MMA show that below a concentration of 300pmol/L of vitamin B_{12}, MMA levels start to rise (Figure 3.2) Refsum et al., (2001), in a study in India reported a low frequency of meat consumption especially lamb amongst non-vegetarians. They have reported cobalamin deficiency and increased MMA concentrations in vegetarians and non-vegetarians. Further, Refsum et al., (2001) reported a high odds ratio for vitamin B_{12} deficiency in subjects who rarely consumed eggs, poultry and lamb. Nevertheless, in the present study diet was the major determinant of vitamin B_{12} status which was apparent from the high MMA concentrations in the non-meat-eaters. Thus, meat consumption or consumption of foods rich in vitamin B_{12} are a feasible way to increase B_{12} in the blood and the next step would be a supplementation study to prove this.

In the present thesis, meat-eaters had a relatively high serum concentration of haemoglobin and serum iron and there was no evidence of anaemia which could be expected in a vitamin B_{12} deficient group. There was no difference between the two groups for serum ferritin concentrations. The lower iron and haemoglobin in the non-meat eaters could be due to lower absorption of non-haem iron. There was no evidence of macrocytosis, since there was no difference in the mean cell volume (MCV) and the concentration of MCV was within the reference value. Thus as suggested by Khanduri et al., (2005) MMA and serum total homocysteine are better indicators of vitamin B_{12} deficiency compared to MCV and lack of signs of anaemia are not necessarily diagnostic of an adequate B_{12} status.

Vegetarianism can lead to iron and vitamin B_{12} deficiency. Since meat and meat products are the richest source of iron and B_{12}. The irregular meat-eating habits (meat not consumed more than four times a week) in the meat eaters and the erratic egg consumption was similar to the observations by Khanduri et al., (2005) in India with "occasional eggs and very occasional meat". In the same study cobalamin deficiency in meat-eating subjects was reported and fortification of the diet called for. Undesired modifications in meat consuming habits amongst vitamin B_{12} deficient meat-eating Caucasians in UK were observed by Narayanan et al., (1991). They reported a decrease in consumption of beef and proportionate increase in consumption of chicken and pork. The latter is reported to have lower amounts of B_{12} compared to the former (Narayanan et al., 1991). In the present thesis in the meat-eaters vitamin B_{12} levels were not appreciably high and in two of the non-meat-eaters vitamin B_{12} deficiency was identified and the remaining four were relatively insufficient.

Low consumption of meat has previously been associated with low vitamin B_{12} levels. Herrmann et al., (2001) have shown significantly low concentrations of vitamin B_{12} in low meat eaters, (abstaining from red meat and consuming white meat or fish once or twice a week only) vegans, lacotovovegetarians and lactovegetarians compared to high meat eaters (consuming a typical omnivores diet) and significantly high MMA and homocysteine in vegans compared to high meat eaters, with no significant difference between lacotovegetarians and lactoovovegetarians.

In the present study serum folate concentrations tended to be higher in non-meat eaters which is similar to the findings of (Krajcovicova-Kudlackova et al., 2000) and this could be contributed to their high consumption of cereals and roti rather than green leafy vegetables which were not often consumed. These researchers reported significantly low serum vitamin B_{12} concentrations and significantly high homocysteine concentrations in vegans, lactovegetarians, lactoovovegetarians compared to omnivores and significantly low serum folate concentrations in omnivores compared to lactovegetarians and lactoovovegetarians. In spite of low folate concentrations, the omnivores in their study had lower homocysteine concentrations which suggests that vitamin B_{12} concentration is more influential in maintaining the homocysteine concentrations. In the present thesis, serum homocysteine concentrations were not measured but if they were measured, the higher folate and significantly lower

concentrations of vitamin B_{12} of the non-meat eaters could be helpful in understanding if lower vitamin B_{12} in the presence of folate is associated with raised homocysteine?

Due to the non linear relationship (Figure 3.2), Spearman rank correlation was used to establish a correlation between vitamin B12, MMA and dietary intake of B12. The serum vitamin B_{12} concentrations of the girls in the present thesis tended to correlate negatively with MMA (r = -0.62, p = 0.03,). Dietary intake of vitamin B_{12} was also significantly negatively correlated with serum MMA concentrations (r = -0.75, p = 0.005). There was a significant positive association between serum vitamin B_{12} concentration and dietary B12 ingested through foods, (r = 0.74, p = 0.006). Thus consuming milk, diary products and eggs should be advantageous for maintaining vitamin B_{12} concentrations.

The low levels of vitamin B_{12} in girls in the present thesis is very likely to have existed from birth due to the low maternal intake of B_{12} during pregnancy by their mothers and then further deteriorated due to non-meat-eating habits (Yajnik et al., 2005). Meat in expensive in developing countries and people from the middle class and lower income bracket in India cannot afford to have meat daily or more than three times a week (Antony, 2003). The girls in the present study were from middle-low by New Zealand income standards classes. They had a similar socio-economic status in India and therefore the mothers did not consume meat regularly and the same pattern is carried forward by the girls. Maintaining the same intake of meat in New Zealand meat-eating girls did not show high levels of vitamin B_{12}. There are emerging cases of greater prevalence of vitamin B_{12} deficiency compared to folate deficiency in India (Chandra et al., 2002; Gomber et al., 1998) and the same may occur in some groups in New Zealand as folate supplementation becomes mandatory.

