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# The Effects of Genes, Environment and Ethnicity on Insulin Resistance and Obesity in Aboriginal and Non-Aboriginal South American Populations

by

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# Abstract

The worldwide prevalence of type 2 diabetes (T2D) has increased dramatically over the past half-century and is continuing to rise at a rapid rate, along with increasing levels of obesity. These changes are having a profound effect on healthcare planning and provision in many countries. Strong environmental effects in T2D risk are clear from longitudinal studies. In addition, groups with traditional lifestyles who migrate to a more 'Westernised' environment and lifestyle suffer increased diabetes prevalence. Environmental factors, however, do not seem to explain all of the variance in type 2 diabetes prevalence, nor all the variance in response to intervention studies.

Offspring of patients with type 2 diabetes have about a three-fold higher risk of developing the disease than those with no diabetes family history. Diabetes prevalence also differs between ethnic groups within countries. South Asian populations living in the UK and US have approximately 4-6 times the risk of developing diabetes compared to those of European descent. This effect may also be evident in other Native American populations such as the Mapuche in Chile. Mapuche populations living a traditional rural lifestyle appear to be relatively protected, on limited data, against diabetes risk (prevalence of ~1% to ~4%; but this risk increases markedly in the urban environment (6.2 to 8.2%). These observations suggest that genetic predisposition is also a factor in determining diabetes risk, but this is complicated by gene-environment interactions, where individuals with different genotypes respond differently in different environments.

The overall aim of this study was to investigate the interplay between genetic and environmental influences on predisposition to T2D and on related physiological traits in the Chilean population. The focus was on particularly indigenous populations that show increased risk of obesity, diabetes and related cardio-metabolic phenotypes when exposed to a 'Westernised' environment, and to characterise the relative contributions of individual genes, and environment and gene-environment interactions to this risk. In doing so, this investigation also allowed comparison between indigenous and immigrant populations that display different risks in different environments. These findings will hopefully add to existing efforts aimed at

finding appropriate treatments and effective preventative programmes, with concomitant improvements in public health in Chile.

In order to explore how environment and genes interact in the development of obesity and cardiometabolic disease, this study focuses on indigenous populations that show increased risk of obesity, diabetes and related cardio-metabolic phenotypes when exposed to a 'Westernised' environment. For this study four groups of participants were recruited: Mapuches (M) living in Rural (MR) and Urban environments (MU), and Chileans of European descent (E) living in Rural (ER) and Urban environments (EU). Detailed characterisation of body composition, fitness, physical activity patterns, nutritional factors, socio-economic, genetics and metabolic factors in these four groups was performed.

The main findings from this thesis were: (a) urbanisation, adiposity, physical activity and sedentary behaviours influence insulin resistance to a greater extent in Chilean Mapuches than in Chileans of European descent. These associations persist after adjustment for a comprehensive range of potential confounding factors; (b) *FTO* genotype influences insulin resistance in the Mapuche but no European Chilean population. These associations persist after adjustment for a comprehensive range of potential confounding factors; (c) *FTO* influences obesity-related traits in the Chilean population. However, these relationships were not all independent of lifestyle factors and these factors should therefore be taken into account in future research studies, which try to address the real genetic effect of *FTO* and its contribution to obesity; (d) that physical activity plays a key role in modulating the genetic predisposition to obesity and insulin resistance. This observation has important public health implications, because the present data indicate that being physically active can overcome, at least in part, the genetic predisposition to obesity induced by variation in the *FTO* gene. However, further studies into the mechanisms underpinning this effect are needed. This has potential implications both on the design as well as on the implementation of lifestyle strategies to reduce metabolic risk in different ethnic groups, and for advancing the basic understanding of the mechanisms behind human obesity and insulin resistance.

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# List of Abbreviations

| | |
|---|---|
| ALT | Alanine Aminotransferase |
| BF | Body Fat |
| BMI | Body Mass Index |
| CHO | Dietary Carbohydrates intake |
| CVD | Cardiovascular Disease |
| *FTO* | Fat Mass Obesity-associated gene |
| GGT | Gamma Glutamyl-tranferase |
| GWAS | Genome Wide Association studies |
| HDL | High Density Lipoprotein |
| $HOMA_{IR}$ | Homeostasis Model Assessment of Insulin Resistance |
| HR | Heart Rate |
| HRmax | Maximal Heart Rate |
| hsCRP | High sensitivity C Reactive Protein |
| Kcal | Dietary Energy Density Intake – kilo calories |
| LDL | Low Density Lipoprotein |
| MVPA | Moderate to Vigorous Physical Activity |
| PA | Physical Activity |
| SES | Socio-economic Status |
| T2D | Type 2 diabetes mellitus |
| TG | Triglyceride |
| $VO_2$ | Oxygen Uptake |
| $VO_{2max}$ | Maximal Oxygen Uptake |

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# Author's declaration

Unless otherwise indicated by the acknowledgment or reference to published literature, the present work in this thesis is the author's own and has not been submitted for a degree at another institution.

**Carlos Alberto Celis Morales** _____ **Date**_____

The findings of some of the studies have been published as follows:

## Publications (in press)

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# 1 Introduction and Literature Review

This chapter aims to provide the relevant scientific background to the studies in this thesis; additionally it aims to establish the theoretical basis for these studies. This chapter begins with an overview of cardiovascular disease, obesity, type 2 diabetes (T2D) and the public health impact of these conditions worldwide and also with a specific overview on population of different ethnic backgrounds. Furthermore, there is a more detailed consideration of the potential contributing factors of obesity and T2D which gives an insight into the lifestyle aspect as well as genetic components of these conditions. Finally the potential role of genes and environment interaction with the environment in the development of obesity and T2D is explored and explained.

## 1.1 Cardiovascular Disease: Worldwide Overview

Over the last half century, cardiovascular disease (CVD) has shifted from a relatively inconsequential disease worldwide to a leading cause of morbidity and mortality. At the beginning of the 21st century, an estimated 16.7 million per year – or 29.2% of total global deaths – result from the various forms of cardiovascular disease (CVD). By 2030, almost 23.6 million people per year will die from CVDs, mainly from heart disease and stroke. These are projected to remain the single leading causes of death and will surpass infectious disease as the world's leading cause of death and disability (WHO 2004c; WHO 2006a; WHO 2011). A more specific picture of this dramatic epidemiological change shows us that of the 16.7 million deaths from CVDs every year, 7.2 million are due to ischaemic heart disease, 5.5 million to cerebrovascular disease, and an additional 3.9 million to hypertensive and other heart conditions. At least 20 million people survive heart attacks and strokes every year, a significant proportion of them requiring costly clinical care, which puts a huge burden on long-term care resources. CVD affects people in their mid-life years, undermining the socioeconomic development, not only of affected individuals, but families and nations. Lower socioeconomic groups generally have a greater

prevalence of risk factors, diseases and mortality in developed countries. Different factors have been identified as the major driving force behind this substantial shift. Some of major contributors of this epidemiological shift are the increase in longevity, as a result of improvements in public health and medical care that are reducing rates of communicable disease, malnutrition, and maternal and infant deaths. The National Health and Nutrition Examination Survey (NHANES III) indicated that the T2D is strongly related to age, and more specifically, people over 45 years show 4-times higher risk of developing diabetes than those of less than 45 years. In addition, patients older than 60 years appear to make up 40% of the diabetic population in the United States. Older diabetics also carry a disproportionate burden from microvascular complications, presumably related to longer duration of diabetes (Alexander et al. 2003b). A second factor, in which we has been focus our attention in this thesis, is the economic, social, cultural and lifestyle changes that have led to increases in risk factors for CVD (WHO 2006a; WHO 2011).