Given, the high prevalence of B_{12} deficiency in vegetarians, girls born to vegetarian mothers are at a high risk of B_{12} deficiency and it is an intergenerational problem. A limited supply of vitamin B_{12} during pregnancy has important consequences for foetal growth. Foetus depends on the maternal nutrition for growth and development. The longer the mother has adhered to a vegetarian diet, the greater the likelihood that she will maintain a low serum and breast milk concentration of vitamin B_{12} that correlate with the B_{12} insufficiency in the infants (Bjorke Monsen et al., 2001). Maternal vitamin B_{12} status in pregnant mothers is an important factor in maintaining optimal total

homocysteine and B_{12} levels during pregnancy (Relton et al., 2005).Experiments with methylated diets in pregnant mammals (rats) have shown desired improvements in the off-spring. Maternal nutritional intakes of micronutrients (folate, iron and vitamin B12) have shown to have profound effects neonatal anthropometry.

Further, in India, children are breast fed for minimum two years (Antony, 2003) and all the children in this study were breastfed, it is highly likely that breast fed infants of vitamin B_{12} deficient mothers would continue to exhibit the same deficiency (Chandra et al., 2002). Thus, the nutritional deficiency is passed over to the infant through breast milk continuing the deficient state.

Another significant effect of vitamin B_{12} deficiency and increased MMA could be increased lipogenesis and insulin resistance due to increased concentration of methylmalonyl CoA (Allen et al., 1998). Malonyl CoA increases because of increased fuel intake of carbohydrate, protein and fat (Winder et al., 1990). In the present thesis, the reported energy intakes of the girls in both the groups were higher than the recommended values. This could be due to over reporting or over consumption. The non-meat eaters tended to report a slightly higher total energy intake with no difference in the energy obtained from carbohydrates. The total respiratory exchange ratio (a marker of fuel mix in the diet, RER) for both the groups was high (0.89) suggesting an increased carbohydrate oxidation and decreased fat oxidation. The non-meat eaters had a higher respiratory exchange ratio compared to meat-eaters. (p= 0.27, ES= 0.78).

4.1.3.2 Fibre

A non-meat-eating diet has more bulk due to a high fibre and water content. Fibre plays an important role in maintaining a healthy digestive system and serum lipid levels (Ballesteros et al., 2001; Bingham et al., 2003). Janelle and Barr (1995) reported a significant but a small difference of approximately 2.3g in the fibre consumption between Canadian non-vegetarians and lactoovovegetarians women (22.4g vs 24.7g, p<0.05). In the popular Oxford Vegetarian Study, Appleby et al., (1998), reported a significant difference of 6.2g in adult British non-meat-eating and meat eating women. The present thesis reported a significantly large difference in the daily fibre consumption between the non-meat-eaters and meat-eaters (30.4g versus 19.9g, p = 0.03, ES= 1.4).The increased lipids in the blood of the meat-eaters in the present thesis

could be associated with the significantly lower consumption of fibre (Ballesteros et al., 2001). This highlights the special nature of the non meat eating Indian population and that findings from other studies of dietary patterns in different cultures do not necessarily apply to the Indian dietary patterns of vegetarianism.

4.1.3.3 Iron

Anemia as characterized by decreased iron stores or decreased serum ferritin was not present in the participants in the following thesis. However it is noted that the non-meat eaters had lower haemoglobin and serum iron. This could be due to decreased absorption of non-haem iron at higher serum ferritin levels as explained by Roughead and Hunt (2000). Also the nutritional factors like phytate present in wheat bran, whole grains, legumes and lentils, polyphenols present in cereals could have reduced the absorption of non-haem iron in a non-meat-eating diet (Hunt, 2002). Hunt and Roughead (1999) observed 70% less absorption of non-haem iron in lactoovevegatarian diet compared to a non-vegetarian diet. Hunt (2002) attributed this increased absorption to unidentified enhancers present in meat, poultry and fish. Vegetarian diets also provide nutrients like; vitamin A and ascorbic acid that promote iron absorption, but the higher consumption of phytates in the vegan diet may counterbalance the effect (Hunt et al., 1999). The higher consumption of white meat in meat-eaters in this study could be a cause of concern, as it contains less total iron and vitamin B_{12} compared to red meat (Narayanan et al., 1991).

4.2 Diabetes and cardiovascular risk

In these cross-sectional measures made in a very young population there was no evidence of any diabetes (glucose and insulin measures) or cardiovascular risk. There was however a higher total cholesterol in the meat eaters which could suggest a protective effect of not eating meat. How this and the other risk factors measured track into adulthood is not known.

4.3 Body composition

In the present thesis, the girls were not overweight or obese in relation to body weight, but when compared with Caucasian counterparts for body fat percentage all but four were obese or overweight according to the classification of McCarthy et al., (2006) and ten of the twelve girls had a high body fat percentage. As a group adjusted for age these girls were 34 percentile points above the British reference range (ibid), which means they had elevated fatness. In spite of the fact that they had a normal BMI, all except two had body fat percentage above 50th percentile. Thus, high body fat percentage at a lower BMI is a peculiar characteristic of Asian Indians (Deurenberg et al., 2002), but the associated health risks remain to be determined.

Therefore, the girls appeared to be 'normal' with respect to obesity when body weight and BMI were considered, but on comparing the body fat percentage on the body fat reference curves suggested by McCarthy et al., (2006) two thirds of the girls could be classified as overweight and obese. Mckeigue et al., (1991), Shelgikar et al., (1991) and Deurenberg et al., (2002) have also shown that Asian adults have a lower BMI and higher body fat percentage compared to Caucasians. Deurenberg-Yap et al., (2000) further reported that, amongst Indians, Chinese and Malay in Singapore, Indians had the highest body fat percentage for the same BMI. The above findings do not undermine the use of BMI in predicting obesity. BMI is an important determinant of generalized obesity but cutoffs may vary by population.

Another striking feature of increasing adiposity in Asians is increased abdominal adiposity, marked by increased waist circumference. In the present thesis, waist circumference correlated positively with subscapular ($r = 0.89$, $p<0.001$) and triceps skinfold thickness ($r = 0.58$, $p<0.001$), this was similar to the finding of Maffeis et al., (2001). Increased waist circumference was also indicative of presence of subcutaneous adiposity (Goran & Gower, 1999). Waist circumference tended to correlate with diastolic blood pressure ($r = 0.53$, $p = 0.08$). Larger waist circumference and increased diastolic blood pressure are salient features of insulin resistance syndrome (Maffeis et al., 2001). Maffeis et al., (2001) showed significant correlations of waist circumference with systolic and diastolic blood pressure compared to correlations of triceps and subscapular skinfold thickness with blood pressure in preadolescent children. But, the

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present thesis showed a stronger association between triceps skinfold and diastolic blood pressure (r = 0.65, p = 0.02) than waist circumference.

Increased waist-to-hip ratio is an important feature of the insulin resistance syndrome and a correlate of hyperglycemia in Indians (Shelgikar et al., 1991). In the present thesis, waist-to-hip ratio did not correlate with fasting glucose, insulin, insulin resistance (HOMA B%) and insulin sensitivity (HOMA S%) but correlated positively with CRP-hs (r = 0.70, *p* = 0.03). which is similar to associations observed by Forouhi et al., (2001) in 113 healthy south Asian men. Also, waist circumference positively correlated with CRP-hs (r = 0.77, p = 0.004). similar association has been reported by Vikram et al., (2004) in postpubertal Indian adolescents aged 14 to 18y. Thus central adiposity related chronic inflammation is a feature of south Asians. Along with increased central obesity, there was decreasing physical fitness, which was apparent from the association between CRP-hs and heart rate (r = 00.60, p = 0.04), this correlation was similar to the findings reported by Cook et al., (2000).

Mckeigue et al., (1991) reported a positive association between waist-to-hip ratio and fasting serum triglycerides, the present thesis did not report a similar association but observed a positive association between waist circumference and fasting serum triglycerides (r = 0.72, p = 0.009). Further, similar to the findings by Forouhi et al., (2001) the present study also found a positive association between CRP-hs and serum triglycerides (r = 0.68, *p*<0.01). CRP-hs is a risk factor associated with features of insulin resistance syndrome (Festa et al., 2000).

With the increasing fat free mass along with the fat mass in obesity there is an increase in the upper body power (Steinbeck, 2001).The present thesis showed a correlation between fat free mass and grip strength of the right hand and left hand, (r = 0.83, p = 0.001) and (r = 0.87, p <0.001) respectively.

The ratio of subscapular triceps skinfold thickness an important feature of central adiposity (Goran et al., 1995) positively correlated with insulin and measure of insulin resistance HOMA B% and negatively with insulin sensitivity HOMA S% (Appendix 8, Table 8.3) serum triglycerides. Similar correlations between subscapular to triceps ratio and insulin resistance have been shown by Misra et al., (2004) in post pubertal Indian males aged 14 to 18y. Central adiposity measured using subscapular to triceps skinfold ratio has been shown to be to associated to adverse concentrations of triglyceride and insulin independent of age, sex, race, weight and height in children aged 5-17y by. Freedman et al., (1999) These correlations signify the presence of symptoms of insulin resistance at a young age.

Further, serum folate tended to correlate positively with waist-to-hip ratio (r = 0.53, p =0.08), this can be explained using the findings reported by Rao et al., (2001), they observed a positive association between the maternal intake of green leafy vegetables and neonatal abdominal circumference. As explained earlier (ibid), the positive association of green leafy vegetables and milk with different neonatal anthropometric measurements explains the significance of various micronutrients on foetal development. The NFHS-2 (1998-99) (International Institute of population sciences and ORC Macro, 2000) reported higher intake of green leafy vegetables compared to milk and milk products in Indian women aged 15-49y.

4.4 Energy expenditure

4.4.1 Resting energy expenditure

REE is a major component of total energy expenditure. As the present thesis does not report the first level validation technique of doubly labeled water to measure total energy expenditure, it was not possible to directly determine the contribution of REE to total energy expenditure. The non-meat eaters had a higher REE compared to the meat-eaters (p = 0.29, ES = 0.63), subsequently they also had a higher energy intake compared to meat-eaters (p = 0.37, ES= 0.56). As reported by Schutz et al., (1999) in obese prepubertal children, REE increases with increase in fat free mass and fat mass the present thesis did not find a clear association. Heavier children did have a higher

metabolic rate (r= 0.489, p=0.11) but FFM and FM did not predict the REE better than body weight. Apart from the small sample size the variance of the bioimpedance measurement could have obscured any relationship.

The non-meat-eating girls had a higher resting energy expenditure and respiratory exchange ratio compared to meat-eating girls (ES = 0.63, p = 0.30) and (ES= 0.78, p = 0.21). The increased respiratory exchange ratio in both the groups indicated higher carbohydrate oxidation and decreased fat oxidation. This finding is similar to the those reported by Rueda-Maza et al., (1996), in obese prepubertal children in whom, oxidation of dietary carbohydrate was greater than the oxidation of endogenous carbohydrate (glycogen stores in liver and muscles). Maffeis et al., (1999)showed a direct correlation between exogenous fat oxidation and fat mass by using sophisticated techniques like ingestion of mixed diet enriched with 13_{Co2}, the present thesis did not use any such technique and therefore it was not possible to establish correlations between indices of obesity (fat mass and body fat percentage) and oxidation of carbohydrate, protein and fat. But the increased carbohydrate oxidation in both the groups in the present thesis was suggestive of suppressed fat oxidation. This could lead to increased deposition of fat in the form of fatty acids and triglycerides as explained by Ruderman et al., (1999) in section 1.1.6.