Cardiovascular disease has typically been viewed as an affliction of wealthy, industrialized societies. In fact, during the past century minimal if any effort aimed at CVD prevention has been allocated to developing countries. This, in part, reflected the higher prevalence of infectious diseases that provided the rationale for not investing time and resources toward chronic diseases. However, these have changed during the last decades due to the emerging body of data, which shows that approximately 80% of the 17 million CVD deaths worldwide in 2003 – took place in developing, low and middle-income countries, while these countries also accounted for 86% of the global CVD disease burden. These give us clear and solid evidence that these are no longer only diseases of the developed world, projecting that by 2010, CVD will be the leading cause of death in developing countries (WHO 2006a; WHO 2011).

## 1.2 Defining Obesity Criteria

Obesity is defined as abnormal or excessive fat accumulation that may impair health (WHO 2000). Body mass index (BMI) is a simple index of weight-for-height that is commonly used in classifying overweight and obesity in adult populations and individuals. It is defined as the weight in kilograms divided by the square of the

height in meters (kg.m$^{-2}$). The World Health Organization (WHO) defines "overweight" as a BMI equal to or more than 25, and "obesity" as a BMI equal to or more than 30 (WHO 2000). BMI values are age-independent and the same for both sexes. However, BMI may not correspond to the same degree of fatness in different populations due, in part, to different body proportions. The health risks associated with increasing BMI are continuous and the interpretation of BMI gradings in relation to risk may differ for different populations (Hall, Sattar, and Gill 2008; WHO 2004a).

In recent years, there has been a growing debate on whether there are possible needs for developing different BMI cut-off points for different ethnic groups due to the increasing evidence that the associations between BMI, percentage of body fat, and body fat distribution differ across populations and therefore, the health risks increase below the cut-off point of 25 kg.m$^{-2}$ that defines overweight in the current WHO classification. There had been two previous attempts to interpret the BMI cut-offs in Asian and Pacific populations (WHO/IASO/IOTF. 2000; James, Chunming, and Inoue 2002) , which contributed to the growing debates. Therefore, to shed the light on this debate, WHO convened the Expert Consultation on BMI in Asian populations (WHO/IASO/IOTF. 2000). The WHO Expert Consultation concluded that the proportion of Asian people with a high risk of T2D and cardiovascular disease is substantial at BMI's lower than the existing WHO cut-off point for overweight (25 kg.m$^{-2}$). However, the cut-off point for increased risk varies between 22 kg.m$^{-2}$ and 25 kg.m$^{-2}$ for different populations in Asia. The Consultation, therefore, recommended that the current WHO BMI cut-off points (Table 1.1) should be retained as the international classification (WHO 2004b). But the cut-off points of 23, 27.5, 32.5 and 37.5 kg.m$^{-2}$ are to be added as points for public health action. It was, therefore, recommended that countries should use all categories (*i.e.* 18.5, 23, 25, 27.5, 30, 32.5 kg.m$^{-2}$, and in many populations, 35, 37.5, and 40 kg.m$^{-2}$) for reporting purposes, with a view to facilitating international comparisons.

**Table 1.1. The international classification of adult underweight, overweight and obesity according to BMI**

| Classification | BMI (kg.m$^{-2}$) | |
|---|---|---|
| | **Principal cut-off points** | **Additional cut-off points** |
| Underweight | < 18.50 | < 18.50 |
| Normal range | 18.50 - 24.99 | 18.50 - 22.99<br>23.00 - 24.99 |
| Overweight | 25.00 | 25.00 |
| Pre-obese | 25.00 - 29.99 | 25.00 - 27.49<br>27.50 - 29.99 |
| Obese | 30.00 | 30.00 |
| Obese class I | 30.00 - 34.99 | 30.00 - 32.49<br>32.50 - 34.99 |
| Obese class II | 35.00 - 39.99 | 35.00 - 37.49<br>37.50 - 39.99 |
| Obese class III | 40.00 | 40.00 |

Another well known and common measure of obesity is waist circumference, a proxy of central obesity; different cut-off values have been proposed to identify and classify individuals with risk of central obesity. The WHO cut-off value is <94 cm for both sexes. The International Diabetes Federation proposes 102 cm and 88 cm for men and women, respectively while the NCEP-ATP III suggests 94 cm and 80 cm for men and women, respectively. Similarly to body mass index cut-off point criteria, the IDF and NCEP-ATP III recommend to use waist circumference thresholds that are ethnic-group specific (Table 1.2). The IDF strongly recommends that for epidemiological studies and, wherever possible, for case detection, specific cut-points should be used for people of the same ethnic group whatever their country of residence.

Table 1.2. Ethnic specific values for waist circumference proposed by the IDF

| Country/Ethnic group | Sex | Waist circumference |
|---|---|---|
| Europeans In the USA, the ATP III values (102 cm male; 88 cm female) are likely to continue to be used for clinical purposes | Male<br>Female | 94 cm<br>80 cm |
| South Asians Based on a Chinese, Malay and Asian-Indian population | Male<br>Female | 90 cm<br>80 cm |
| Chinese | Male<br>Female | 90 cm<br>80 cm |
| Japanese | Male<br>Female | 85 cm<br>90 cm |
| Ethnic South and Central Americans, | Use South Asian recommendations until more specific data are available | |
| Sub-Saharan Africans and Eastern Mediterranean and Middle East (Arab) populations | Use European data until more specific data are available | |

## 1.3 Defining Type 2 Diabetes and Other States of Impaired Glucose Homeostasis

Diabetes mellitus is a disease of metabolic dysfunction, characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO 1999) (Table 1.3). Insulin resistance and defective glucose sensing at the  -cell are the central pathophysiologic determinants that together cause hyperglycemia (Tripathy and Chavez 2010; Ferrannini 1998; Kahn 2003b). Regardless of the cellular origin of insulin resistance, excessive tissue fat utilization is a consistent metabolic mechanism. Although genetic influences affect  -cell function, becoming overweight is the main acquired challenge to insulin action (Ferrannini, Gastaldelli, and Iozzo 2011).

Table 1.3. Diagnostic criteria for diabetes mellitus, IFG and IGT (WHO, 1999).

| Classification | Fasting plasma glucose (mmol.l$^{-1}$) | 2 – hour post-glucose load plasma glucose (mmol.l$^{-1}$) |
|---|---|---|
| Diabetes mellitus | 7.0 | 11.1 |
| Isolated IFG | 6.1 – 6.9 | |
| Isolated IGT | 6.1 | 7.8 – 11.0 |
| Combined IFG & IGT | 6.1 – 6.9 | 7.8 – 11.0 |

## 1.4 Obesity and Type 2 Diabetes: A Worldwide Overview

Globally, the prevalence of chronic, non-communicable disease is increasing at an alarming rate. About ~17 million people die every year from CVDs, for which T2D is one of the major predisposing factors. Propelling the upsurge in cases of diabetes is the growing prevalence of overweight and obesity – which have, during the last past decade, turned into one of the major public health problems (WHO 2006b). Both conditions recently have been merged into a new epidemiological concept named "diabesity" which describe mainly diabetes in the context of obesity. WHO's latest estimates indicate that globally in 2008: the number of overweight adults (age 20+) (BMI 25-29.9 kg.m$^{-2}$) was ~1.5 billion, of these, more than 200 million men and nearly 300 million women were obese (BMI    30 kg.m$^{-2}$) (WHO 2006d). This accounts for one quarter of the world's total population being at increased risk for developing cardiovascular disease and diabetes. The projections for the year 2015 expect the prevalence of overweight adults to be as high as 2.3 billion and that of obese adults to be ~700 million (WHO 2006d).