4.4.2 Energy expenditure in physical activity

Both the groups in the present thesis reported very little time devoted to moderate to intense physical activity. Girls in both the groups were involved in moving activities for only three hours a day and the rest of 21 hours of the day were spent in sedentary activities. Decreased physical activity increases malonyl-CoA concentrations in the skeletal muscle. Increased malonyl-CoA suppresses fatty acid oxidation and lipogenesis occurs (Dean et al., 2000; Pan et al., 1997). Therefore, the decreased physical activity and the poor protein intake in these girls would support poor muscle development and lead to the progression of increased body fatness and insulin resistance. Non-meat eaters are at a greater risks due to the accumulation of malonyl-CoA as a result of vitamin B_{12} deficiency (Allen et al., 1998).

Physical activity or exercise also has the effect of improving muscle function and driving an increase the muscle mass. Increased muscle mass requires an increased influx of protein through increased amino acids from the diet (Wolfe, 2006).

In the present thesis, girls in both the groups did not have a particularly high protein intake and the plant proteins may be less digestible Millward and Jackson, (2003) resulting in lack of some of the essential amino acids (Young et al., 1994). Participation in constructed physical activity such as sports was not very popular in this study group. Cultural attitudes and values in the Indian people does not encourage participation in sports and physical activity (Hayes et al., 2002).

Only one of the twelve participants had organized sport outside school (swimming). Amongst children in Indian cities, Pathmanathan and Prakash (1994) went on to observe a lack of physical activity in 589 boys and 566 girls aged 6-16y, in relation to time spent in mandatory physical activity like sports and games. The limited time dedicated to physical activity was attributed to the increased burden of studying and passive entertainment through media. Others (Ramachandran et al., 2003) have reported reduced physical activity in children with type 2 diabetes and age of diagnosis was <15 years, out of the 18 children, 17 of them had a sedentary lifestyle, 50% had ≥ 120% of ideal body weight and 12 out of 13 children had increased WHR.

Various factors that influence childhood physical activity include gender, ethnicity, and parental activity. Goran et al., (1998) observed a 50% reduction in physical activity as girls reached prepuberty and attributed the change to energy conserving mechanisms prior to puberty. The present thesis did not include information on parental or past activity, but with the observed low levels of physical activity in the girls monitoring the parental physical activity to establish a correlation could be helpful in the future. Further, in relation to ethnic differences, studies by Bhopal et al., (1999) and Hayes et al., (2002) have reported significantly less physical activity in South Asians compared to Europeans in Britain. Women in both studies were less active than men and it is likely to follow that girls are less active than boys. It is reported that obese children are physically inactive (Brown et al., 2001; Trost et al., 2001). Therefore girls such as in this study reaching puberty and with increased body fatness are likely to become less active. Any intervention study should look at maintaining or increasing and sustaining activity in young girls.

Figure 4.1: Interrelations

DNA and RNA synthesis. Purines and thymidylate synthesis

5, 10- methylene- THF

5- methyl THF

THF

Homocysteine

MS

B12

B_6 CβS

Cysteine

SAH

Sulfate (excreted in urine)

R–CH3 R

DNA hypomethylation & altered gene express

Folic acid supplement

Vegetarian diet

Folate

Protein

Methionine

SAM

MMA

Succinyl- CoA

MCM B_{12} b

MMA-CoA

Propionyl-CoA

Other amino acids

Decreased net protein utilisation

β-oxidation

a

CPT1

Fatty acids + Glucose

c

Lipogenesis Insulin Resistance

c

c

❌ Pathway blocked due to vitamin B_{12} deficiency

I Pathway secondarily inhibited

→ Pathways which are stimulated

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4.5 Interrelations

The pattern (Figure 4.1) of metabolism investigated factors associated with the Indian dietary pattern (non-meat-eating and meat-eating habits) and B_{12} deficiency that may result in long-term risk for chronic disease. These factors were glucose, insulin, lipids, substrate metabolism, body fatness and poor muscle mass. The present thesis did not show hyperglycemia, hyperinsulinemia or hyperlipidaemia but the increased RER was indicative of increased carbohydrate oxidation and decreased fat oxidation (Step a, Figure 4.1). This was in line with the reported diet which was high in carbohydrate and fat and relatively low in protein. Overall the energy intake of the girls was high in relation to their resting energy expenditure. The model (Step a, Figure 4.1) of decreased fat oxidation long term could be associated with increased fat deposition (lipogenesis). This may explain why Indian babies who are born thin have reduced muscle mass but increased body fat. This fat appears to be preferentially deposited in the subscapular area – a marker of increased central fat (Yajnik et al., 2003).. The waist-to-hip ratio of the girls in the present thesis was on the higher level of normality.

Body composition is mediated by the relationship between energy intake and energy expenditure. Low energy expenditure as reported by the study group is also associated with decreased fatty acid oxidation (through increase in Malonyl-CoA) and increased body fat percentage (Step b, Figure 4.1). Methylmalonic acid was also raised in association with low dietary and serum vitamin B_{12}. B_{12} plays a role in maintaining the homocysteine levels (even in the presence of adequate folate) and the methylation processes association DNA and RNA synthesis and expression.

The poor protein intake reported in the study group is also of concern as at critical periods of growth in particular decreased formation of muscle mass could have life-long implications (Step c, Figure 4.1). This effect could result in a positive but debilitating feedback loop. Poor muscle mass could have also affected the drive and ability to participate in physical activity- to keep up. Muscle also has a vital role in the insulin uptake and glucose homeostasis (Step c, Figure 4.1). Although overt hyperinsulinemia was absent in this group, it cannot be assumed that it might not be a problem in the future. The girls were at a developing stage and could be progressing towards insulin resistance due to their lifestyle pattern particularly dietary and activity habits.