Along with this growing obesity problem, the prevalence of diabetes has been persistently rising for the last few decades and it is being recognized as a worldwide epidemic (Shaw, Sicree, and Zimmet 2010a). It shows that the worldwide prevalence in the year 2010 is estimated to be 6.4%. Between 2010 and 2030, there will be a net increase in the prevalence of diabetes among adults, as reflected by the 73% increase in adult diabetes numbers in developing countries, compared to 20% increase in developed countries (Shaw, Sicree, and Zimmet 2010c). Furthermore, the projections for 2030 show the prevalence to reach 439 million individuals comprising ~7.7% of the world population (Shaw, Sicree, and Zimmet 2010b).

## 1.5 Health Consequences of Obesity and Type 2 Diabetes

Obesity is associated with increased risk for several chronic diseases including diabetes, hypertension, heart disease and stroke (Field et al. 2001). Obesity is also associated with a range of diseases including gastroesophageal reflux disease, colon

cancer and liver diseases such as non-alcoholic fatty liver disease, cirrhosis and hepatocellular carcinoma (ACG 2008). Furthermore, obesity and overweight are associated with significantly reduced quality of life (Hlatky et al. 2010). Adams *et al.* reported that the risk of death is increased approximately by 20–40% in overweight individuals and by 2.5-fold in obese individuals compared to normal weight individuals (Adams et al. 2006).

The major health impact of T2D is due to its long-term complications including retinopathy, nephropathy, neuropathy, CVD, peripheral vascular diseases, stroke and periodontal pathologies (ADA 2003). Recent studies demonstrated that there is a reduction in health-related functioning (increased bodily pain, reduce mental health and vitality) associated to individuals with impaired fasting glucose, impaired glucose tolerance and T2D when compared to those with normal glucose tolerance (Tapp et al. 2010).

## 1.6 The Economic Cost of Obesity and Type 2 Diabetes

Concomitant with the rapid increase in obesity, there has been an important rise in health problems associated with obesity and the burden these place on healthcare systems. It has recently been estimated that obesity accounts for between 0.7% and 2.8% of a country's total healthcare expenditure. (Withrow and Alter 2011b). Furthermore, obese individuals were found to have medical costs that were approximately 30% greater than their normal weight peers (Withrow and Alter 2011a). Other studies showed that the health expenditure attributed to obesity alone in relation to a country's total health expenditure is not easily estimated, due to the strong link between obesity and cardiovascular and other non-communicable diseases. When costs associated with being overweight were also included, the upper limit of this range rises to reach 9.1% of a country's total healthcare expenditure (Finkelstein, Fiebelkorn, and Wang 2003). Moreover, healthcare expenditures for morbidly obese individuals (BMI $40 kg.m^{-2}$) are 81% higher than normal weight individuals (BMI = $18.5$-$24.9 kg.m^{-2}$), 65% higher than overweight individuals (BMI $25$-$29.9 kg.m^{-2}$) and 47% higher than individuals with class I obesity (BMI = $30$-$34.9 kg.m^{-2}$). This higher expenditure was attributed to more frequent office-based visits,

outpatient hospital care, inpatient care and prescription drugs (Finkelstein, Fiebelkorn, and Wang 2004; Arterburn, Maciejewski, and Tsevat 2005).

The burden of diabetes on the world economy has been increasing lately to reach at least $376 billion in 2010 and is expected to reach $490 billion in 2030. In 2010, it is estimated that approximately 12% of the health-related expenditures per person worldwide are expected to be spent on diabetes to reach $1330 per person (Zhang et al. 2010b). This represents an important economic burden as reflected by its consumption of 21, 16, 15 and 14% of the country's total health expenditure in Saudi Arabia, Egypt, Mexico and USA, respectively (Farag and Gaballa 2011). In contrast, hypertension and its main sequelae, stroke and myocardial infarction, cost ~10% of the world's overall health expenditure (Gaziano et al. 2009). Although developing countries are anticipated to have a 69% increase in prevalence by 2030 (Shaw, Sicree, and Zimmet 2010d), 91% of the world total health expenditure on diabetes will be in developed countries while only 9% of the total will be in the developing countries (Zhang et al. 2010a).

## 1.7 Obesity and Type 2 Diabetes: a Multiethnic Perspective

### 1.7.1 Definition of Ethnicity and Indigenous Population

When we talk about medicine and public health in a multiethnic world, it becomes relevant to define the concept behind this. Several terminologies have been used to describe these specific population, such as ethnicity, race, ethnic minorities groups, indigenous, aboriginal or natives (Bhopal 2004). Through this thesis the expression "ethnicity" will be generally used, this term is usually used to mean the social group a person belongs to, and either identifies with or is identified with by others, as a result of a mix of cultural and other factors including language, diet, religion, ancestry, and physical features traditionally associated with race. A second common term used through this thesis is indigenous as a synonym of aboriginal or natives, this term is usually used to define a person who belongs naturally to a place in the

sense of long term family origins. Now that these concepts have been clarified, we can move forward to ethnicity-related health.

Of the estimated 6,000 cultures in the world, around 4,000 of them are aboriginals, with a total population numbering ~300 million spread worldwide. Although, there is a wide geographic distribution and major differences in culture and history between these indigenous groups, there seems to be a shared susceptibility of increasing obesity and T2D rates in comparison to the local dominant culture (Yu and Zinman 2007h).

Overall obesity and diabetes estimates are likely to mask important differences in mortality and morbidity between different population groups. Significant changes in ethnic composition worldwide have already taken place following substantial emigration and immigration throughout the 20th century, undoubtedly affecting the epidemiology of chronic disease and their determinants (Carballo, Divino, and Zeric 1998a; Rafnsson and Bhopal 2009b). In fact the increasing burden of chronic diseases, especially CVD and diabetes, in ethnic minority populations, is a major public health challenge worldwide (NHS 2004; Maffla 2008; Carballo, Divino, and Zeric 1998b).

### 1.7.2 Obesity in a Multiethnic Population

It is well recognized that the prevalence of obesity varies drastically by ethnicity (Yu and Zinman 2007a; NHS 2004; Bhopal 2009). Minority non-white ethnic groups in Western countries (including Blacks, non-White Hispanics and Native Americans) exhibit a higher prevalence of obesity and obesity-related complications compared to people of white European ancestry (Yu and Zinman 2007f; NHS 2004). In the U.S. the prevalence of overweight or obesity among adults between 2003 and 2004 was 66.3% in the general population, 64.2% in white Caucasians, 76.1% in blacks, and 75.8% in Mexican Americans (Ogden et al. 2006). Similarly, data from the 2004 Health Survey for England showed that black Caribbeans had the highest prevalence of obesity (25%), while for women; black African (38%), black Caribbean (32%) and Pakistani ethnic groups (28%) had higher obesity prevalence rates compared to the general adult population (23-25%) (NHS 2004). Another example of this link

between ethnicity and obesity, are Pima Indians living in U.S.; Schultz, *et al.* (2006) reported a prevalence of obesity in this indigenous population by ~64% and ~75% for men and women, respectively. Obesity in this indigenous group was 10-times more frequent in U.S. Pima men and 3-times more frequent in the women than in their Mexican Pima counterparts (Schulz et al. 2006d).

### 1.7.3 Type 2 Diabetes in a Multiethnic Population

Along with the increased prevalence of obesity, the prevalence of diabetes in Western societies is rapidly rising; with ethnicity as an important determinant of this substantial increase (Ujcic-Voortman et al. 2009; Rafnsson and Bhopal 2009a; Weijers, Bekedam, and Oosting 1998a). In particular, migrants to Western societies have an increased risk of diabetes. This has been clearly demonstrated for African, in particular groups of West African (such as African-American and African-Caribbeans), Hispanic-American and Asian migrants to the USA or the UK, who have a far higher prevalence of diabetes than the native white population (Cappuccio et al. 1997; Weijers, Bekedam, and Oosting 1998b). Additional studies in other ethnic groups in Europe, showed that the prevalence of diabetes in other ethnic minorities such as Turkish (5.6%) and Moroccan (8.0%) living in Amsterdam experienced significantly higher prevalence of T2D compared to Dutch natives individuals (3.1%). Similar, increased T2D prevalence has been reported for South Asian populations. The Southall study in 1991 was the first large cross sectional study to compare diabetes prevalence from Indian and Pakistani subjects with that from age and BMI-matched white European subjects. The aforementioned study confirmed that South Asians have a higher prevalence on T2D (19% vs 4%) and allied to this they also exhibited hyperinsulinemia (McKeigue, Shah, and Marmot 1991b). Additionally, high prevalence of T2D was found in several American indigenous populations, Dakota Sioux, Apaches, Caddo, Comanche, Kiowa, Wichita and Pima Indians; all of them shared a similar but substantially higher prevalence of T2D (~40%) than the non-aboriginal population (Valencia et al. 1999; Yu and Zinman 2007g; Lee et al. 1995). Other ethnic indigenous groups that have significant increase in the prevalence of T2D, are Mapuches (8.2%) and Aymaras (6.9%) from Chile, who, in contrast to other ethnic groups in South America, have a large proportion migrated to a more westernised lifestyle. However, not all indigenous

population have reported this drastic increase; low prevalence of T2D has been found in South American indigenous groups living in remote areas of Bolivia, Peru, Brazil, Chile and Colombia (<1%). Similar levels were reported in Navajos Indians in Mexico (0.4%) and Malaysian Orang Asli (0.3%) (Yu and Zinman 2007b; Kim et al. 1999; Ali et al. 1993; Briceno et al. 1996; Franco 1992).

## 1.8 Obesity, Type 2 Diabetes and Cardiovascular Disease in South America

During the past three decades, South America has experienced a transition with a significant impact on lifestyle and health profile leading to demographic, socioeconomic, epidemiologic and nutritional changes (Albala, Vio, and Yanez 1997a; Kain, Vio, and Albala 2003). Dietary intake has experienced a shift to a higher intake of high-density food and sweetened beverages (Kain, Vio, and Albala 2003). A wide variety of high-energy food products are more readily available, in larger portions (Kain, Vio, and Albala 2003; Barria and Amigo 2006; Romieu et al. 1997b). Another important change derived from urbanisation and lifestyle modifications is the decrease in physical activity. Indicators for sedentary life such as number of cars, televisions and computers have increased across Latin American countries (Cuevas, Alvarez, and Olivos 2009b). In South America the rates of obesity are alarming (~30%), with Chile, Mexico, Peru, Guatemala and Argentina leading the ranking of obesity (Cuevas, Alvarez, and Olivos 2009a). According to the INTERHEART study involving 52 countries worldwide, the attributable risk for first acute myocardial infarction in Latin America for central obesity was higher than in other countries (Romieu et al. 1997a). The included Latin American countries were Argentina, Brazil, Colombia, Chile, Guatemala and Mexico. The population attributable risk (PAR) for abdominal obesity was 48.5%, followed by dyslipidemia (40.8%) and smoking (38.4%). Important differences by gender were observed; women are more severely affected by this trait. This increase in obesity is leading to higher morbidity and mortality due to T2D and cardiovascular disease (Tejero 2010a).

Along with this remarkable epidemiological transformation in South America, T2D is now leading health problems. Brazil, Chile and Mexico are among the 10 countries

with the highest rate of diabetes in South America (Barcelo et al. 2003; Barcelo and Rajpathak 2001a). About ~10% of Chilean and Mexican population in the Americas has diabetes (MINSAL 2010; Barcelo 2006a). The multinational study, Cardiovascular Risk factors Multiple Evaluation in Latin America (CARMELA), investigated the prevalence of risk factors for CVD in seven Latin American cities (Escobedo et al. 2009b). Overall, the prevalence of diabetes was 7.0% while impaired fasting glucose was found in a further 2% of the population. The highest prevalence of diabetes was in Mexico (8.9%) and Bogota (8.1%) and the lowest was in Lima (4.4%) (Escobedo et al. 2009c).

Similar to obesity and T2D, cardiovascular diseases has also increased amongst South American countries. At the moment, mortality has decreased from infant and infectious diseases, and life expectancy has increased. This shift has occurred in a rapid manner, leading to a complex epidemiological profile within this region, characterised by wide socioeconomic differences (Kain, Vio, and Albala 2003; Albala, Vio, and Yanez 1997f). Along with this, CVD is now the leading cause of death in most countries of South America; from all causes of mortality ~31% of deaths have been attributable to CVDs (Barcelo et al. 2003; Schargrodsky, Escobar, and Escobar 1998a). WHO forecasts indicate that the number of deaths in the region attributed to CVDs will increase by more than 60% between 2000 and 2020, unless preventive measures are introduced (Murray and Lopez 1996). Despite its relevance, the prevention and control of cardiovascular and other chronic diseases do not have a prominent place in the public health agenda in South America.

## 1.8.1 Health Epidemiological Transition in Chile: Overview

Additionally to the predicted increase in CVDs, it is also relevant to consider the significant variation across South American countries regarding the socioeconomic indicators, ethnicity, cultural background and lifestyle that could impact to a different extent on the health indicators. Chile is one of the countries of South America that is simultaneously experiencing a demographic and epidemiological transition, resulting in an ageing population and shift from infectious to chronic disease (Albala, Vio, and Yanez 1997b). Chile underwent rapid modernisation in the 1990s as a consequence of economic growth. This economic improvement produced a positive effect in

relation to the reduction of infectious disease, malnutrition and infant mortality rate. On the other hand, this growth produced a negative effect on lifestyle, such as the turning to a more "Western diet" and decrease in physical activity (Vio, Albala, and Kain 2008a). With respect to mortality rate, CVDs related deaths have increased from 23% in 1970 to 29% in 1992 (INE 2002a).

Along with this mortality increase, Chile's National Health Survey (2003) showed that the prevalence of overweight, obesity and morbid obesity in the adult population was 38%, 22% and 1.3% respectively, varying widely by education level and socio-economic status. Individuals with lower socioeconomic status or lower educational levels appear to have a higher prevalence of obesity compared to those with higher education and socioeconomic status. In the most recent National Health Survey (2010) the prevalence of overweight and obesity had slightly increased (39.3% and 25.1%, respectively), and prevalence of morbid obesity had almost doubled (2.3%). Central obesity, measured by waist circumference, was included for first time in the 2010 Survey, revealing that ~33% of the adult Chilean population have a waist circumference above 102 cm for men and 88 cm for women. Additionally, the prevalence of metabolic syndrome (assessed by ATPIII criteria) was substantially increased from 2003 to 2010 (22.6% to 35.3%) (INE 2002a).

Along with this high rate of overweight and obesity reported in 2010 (66.7%), the adult prevalence of T2D also considerably increased; from 4.2% in 2003 to 9.4% in 2010. The 2010 Health Survey also included for first the time the gamma glutamyl transferase (GGT) level, showing that ~17% of the adult population have GGT above the normal range (50 U/L). Despite these increases in overweight, obesity and T2D, the prevalence of self-reported inactivity (less than three times 30 minutes per week of moderate physical activity) did not show this increase; rates were high at both time points (~88%) in the adult population (MINSAL 2010).

## 1.8.2 Obesity and Type 2 Diabetes in Multiethnic Chilean Population

It is important to note that these Chilean Health Survey diabetes prevalence figures do not tell the full story, given that we previously showed that the prevalence of

diabetes differs widely by both ethnicity and environment (Yu and Zinman 2007d). The information provided by this Survey should be interpreted with caution since Chile's population (~18 millions) is comprised for an important number of Native American groups (~6% of the total population,) as well as a large proportion of people with White European backgrounds (INE 2002a; INE 2002b). This feature was not taken into account in the National Health Survey.