4.6 Limitations

The small sample size is the major limitation of the present study. Firstly, the small number increases the likelihood of Type I and Type II errors in determining the associations and finding differences between the non-meat-eaters and meat-eaters. As this was a cross-sectional study and most measurements were made only once in a short time frame intraindividual variation is not known. Secondly, time and financial constraints limited the number of subjects to be recruited and the number of interactions for this intensive pilot study. The number and measurements of blood biomarkers was limited by expense and also by the amount of blood required. Lastly, recruiting Indian prepubertal girls was difficult because sometimes the mother's felt they were too young for a blood test or the girls themselves were not keen. None of the girls had ever had a blood test previously. Often both parents were working so it was difficult to arrange convenient visits. Some of the difficulties were reduced as the researcher (PC) spoke Hindi, had a degree in dietetics from India and was able to appropriately and culturally interact with the family.

Reporting of diet was subject to many errors including over or under-representing a portion size by the girls or their mother or over representation by me when entering the data for analysis. Similarly, reporting of physical activity is likely to be over reported.

4.7 Recommendations

This study has piloted a number of tools in this subset of the New Zealand Indian population. They were found to be acceptable and both parents and children reported general satisfaction with the study and appreciated the information that they received in return. This has paved the way for a longitudinal study in association with a dietary intervention whose main target would be in the short term to increase dietary and therefore serum vitamin B_{12} which will in-turn, change other biomarkers such as MMA. Secondary and longer term goals would be to measure changes in mental and psychometric performance, physical fitness, diabetes and cardiovascular risk factors and body composition.

Other recommendations include:

1. Homocysteine could be measured along with MMA to understand the role of vitamin B_{12} in maintaining the homocysteine concentration in presence of adequate folate

2. Longitudinal studies in association with physical activity intervention to track the changes in body fatness, fitness and muscle strength.

3. Interventions for the whole community to reduce obesity can include creating a supportive environment to promote physical activity. Some dietary modifications in relation to low-fat milk consumption, quantity and quality of oil consumption, promoting consumption of wholemeal bread and cereals. Girls and women should be educated in good nutritional habits tailored according to their cultural practices.

4. From the perspective of health objectives outlined by Ministry of Health (2001) for the New Zealand population, it is important to reduce obesity, improve nutrition and promote physical activity in the Indian children. Today's' children are budding to be tomorrows' adults therefore it is important to safeguard their health at this stage.

5. Ensure that the community has an easy access to nutritious food.

6. From limited experience of the researcher (PC) in New Zealand, it was observed that, it is not a common practice amongst Indians to be registered with a general practice. It should be ensured that registering with a general practice is emphasized in this community, as it will provide appropriate preventive health care.

4.8 Conclusions

This study is unique in New Zealand because it looks at a dietary pattern and its relationships to body composition, blood biochemistry, and other physiological aspects measured in the study in a group of Indian girls whose mothers and their mothers have followed the same dietary pattern.

In twelve girls, selected by ethnicity, tanner stage and dietary pattern there was a high average fatness which from their height and weight was not expected. The converse of this is that there is a relatively low lean mass which has implications for physical fitness.

The most significant finding is that five of the six non-meat-eating were either borderline or deficient (n=2) in vitamin B_{12}. While there were clear differences between meat-eating and non-meat eating profile the whole group was skewed towards low B_{12} and high MMA.

Dietary information supports a low B_{12} intake especially in the non-meat-eating girls. The current dietary and physical activity information demonstrate a diet that could be low in protein and high in carbohydrate and a pattern of reduced energy expenditure. The apparently low protein intake in the non-meat-eaters is a cause of concern, since it may compromise muscle quality, physical fitness and growth particularly at critical growth periods such as adolescence. There are also concerns about cognitive impairment with low B_{12} status in the presence of high folate. Given that this imbalance in one carbon metabolism has likely been the situation since conception long term adverse metabolic consequences are likely.

The present attention to folate supplementation by policy makers needs to be moderated by considerations of interactions with the status of other micronutrients. While the New Zealand Indian population is small and the vegetarianism practiced is not the norm in New Zealand it should not be ignored. Vitamin B_{12} monitoring, dietary recommendations and if necessary supplementation in New Zealand Indian people should be considered and where possible intervention before pregnancy (as for folate) be a priority.

It is time for serious action in this area of health so that the risk accumulated through an imbalance in nutrition and physical activity is reduced and the health of those as yet unborn is improved.

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APPENDICES

Appendix 1: Ethics approval

MEMORANDUM

To: Elaine Rush
From: Madeline Banda Executive Secretary, AUTEC

Date: 7 April 2006
Subject: Ethics Application Number 06/36 **Effects of diet on future health:**
            interrelationships among diet, energy expenditure, body composition
            and risk factors for lifestyle disease in Indian pre-adolescents.

Dear Elaine

Thank you for providing written evidence as requested. I am pleased to advise that it satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC) at their meeting on 13 March 2006. Your ethics application is now approved for a period of three years until 7 April 2009.

I advise that as part of the ethics approval process, you are required to submit to AUTEC the following:

- A brief annual progress report indicating compliance with the ethical approval given using form EA2, which is available online through *http://www.aut.ac.nz/research/ethics*, including a request for extension of the approval if the project will not be completed by the above expiry date;

- A brief report on the status of the project using form EA3, which is available online through *http://www.aut.ac.nz/research/ethics*. This report is to be submitted either when the approval expires on 7 April 2009 or on completion of the project, whichever comes sooner;

You are reminded that, as applicant, you are responsible for ensuring that any research undertaken under this approval is carried out within the parameters approved for your application. Any change to the research outside the parameters of this approval must be submitted to AUTEC for approval before that change is implemented.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this. Also, should your research be undertaken within a jurisdiction outside New Zealand, you will need to make the arrangements necessary to meet the legal and ethical requirements that apply within that jurisdiction.