Mapuches, an indigenous Native American population from Chile, appear to follow a pattern of disproportionate increase in the risk of diabetes (compared to Chileans of White European population descent) when they move from a traditional rural lifestyle to an urban one. Data from cross-sectional studies suggest that diabetes prevalence for Mapuches living in traditional rural environments is low, at between 1% and 4% of the adult population (Perez-Bravo et al. 2001e; Larenas et al. 1985d), but rises to ~8% amongst urban Mapuches living in westernised environments (Perez-Bravo et al. 2006e; Carrasco et al. 2004f). In contrast, Chileans of European descent had a smaller difference between rural and urban settings (4.5% and 5.8%), respectively (Baechler et al. 2002a). Interestingly, in these cohorts obesity levels are high but quite similar between both environments in both ethnic groups (~28%) (INE 2002b; Perez-Bravo et al. 1998; Perez-Bravo et al. 2001d).

## 1.9 Type 2 Diabetes, Insulin Resistance and  -cell Dysfunction

The previous section showed evidence that T2D is increasing worldwide in epidemic proportions. However, behind T2D, insulin resistance and  -cell dysfunction are recognised as major factors involved in its development (Kahn 2003a). The pancreatic beta-cell has been traditionally considered to display adaptive secretory responses to insulin resistance, by augmenting insulin secretion to maintain normoglycaemia (Cerasi 1995; Taylor, Accili, and Imai 1994). Beta-cell failure has therefore been thought to be a relatively late occurrence in T2D, signifying a failure of both this adaptive response and the ability to maintain normal blood glucose. Longitudinal studies have clearly shown that insulin resistance is a major risk factor for the development of T2D (Lillioja et al. 1993a; Lillioja et al. 1988; Warram et al. 1990b). In a prospective study of Pima Indians, Lillioja *et al.* reported that insulin

resistance was the strongest single predictor for diabetes, with a 27% cumulative incidence of diabetes over 6 years (Lillioja et al. 1993b). Warram *et al.* followed for 25 years 155 offspring of couples who both had T2D. Subjects who developed diabetes had insulin resistance >10 years before they developed the disease (Warram et al. 1990a). These observations argue in favour of insulin resistance as the primary defect in the development of diabetes.

On the other hand, studies in monozygotic twins, where one of whom already had diabetes and one of whom had either normal glucose tolerance or IGT (Vaag et al. 1995a), those with normal glucose tolerance or IGT had decreased first-phase insulin release, while only those with IGT also had a significant reduction in insulin sensitivity compared with appropriate control. This study provides some evidence that impairment of -cells can occur before insulin resistance is detectable (Vaag et al. 1995b). However, both seem to be present by the time hyperglycemia appears. Other reports have confirmed those observations in offspring of two parents with T2D or first-degree relatives of someone with T2D (Pimenta et al. 1995; van Haeften et al. 1998). Although these observations provide some evidence that -cell dysfunction is already present in normal glucose-tolerant individuals genetically predisposed to develop T2D. There is not clear consensus in the diabetes field about this topic. In summary, it is likely therefore, that insulin resistance remains the key metabolic abnormality in the development of T2D and that this, in concert with the metabolic effects of obesity, drives early beta-cell dysfunction. But both are present in IGT stage before T2D.

## 1.10 Cardiovascular Risk and Insulin Resistance

Type 2 diabetes is often preceded by insulin resistance and development of cardiovascular disease starts long before diabetes is diagnosed (Haffner et al. 1990). Insulin resistance has been linked to a number of cardiovascular risk factors. For example, high VLDL triglycerides, low HDL-C and a shift in LDL particle size from large buoyant to small dense LDL are characteristic of the dyslipidaemia observed in insulin-resistant subjects (Karhapaa, Malkki, and Laakso 1994; Lewis et al. 2002). Studies have also suggested that elevated inflammatory and thrombosis markers, such as C-reactive protein (CRP), plasminogen-activator inhibitor-1 (PAI-1),

fibrinogen and endothelial dysfunction, are associated with insulin resistance and confer increased cardiovascular risk (Festa et al. 2000; Haffner 2003; Sobel et al. 1998; Howard et al. 1996).

Additionally, markers of liver function, specifically gamma-glutamyltransferase (GGT) and alanine amino-transferase (ALT), predict incident type 2 diabetes in various populations and are associated with insulin resistance and confer increased cardiovascular risk (Lee et al. 2004; Bonnet et al. 2011). This has been confirmed by a recent meta-analysis that suggested that GGT may be a better diabetes predictor than ALT (Fraser et al. 2009). Gautier *et al.* recently reported that a moderate elevation of GGT concentration within the normal range is a strong risk marker for incident type 2 diabetes in a large nonobese population, independently of the homeostasis model assessment index (Gautier et al. 2010). However, the physiopathological mechanisms that underlie the association between GGT, ALT, and the risk of diabetes remain poorly understood. Studies have shown that elevated levels of ALT and GGT reflect peripheral insulin resistance (Hanley et al. 2004), but specific assessment of hepatic insulin sensitivity with appropriate methods is lacking.

The previous section described the strong link between insulin resistance and T2D. Considering this, the diagnosis of T2D is understood to confer up to a four-fold increase in cardiovascular risk (Stamler et al. 1993). Additionally, there is some evidence that T2D may have a relatively greater CVD risk impact in women compared to men (Hu et al. 2001e; Huxley, Barzi, and Woodward 2006a; Oterdoom et al. 2009; Mak and Haffner 2003), Huxley *et al.* reported that the relative risk for fatal coronary heart disease associated with diabetes is 50% higher in women than it is in men (Huxley, Barzi, and Woodward 2006b). Insulin resistance appears to have a greater impact on cardiovascular risk in women, both prior to and after the development of T2D (Becker *et al.* 2003,Hu *et al.* 2001). Tailored public health strategies have recently been suggested to tackle cardiovascular risk in women (Engberding & Wenger 2008,Evangelista & McLaughlin 2009). The presence of insulin resistance in women may be associated with other metabolic disturbances which are not present to the same extent in men, and which may confer increased risk (Regitz-Zagrosek *et al*. 2006). In insulin resistant subjects, higher inflammatory markers have been reported in women compared to men, and remain significantly

higher in women even after adjustment for BMI (Lakoski *et al*. 2006,Saltevo *et al*. 2008). This observation may simply reflect a greater amount of adipose tissue in women compared to men of a given BMI, although Saltevo and colleagues have also suggested that with increasing insulin resistance women display relatively greater reductions in adiponectin compared to men and that this may be indicative of increased adipose tissue macrophage infiltration (Saltevo *et al.* 2009). The influence of circulating oestrogen in pre-menopausal women is recognised to have metabolic benefit; promoting fat oxidation, reduced inflammatory cytokines, reduced oxidative stress, reduced hepatic glucose output and subcutaneous, rather than visceral fat accumulation (Geer & Shen 2009). The development of insulin resistance in women is associated with attenuation of these protective mechanisms (Geer & Shen 2009). It is therefore conceivable, that the development of insulin resistance in women is mediated by different mechanisms than those observed in men, and that low-grade inflammation may have greater influence on metabolic health in insulin resistant women compared to men.

Increased cardiovascular risk is also observed in other insulin resistant states. The metabolic syndrome, a condition where insulin resistance and adiposity are one of the main subcomponents, is associated with increased prevalence of coronary heart disease (Alexander et al. 2003a; Church et al. 2009b). Malik and colleagues (2004) showed that cardiovascular disease and total mortality was increased in patients with metabolic syndrome, and that risk was increased even when only one or two of the diagnostic criteria (insulin resistance and central adiposity) were met (Malik et al. 2004). The link between adipose tissue, metabolic syndrome and CVD is thought to be partially mediated by adipose tissue secreted factors known as adipokines (Tilg and Moschen 2006). Two well-known adipokines are adiponectin and leptin. Adiponectin is known to have anti-inflammatory and insulin-sensitizing effects and is inversely related to the metabolic syndrome and type 2 diabetes mellitus (Berg and Scherer 2005; Lago et al. 2007a). On the other hand, leptin normally has insulin sensitizing and anorexigenic effects (Rabe et al. 2008b). However, obese people develop hyperleptinemia and become leptin resistant (Considine et al. 1996a) and leptin is positively correlated with the metabolic syndrome in obese subjects (Schulze et al. 2004b; Rabe et al. 2008a). Visceral adiposity is predictive of the development of insulin resistance, as it appears to be a more active endocrine organ

secreting more pro-inflammatory adipokines and less adiponectin (Mohan et al. 2005a). Studies of South Asians have shown that lower levels of adiponectin are correlated with insulin resistance and metabolic syndrome, while leptin levels appear to be positively correlated with impaired glucose tolerance and type 2 diabetes mellitus (Mohan et al. 2005b; Carantoni et al. 1999; Lago et al. 2007b).

## 1.11 The Aetiology of Insulin Resistance

Considering the strong link between insulin resistance and T2D, and its associations with cardiovascular disease, early intervention to prevent or reverse its progression may have significant public health benefits by the reduction of these chronic diseases. The state of insulin resistance is characterised by very high circulating levels of insulin as well as a reduced ability of insulin to suppress hepatic glucose production and to promote peripheral glucose disposal. Type 2 diabetes occurs when this condition is accompanied by a failure of the pancreatic beta-cell to secrete enough insulin to overcome the degree of insulin resistance (Abdul-Ghani, Tripathy, and DeFronzo 2006). The insulin resistant state is associated with metabolic dysfunction in adipose tissue, hepatic, skeletal muscle tissue and the vascular endothelium (McGarry 2002). These changes are both a consequence of, and contribute to insulin resistance and it is difficult to separate metabolic cause from effect.

## 1.12 The Aetiology of Insulin Resistance and Type 2 Diabetes: a Matter of Lifestyle?

The previous section provided evidence that obesity is strongly related to insulin resistance and the development of T2D, an epidemic condition that shows no sign of abating. The complexity of their aetiology involve the interaction of several environmental and lifestyle factors. Strong evidence suggests that an epidemiological transition, characterised by an important migration and urbanization tendency is one of the major risk factors involved in the increasing level of obesity and diabetes. Along with this urbanization and migration, it is the change in dietary intake patterns,

with populations having more access to a wide variety of higher energy dense food. Other changes stimulated by the environment are related to the reduction of the energy expenditure and promotion of a more sedentary lifestyle; this includes reduction in jobs requiring physical labour, reduction in energy expenditure in the daily living, and increasing time spent sitting. Finally, socio-economic factors, low or higher socio-economic status that could change your lifestyle patterns and behaviours mentioned above, in a healthy or not healthy way. All these factors and the evidence of their association with insulin resistance and T2D are discussed in the subsequent paragraph.

### 1.12.1    Migration and Urbanisation as a Potential Contributor of Insulin Resistance and Type 2 Diabetes

Chronic disease including CVDs and diabetes, are currently the major causes of morbidity and mortality in Europe. In Europe almost 2 million deaths are attributed to CVD every year and diabetes is the major risk factor for CVD which affects over 48 million adults in Europe, with an overall estimated prevalence of 7.2% for the European union (BHF 2008). The burdens of disease estimates are likely to mask important differences in mortality and morbidity between different population groups within regions and countries, including migrant and ethnic minorities. Migration and urbanization appears to be important contributors to the epidemiology of diabetes and its determinants.

A good example of migration and urbanization as risk factors of insulin resistance and diabetes, are provided in studies of South Asian populations. The prevalence of T2D in urban India is around 5-times as high as that in rural setting (Ramachandran et al. 1992; Ramachandran et al. 2008a), and this observation has been mirrored in Pakistan, Bangladesh, and Nepal (Shera, Jawad, and Maqsood 2007; Hall, Sattar, and Gill 2008). A recent study, in South Asian populations clearly showed that urbanization has a strong impact in the prevalence of T2D. This study compared the prevalence of diabetes in three locations with different degrees of urbanization, they found that peri-urban or rural traditional setting had a lower prevalence of diabetes (9.2%) than the main urban city (18.6%) and town (16.4%) demonstrating that

urbanization has a strong impact in the develop of the disease (Ramachandran et al. 2008b).

The Pima Indians are another well known example of the urbanization and migration impact on T2D. Schulz and collaborators reported a substantially lower prevalence of T2D in Pima Indians living in the traditional environment in Mexico (6.9%) compare to the Pimas living in urban setting in U.S. (38%), but similar to non-Pimas living in Mexico (2.6%) (Schulz et al. 2006c). Similarly, to Pima Indians and South Asians population, Mapuches – an indigenous Native American population from Chile – appear to follow the same pattern of a disproportionate increase in risk of diabetes (compared to Chileans of white European descent) when they move from a traditional rural setting to an urban one. A first study in Rural Mapuches showed that in 1985 the prevalence of T2D in this native group was ~1% to 4% (Larenas et al. 1985c), and that prevalence rises to ~8% in urban-dwelling Mapuches (Carrasco et al. 2004e). However, no studies have been conducted in this population to identify and determine if changes in lifestyle factors could explain this large increase in T2D prevalence between 1985 and 2004. Thus, urbanization and migration, appears to have a disproportionately adverse affect on the risk of diabetes. However, the effect of migration and levels of urbanization on risk of T2D are probably mediated by other potential risk factors, such as decrease in physical activity level, changes in dietary intake patterns and socio-economic factors.

### 1.12.2  *Dietary Intake and Nutritional Transition as a Potential Contributor of Insulin Resistance and Type 2 Diabetes*

Diet modification due to migration or urbanization, is considered to be one potential risk factor associated with an increase risk of insulin resistance and T2D. Changes in dietary intake, such as excess of energy intake and positive energy balance are associated with the development of obesity and insulin resistance, which is a key feature underlying the pathophysiology of T2D. However, reports show that over time the dietary intake patterns in some populations, changes to closely resemble that of the local dominant culture. For example, South Asians living in the U.S. did not reveal a different dietary intake compared to that of the white American population

(Raj, Ganganna, and Bowering 1999). On the other hand, those south Asians living in the UK, showed a healthy dietary intake pattern compared to the UK population as a whole, with lower total energy intake, higher intake of complex carbohydrates, vegetable fiber and polyunsaturated fatty acid (McKeigue et al. 1985; Sevak, McKeigue, and Marmot 1994). Another study reported by Schultz and collaborators (2006), compared the dietary intake patterns of Pima Indians and non Pimas living in Mexico. No significant differences were found in dietary intake patterns between both groups. This may explain a previous study in pima Indians from Arizona U.S, which reported a similar energy intake to Pimas living in Mexico, but a considerably higher percentage of calories derived from fat and a lower fiber intake for Arizona Pimas compared to Mexican Pimas studied by Schultz (Smith et al. 1996; Schulz et al. 2006b). Another study in U.S. Pima Indians showed that the preference for a "Western-diet" compared to the "traditional" Pima Indian diet was associated with a 2.5-fold increase in the risk of developing T2D (Williams et al. 2001). However, this study diod not account for measurement error that could strongly influence the relationship between dietary intake patterns and cardio-metabolic risk. One of the main Issues of diet measurement is the error associated to the estimation. Most of the diet measurements nowadays are determinate using self-reported questionarries, which involve a large bias and poor accuracy in determinate caloric intake and macornutrients compsumption (Freedman et al. 2011).