To enable us to provide you with efficient service, we ask that you use the application number and study title in all written and verbal correspondence with us. Should you have any further enquiries regarding this matter, you are welcome to contact Charles

Grinter, Ethics Coordinator, by email at charles.grinter@aut.ac.nz or by telephone on 921 9999 at extension 8860.

On behalf of the Committee and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Madeline Banda

Executive Secretary

Auckland University of Technology Ethics Committee

Cc: Purvi Chhichhia drb3870@aut.ac.nz

Effects of diet on future health

Why are we doing this research?

Throughout the lifecycle future health is determined by what is eaten, how much physical activity is done and the environment we live in. In New Zealand and throughout the world Indian people are at relatively high risk of diabetes and cardiovascular disease in later life. Adolescence is a critical growth period and in girls determines the health of their future children.

By participating in this study you will help us understand more about the different effects of diet and activity on growth.

Who is in this study?

We will invite 12 Indian girls aged 9-11 years who have not yet undergone any changes of adolescence to participate. Six who eat a vegetarian diet only and six who eat meat at least three times a week.

What is involved?

You will be visited three times over a period of two weeks should you agree to take part in this project. The first visit will take about an hour and the last two about half an hour each. At the first visit you and your child will be shown how to measure and record what your child eats and drinks. We will also ask you questions about your child's birth and childhood growth, general eating habits and activity levels and leave you some diaries about food and activity to fill in. During this **first visit** the interviewer will measure and weigh your child and either collect a urine sample from your child or leave a container for this. You will also be given a form to go (at your convenience) to the local laboratory for your child to have a blood test that will look at the levels of iron and fats in your child's blood. The time for the **second visit** will be arranged with you. At the second visit your child will drink a specially prepared drink. This drink contains water that has been specially distilled to increase the concentrations of oxygen-18 and deuterium. This water is being used in accordance with internationally recognised procedures and will give a measure of how much energy your child uses over the next 14 days. The air your child breathes out gives a measure of the amount of energy used at rest. The child will lie on the couch and will listen to music or read a book. Fresh air

comes into a mask placed over your child's nose and mouth and the air leaving the mask is analysed by another interviewer. Measurements of your child's body size and blood pressure will also be made and the interviewer will return to collect a urine sample 5 hours after the water was drunk. Over the next days the interviewer will ring and remind you about collecting more urine samples and will visit again 7 days after the last visit. At this **third visit** more questions about diet and activity will be asked and the questionnaires you have filled in collected. Another urine sample will be collected. The **fourth** visit will be a week later another urine sample will be collected and the diet and activity analysis discussed with you.

Do I have to take part in this survey?

Your participation and the participation of your child is **entirely voluntary**. If you agree to take part in the study, you are free to withdraw at any time and this will not disadvantage you in any way or affect the future health care of your child. You do not have to answer all the questions and you may stop the interview at any time. It will not cost you anything to take part in this study.

What will happen to the results?

The information about your child and family is completely confidential. No information which could identify you will be used in any reports on this study. The results will be stored by a code number in a computer at the Auckland University of Technology. The questionnaires will be stored in a locked room. They will be stored for 10 years and then destroyed. You will receive a copy of the analysis of your child's diet, blood, body measurements and energy use as they become available.**……..If you have any Questions?** If you have any questions about our project, either now or in the future, please feel free to call us at AUT.

Contact numbers

Elaine Rush 921 9999 x 8091

Elaine.rush@aut.ac.nz

Purvi Chhichhia 9219999 × 7119

 Drb3970@aut.ac.nz

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, *madeline.banda@aut.ac.nz* , 921 9999 ext 8044.

This study has received ethical approval from the Auckland University of Technology Ethic Committee

Appendix 3: Description of Tanner stage and procedures.

TANNER STAGE

Tanner stage 1 Preadolescent

 Small elevated nipple with no significant underlying breast tissue.

no pubic hair except for a fine "peach fuzz" of body hair
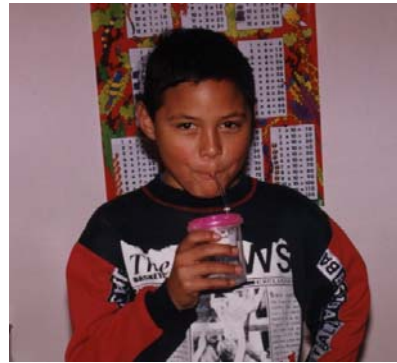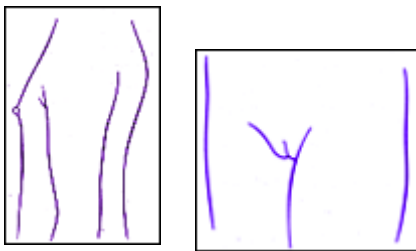
Drinking the

doubly labeled

water

Measurement of resting metabolic rate by analysis of expired air.

Appendix 4: Consent and assent forms.

CONSENT FORM

Parent/guardian consent to Participation in Research

Title of Project:

Effects of diet on future health: (Interrelationships among diet, energy expenditure, body composition and risk factors for lifestyle disease in Indian pre adolescents).

Project Supervisor: Professor Elaine Rush

Researchers:Purvi Chhichhia, Christine Nabiryo, Jennifer Crowley

- I have had an opportunity to ask questions and to have them answered.
- I understand that I may withdraw my child or any information that he/she has provided   for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- I agree for my child to take part in this research.
- I wish to receive a copy of the report from the research: tick one: Yes   O   No   O

Participant signature: ....……………………..

Participant name: …………………………………………………………….

Participant Contact Details (if appropriate):

Date:

This study has been approved by the Auckland University of Technology Ethics Committee on

 7 April 2006 AUTEC Reference number 06/36

CHILD ASSENT FORM

Title of Project

Effects of diet on future health: (Interrelationships among diet, energy expenditure, body composition and risk factors for lifestyle disease in Indian pre adolescents).

Project Team: Purvi Chhichhia and Professor Elaine Rush

1.1 Statement of Assent

I have read and understood the information provided about this research project. I have talked about what will happen, been shown pictures and examples of what I need to do and understand that

- I will be visited 4 times

- I will be asked to breathe into a special mask so that how much air I am using can be measured

- I will write down with the help of my parents the food I eat over a week and also how much I am moving

- I will be taken by my parents to a clinic to have a small sample of blood taken from my arm

- I will be asked to provide urine samples over a period of two weeks.

- I have had the opportunity to ask questions and to have them answered.

- I understand that I may withdraw from the study at any time without being disadvantaged in any way.

- I agree for the confidential information collected in this study to be used by AUT for research purposes, with the understanding that my name will not be seen on any reports or records.

Name of Child

Signature of Child

Date

Name of Parent or Primary Caregiver or

Signature of Parent or Primary Caregiver

Date

 This study has been approved by the Auckland University of Technology Ethics

Committee on

 7 April 2006 AUTEC Reference number 06/36

Appendix 5: Diet diary

Please start each day on a new page

Day   Date

Time	Type of Food/Drink	Amount	Time	Type of Food/Drink	Amount

Appendix 6 : Personal communication with Crop and Food Institute

"Elaine Rush" <elaine.rush@aut.ac.nz**> 28/09/2006 12:35 p.m. >>>**

Dear Julian

I am confused about the relationship between the food composition tables for cereals and what is actually in them. Sanitarium assure me that no B12 is added to their skippy cornflakes and therefore there should be none there - unless there is contamination by microorganisms. But consistently for all cornflakes there is Vitamin B12 at a rate of 2 microgram per 100g - the same for rice bubbles.

There is no claim on the food label for presence or addition of B12.

My particular concern is that I am involved with a small study of vegetarian versus non vegetarian young girls and according to the food diary analysis including cereal there is not a shortage of B12 in the diet - and apparently the parents of these children believe that there is B12 in these cereals. One third of the vegetarians are vitamin B12 deficient-from blood analysis!

 Please explain why this anomaly?

Many thanks

 Elaine

  Elaine Rush PhD

Professor of Nutrition

Faculty of Health and Environmental Science

Auckland University of Technology

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90 Akoranga Drive

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64 9 921 9999 x 8091

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Cell phone 64 21 624 077

Hi Elaine

I discussed this with Jason McLaughlin, and he gives the following explanation -

The revision of B12 in cereal chapter of the database was on my to do list for FF2006 (that is Food files 2006 will have correct data, I presume - Julian, see below). Unfortunately, I noted the data was incorrect before printing the Concise tables, but obviously didn't get around to changing the values before the file went to print. I believe Elaine will be using the FF2004 dataset which will also have incorrect values for the breakfast cereals.

To explain to Elaine. The current obsolete database management system doesn't record who or why the data was entered, however the new system will document this information. We have spoken with cereal manufacturers to confirm the fact that no breakfast cereals in NZ are fortified with B12. So I can't explain why the data was entered into the NZFCD. However, we are about to release FOODfiles 2006, which will have this data corrected. It was identified through data quality checks as needing correction.

We will notify users of the anomaly in the Concise Tables by applying a small sticker to alert users of the errors. Also, CFR have developed this website on the NZFCD,

   http://www.crop.cri.nz/home/products-services/nutrition/foodcompdata/fcd-information/fcd-errors.jsp. Which lists errors such as this.   In the next few months we will be promoting this website to dietitians, so that they can be aware of such issues/comment on the database etc.

Elaine - hopes this helps, but give Jason a cal if you need further explanation..

Regards

Julian

 Julian Lee PhD

Team Leader Nutrition and Health

Crop & Food Research

Food Industry Science Centre

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Email leejx@crop.cri.nz

ACTIVITY DIARY

Name/ID number

Day   **Date**

Write in the empty squares the
factor which corresponds best
to the main activity of each
15 min period. If not sure
write in the empty squares a
alphabetical code and write this
code together with a description
of your activity in a few words in
the section for notes below.

Notes

	minutes			
Hour	**0-15**	**16-30**	**31-45**	**46-60**
midnight				
1:00 a.m.				
2:00 a.m.				
3:00 a.m.				
4:00 a.m.				
5:00 a.m.				
6:00 a.m.				
7:00 a.m.				
8:00 a.m.				
9:00 a.m.				
10:00 a.m.				
11:00 a.m.				
12:00 a.m.				
1:00 p.m.				
2:00 p.m.				
3:00 p.m.				
4:00 p.m.				
5:00 p.m.				
6:00 p.m.				
7:00 p.m.				
8:00 p.m.				
9:00 p.m.				
10:00 p.m.				
11:00 p.m.				