Additionally, the nutrition transition in Chile brings a progressive rise in overweight and obesity, with direct consequences in the prevalence of T2D (Vio, Albala, and Kain 2008b). Analysis of the Food and Agriculture Organization's (FAO) Food Balance Sheets demonstrates that the availability of total calories and calories from fat have increased in the last two decades, with a major increase in saturated fats. In fact, in 1980, the Chilean average per capita availability for calories and fat was 2667 kcal (21% from fat), increasing to 2,844 kcal (28% from fat) in 1998 (Albala, Vio, and Yanez 1997c). Existing data from studies done between 1960 and 1989 estimating food intake in Chile, show that the average caloric contribution of macronutrients in the Chilean diet consisted of 10-13% protein, 20-25% fat and 60-70% carbohydrates, without any relevant changes in the period (Kain, Vio, and Albala 2003). A comparative analysis of the National Household Surveys on Food Expenditure conducted between 1988 and 1998 revealed that the main expenditure of

the lower-socioeconomic class is for bread, meat and soft drinks, meaning that the preferences of this social class are in the first place for the staple food in Chile (bread) and then for food with a high proportion of saturated fat (meat) and sugar (soft drinks). Converted into energy and macronutrients, an increase of 22% in average total calories and an increase in average fat consumption of 26% were observed on dietary intake patterns of the Chilean population between 1988 and 1998 (INE 2011). Although no prospective data about diet intake and risk of T2D in Chilean population is available, we could speculate that the nutritional transition described before could drive the higher level of overweight and obesity in Chile (64.5%) that could be reflected in the increased prevalence of T2D observed from 2003 to 2010 (4.2% to 9.4%).

### 1.12.3 Physical Activity, Sedentary Behaviours and Fitness: Their Role on Insulin Resistance and Type 2 Diabetes

The American College of Sport Medicine (ACSM) and the American Heart Association (AHA) and the UK Chief Medical Officer, all recommended that adults participate in at least 150 minutes of moderate intensity physical activity (or at least 60 minutes of vigorous intensity physical activity) per week, to reduce the risk of cardiovascular diseases and T2D (Sigal et al. 2006a; Haskell et al. 2007b; Chief Medical Officer 2004). The most recent ACSM/AHA guideline recommended for adult individuals, that they should accumulate 30 minutes or more of moderate intensity physical activity on five days of the week or at least 20 minutes or more of vigorous intensity physical activity on three days of the week, as well as undertaking muscle strengthening exercises on two days of the week (Haskell et al. 2007a). This updated the previous ACSM guideline by clarifying the definition of moderate intensity activities and incorporating vigorous physical activity and muscle strengthening activities, and specifying that a combination of these activities is complementary in production of health benefits. However, this as well the other guidelines has adopted a "one size fits all" approach, and for physical activity and T2D prevention is possible that one size dose not fit all (Gill and Cooper 2008). The efficacy of physical activity in modulating diabetes could conceivably be influenced by factors that influence risk of the disease such as family history, sex, ethnicity,

obesity status and degree of glucose tolerance/insulin resistance. The associations of physical activity, cardiorespiratory fitness and sedentary behaviours with the risk of T2D have been addressed by a number of prospective studies and clinical trials in the past decade and summarized in a number of reviews (Gill and Cooper 2008; Gill and Malkova 2006h; Gill 2007; Hawley and Lessard 2008; Ostergard et al. 2006). The following sections will summarise the relationship of physical activity and fitness with insulin resistance and T2D.

### 1.12.3.1 Cardiorespiratory Fitness, Insulin Resistance and Type 2 Diabetes

Physical fitness is a set of attributes, such as cardiorespiratory fitness that people have or can achieve. The measurement of fitness is common practice in both preventive and rehabilitative exercise programs. Maximal oxygen intake or $VO_{2max}$ is the most accurate measure of cardiorespiratory (aerobic) fitness. Cardiorespiratory fitness relates to how well the cardiorespiratory system works to transport and utilise oxygen in the body. $VO_{2max}$ can be defined as the highest level of oxygen consumption that is utilised by the body during peak physical exertion (I-Min Lee et al. 2009). Cardiorespiratory fitness is also one physiological characteristic conferred by physical activity patterns, higher level of physical activity generally leading to high levels of fitness and vice versa. A number of studies have reported associations between fitness and insulin sensitivity of varying strength (Racette et al. 2006; Christou et al. 2005; Thamer et al. 2003). The Aerobic Center Longitudinal prospective study, conducted among 8633 men 30 - 79 years of age without diabetes at baseline, found that men with low cardiorespiratory fitness (the least fit 20%) had a 3.7-fold higher risk of developing T2D than those with higher fitness (the most fit 40%) (Wei et al. 1999d). This association remained significant after accounting for age, parental history of diabetes, alcohol consumption, and cigarette smoking, body mass index, waist circumference, high levels of high-density lipoprotein cholesterol and triglycerides, and high blood pressure. However, limitations of this study must be considered. This study assessed cardiorespiratory fitness by using a maximal exercise test that followed a standard protocol, however maximal oxygen uptake was not measured directly, and  this could be a bias on fitness level classification. Additionally, the authors did not report whether the risk of developing diabetes was similar when individuals with moderate fitness level were compared to lower or

upper fitness groups. This does not allow to determinate if the protective effect of fitness on diabetes followed an additive pattern. Another study, the Coronary Artery Development in Young Adults (CARDIA) study conducted on 4,487 U.S. individuals which included men and women, aged 18-30 years, assessed whether low fitness predicted the development of diabetes or insulin resistance/metabolic syndrome and whether increasing fitness was associated with a risk reduction. This study reported that those subjects with low fitness were about two times more likely to develop T2D or metabolic syndrome than those with higher fitness levels. They also reported that increasing fitness during the 7-year study was associated with a 60% reduction in risk for T2D and 50% reduction in risk for metabolic syndrome (Carnethon et al. 2003).

### 1.12.3.2 Physical Activity, Insulin Resistance and Type 2 Diabetes

It is clear, however that different patterns of physical activity have different effects on fitness for the same total volume of activity. Current U.S and UK physical activity guidelines recommend that adults accumulate 30 minutes of moderate physical activity 5 or on most days of a week; or 20 minutes of vigorous intensity physical activity no less than 3 days per week (Chief Medical Officer 2004; Haskell et al. 2007c; Sigal et al. 2006b). However, the recommended intensity of exercise has been a constant debate with some experts suggesting that exercise does not need to be necessarily vigorous to induce reduction in the risk of CVD (Pate et al. 1995b). However other studies suggest that vigorous physical activity may reduce CVD to greater extent than moderate intensity (Lee and Paffenbarger, Jr. 2000; Yu et al. 2003). So far, a few studies have examined the effect of exercise programmes with different exercise intensities, but the same total energy expenditure, on insulin sensitivity. One of these studies (O'Donovan et al. 2005) examined the effect of a 24-week high (80% $VO_{2max}$) or moderate (60% $VO_{2max}$) intensity exercise training intervention in sedentary men aged 35-45 years. Men in both groups exercised 3 times per week, expending 400 kcal per session. The result of this intervention was an increase in fitness by ~21% and ~15% in the high and moderate intensity group, respectively. However, both groups showed a similar decrease in insulin resistance and increase in insulin sensitivity compared to the control group. In contrast to the previous study, Coker and colleagues reported a similar increase effect in fitness

level (~14%) after a 12-week moderate (50% $VO_{2max}$) and vigorous (75% $VO_{2max}$) intensity exercise intervention. These groups did not find differences in body fat, but reported a 28% increase in insulin sensitivity in those subjects enrolled in the high intensity exercise programme (Coker et al. 2006). Similarly, DiPietro and collaborators performed an intervention study where older women (aged 62-84 years) were randomized to either higher (80% $VO_{2max}$), moderate (65% $VO_{2max}$) or control (light resistance exercise) exercise intensity group, undertaking exercise 4 days per week for 9 months. The main finding of this study was that the higher the intensity program, the more significant increase in insulin sensitivity (~21%), whereas the moderate intensity sessions induced a smaller and not significant improvement in insulin sensitivity (16%). No significant changes were reported for body fat or waist circumference in any group (DiPietro et al. 2006).