Activity Factor	**Examples of Activities**
1	Sleeping, resting in bed
2	Sitting, eating, writing, listening, sitting in a car or bus, watching TV etc
3	Standing, washing
4	Walking indoors, light home activities
5	Walking outdoors, light work - e.g. carrying small bag
6	Leisure activities, sports, running and relaxed movement i.e. light intensity
7	Leisure activities, sports and manual work of moderate intensity
8	Leisure activities, sports and manual work of high intensity - sweating and breathing hard
9	Sports activities and work of very high to maximal intensity, competitive running

Appendix 8 :Correlations

Table 8.1: Selected significant correlations of subscapular skinfold thickness

Parameter	Pearson's correlation coefficient (r)	p value
Fat free mass (kg)	0.90	<0.001
Fat Mass (kg)	0.81	0.001
Left hand grip strength (kg)	0.83	0.001
Right hand grip strength (kg)	0.70	0.01
Fasting blood triglyceride (mmol/L	0.84	0.001
Waist circumference (cm)	0.89	<0.001
Hip circumference (cm)	0.70	0.001
BMI (kg/m^{-2})	0.92	0.02
Total/HDL ratio	0.63	0.03

Pearson's correlation co-effecient r calculated using bivariate correlation; correlations signficant at p<0.05; BMI, body mass index

Table 8.2: Selected significant correlations of triceps skinfold thickness

Parameter	Pearson's correlation coefficient (r)	p value
Fat mass (kg)	0.90	<0.001
Fat free mass (kg)	0.67	0.01
MUAC (cm)	0.93	<0.001
Body fat %	0.78	0.003
Waist circumference (cm)	0.85	<0.001
Hip circumference (cm)	0.73	0.007
BMI	0.91	<0.001
Diastolic blood pressure (mmHg)	0.62	0.03

Pearson's correlation co-effecient r calculated using bivariate correlation; correlations signficant at p<0.05; BMI, body mass index; MUAC, mid upper arm circumference

Table 8.3: Selected significant correlations of subscapular to triceps skinfold ratio

Parameter	Pearson's correlation coefficient (r)	p value
Insulin (pmol/L)	0.82	0.001
HOMA S%	-0.77	0.004
HOMA B%	0.80	0.002
Serum triglycerides (mmol/L)	0.69	0.01
Left hand grip strength (kg)	0.71	0.01
Right hand grip strength (kg)	0.74	0.006
AMA (cm^2)	0.62	0.03

Pearson's correlation co-effecient r calculated using bivariate correlation; correlations signficant at p<0.05; HOMAB%, Homeostasis model assessment β cell function; HOMA S%, Homeostasis model assessment; , AMA, Upper arm Muscle Area

Table 8.4: Selected significant correlations of BMI

Parameter	Pearson's correlation coefficient (r)	p value
MUAC (cm)	0.94	<0.001
Total/HDL ratio	0.60	0.03
TAA (cm^2)	0.93	<0.001
AFA (cm^2)	0.91	<0.001
Serum triglycerides (mmol/L)	0.75	0.005
Fat mass (kg)	0.94	<0.001
Fat free mass (kg)	0.87	0.01
Subscapular skinfold thickness (mm)	0.93	<0.001
Triceps skinfold (mm)	0.91	<0.001
Waist circumference (mm)	0.97	<0.001

Pearson's correlation co-efficient r calculated using bivariate correlation; correlations signficant at p<0.05; TAA, Total upper arm area; AFA, Upper arm fat Area

Table 8.5: Selected significant correlations of grip strength

Parameter	Pearson's correlation coefficient (r)	p value
Left hand (kg)		
Serum triglycerides (mmol/L)	0.87	<0.001
MUAC (cm)	0.62	0.03
TAA (cm^2)	0.61	0.03
WHR	0.57	0.05
BMI (kg/m^2)	0.72	0.008
LDL cholesterol (mmol/L)	0.61	0.03
Protein energy percentage	0.61	0.03
Fat free mass (kg)	0.87	<0.001
Right Hand (kg)		
WHR	0.59	0.04
Serum triglycerides (mmol/L)	0.76	0.004
Fat free mass (kg)	0.83	0.001

Pearson's correlation co-efficient r calculated using bivariate correlation; correlations signficant at p<0.05; MUAC, Mid Upper Arm Circumference; TAA, Total upper arm area; WHR, Waist to Hip Ratio;BMI, Body mass index.

Table 8.6: Selected significant correlations among other variables.

Parameter 1	Parameter 2	Pearson's correlation coefficient (r)	p value
Leg length (cm)	MUAC (cm)	0.64	0.02
Leg length (cm)	TAA (cm^2)	0.63	0.02
Energy consumption (MJ/day)	AMA (cm^2)	-0.64	0.03
AFA (cm^2)	Waist circumference (cm)	0.86	<0.001
CRP-hs (mg/L)	Waist –to- hip ratio	0.70	0.03
TAA (cm^2)	Serum triglycerides (mmol/L)	0.60	0.04
Serum triglycerides (mmol/L)	Fat mass (kg)	0.62	0.03
Serum triglycerides (mmol/L)	Fat free mass (kg)	0.72	0.009
Waist circumference (cm)	CRP-hs (mg/L)	0.77	0.004
CRP-hs (mg/L)	Serum triglyceride (mmol/L)	0.68	0.01
Waist circumference (cm)	Diastolic blood pressure (mmHg)	0.53	0.08
Waist circumference (cm)	Serum triglycerides (mmol/L)	0.72	0.009
CRP-hs (mg/L)	BF%	0.75	0.005
CRP-hs (mg/L)	Heart rate (bpm)	0.75	0.005
CRP-hs (mg/L)	Hip circumference (cm)	0.60	0.04

Pearson's correlation co-effecient r calculated using bivariate correlation; correlations signficant at p<0.05; BF%, body fat percentage; TAA, Total upper arm area; AFA, upper arm fat area; AMA, upper arm muscle area; CRP-hs, C-reactive protein high sensitivty;