Given the relevance of fitness and physical activity in enhancing insulin sensitivity and reducing the risk to develop T2D, there is some evidence to suggest that the potential protective effect of physical activity may be greater in groups who are more insulin resistan or susceptible to insulin resistance when sedentary (Gill and Malkova 2006g). For example, sedentary offspring of patients with T2D are often more insulin-resistant than persons with no family history of diabetes, but when active or fit offspring of type 2 diabetic patients are compared with non-diabetic persons, differences in insulin resistance are less evident. A study conducted by Barwell and collaborators (2008), in women offspring of type 2 diabetic patients and 36 matched (age, BMI) female controls. This group underwent a 7 week endurance-type exercise training programme starting with $3 \times 30$ min of exercise in the first week and building progressively to $5 \times 60$ min of exercise in weeks 6 and 7 of the intervention. Prior to the intervention, insulin sensitivity index (ISI) was 22% lower in offspring than controls, despite similar body fat and $VO_{2max}$ values in the two groups. ISI increased by 23% in offspring following the exercise intervention, compared with 7% in the controls. These data suggest that insulin sensitivity is more highly modulated by physical activity in daughters of patients with T2D than in women with no family history of the disease (Barwell et al. 2008b). Thus taken together, the lifestyle changes associated with a worldwide tendency to reduce physical activity levels, plus the higher susceptibility to insulin resistance and T2D of some populations, the public health guidelines should keep the promotion of increased

physical activity. However, further studies are needed to confirm which exercise intensities could have a greater extent in improving insulin sensitivity and reducing the risk of developing T2D.

### 1.12.3.3 Sedentary Behaviour, Insulin Resistance and Type 2 Diabetes

Previously in this section, evidence of the magnitude of T2D was given. The reason for this substantial increase in the prevalence of T2D is complex, but modern lifestyle, such as adoption of new patterns of physical activity are thought to have played the major role (Zimmet, Alberti, and Shaw 2001). In response, public health recommendations on participation in moderate intensity physical activity have been widely promulgated, with the aim of reducing risk of T2D, CVDs and some cancers (Chief Medical Officer 2004; Haskell et al. 2007d; Sigal et al. 2006c). However there is emerging evidence that another set of behaviours, involving prolonged periods of inactivity and absence of whole body movements, is distinctly related to risk of chronic disease independent of physical activity levels (Dunstan et al. 2010; Hamilton, Hamilton, and Zderic 2007c; Healy et al. 2008g; Healy et al. 2008a; Healy et al. 2008j). Self-reported sedentary time (particularly television-viewing time, and total sitting time) have been associated with obesity, abnormal glucose metabolism, T2D, metabolic syndrome and cancer (Helmerhorst et al. 2009c; Bankoski et al. 2011b; Healy et al. 2011a; Healy and Owen 2010a; Hu et al. 2001d; Owen et al. 2010d; Owen et al. 2010a; Tremblay et al. 2010b). Similarly, other studies showed significant association with metabolic markers (Bankoski et al. 2011a; Healy et al. 2008i; Healy and Owen 2010b). Additionally, large cross-sectional studies using objective measurements of sedentary time, reported that sedentary behaviour was positively associated with insulin resistance in European adults (Healy et al. 2011b; Helmerhorst et al. 2009b; Healy et al. 2008h). The magnitude and direction of the association between inactivity and insulin resistance has been reported by Helmerhorst *et al.* in a European adult population, using fasting insulin as a proxy of IR, with coefficients ranging between 0.005 and 0.003 log unit per min.day$^{-1}$ spent in sedentary activities (Helmerhorst et al. 2009a). Taking into consideration the apparent T2D susceptibility of ethnic minorities, the potential impact of increasingly inactive lifestyles could have different clinical implications. A prospective study conducted by Hu *et al.* in ~68500 women that at baseline were free from T2D shows

that, after adjusting for confounding factors such as age and socio-economic status, 2 hours per day increment in TV watching (a potential major component of sedentary behaviour) was associated with a 14% increase in risk of diabetes (Hu et al. 2003e). A similar increase in the risk of T2D was reported in a male population, after 10 years follow up. This study reported that an increment of 2 hours per day spent watching TV was associated with a 20% increase in the risk of diabetes (Hu et al. 2001c). These results make clear the important clinical implications of increasing inactivity or sedentary lifestyle in the population. Although the detrimental association between sedentary-related behaviours and cardio-metabolic risk have been extensively reported in large cohorts, there are still some limitiation associated with these studies that should be taken into account. It is well know that time spent watching TV is one of the majors risk factors for cardio-metabolic disease. However, watching TV is also strongly associated with other risk behaviours such as increased intake of high energy density food and higher caloric drinks. However, most of these studies did not account for these confounding effects when the relationship between watching TV and cardiometabolic disease is examined.

Clinical studies in recent years have examined more carefully the pathways through which inactivity could lead to insulin resistance and its comorbidities. Potential negative effects of inactivity on insulin resistance include: vascular changes, dysfunction of the vascular endothelium which contributes to atherogenesis, reduced blood flow, decreased peripheral insulin-stimulated glucose uptake, and reduced glucose-stimulated insulin secretion (Hamburg et al. 2007; Houmard et al. 2004). Sedentary individuals have impaired endothelial vasomotor function compared to those who are physically active (DeSouza et al. 2000). A sedentary lifestyle also has a direct effect on inactivity-induced factors including deep venous thrombosis and poor lipid metabolism (Hamilton, Hamilton, and Zderic 2007b).

### 1.12.3.4 Assessment of Physical Activity and Sedentary Behaviour by Objective and Subjective Measures of Physical Activity

Physical activity levels can be monitored using different techniques in order to assess the health behaviours of the population and their association with morbidity and mortality rates. Accurate assessment is required to assess current and changing physical activity levels within the population, and to evaluate the effectiveness of

interventions designed to increase activity levels. However, the measurement of physical activity in epidemiological studies and surveillance systems is difficult due to its complex nature. Measurement of physical activity, and energy expenditure associated with it, comprise of time (duration), number of sessions (frequency) and intensity. One of the instruments widely used is self-reporting questionnaires, which provide a subjective measure of physical activity. Questionnaires vary in their complexity, time frame and type of activity that want to be assessed. From the large number of physical activity questionnaires available today, just some of them have been used with more frequency in different populations worldwide, this make it available for cross-cultural comparison of physical activity patterns. One of the most well known subjective instruments is the International Physical Activity Questionnaire (IPAQ). This questionnaire was developed in an attempt to standardise assessment of the prevalence of physical activity in different countries and cultures around the world. However, despite globally acceptable measurement properties, the results from some validation studies worldwide indicated reasonable validity of the IPAQ instrument to determine physical activity patterns in the population (Hagstromer, Oja, and Sjostrom 2006d). A second approach to measure physical activity is through the implementation of objective instruments. Objective assessment tools have the ability to capture components of physical activity that subjective measures often cannot determine accurately, such as unstructured activities (house work duties, or lower intensity activities). The most common instrument nowadays are movement monitors such as accelerometers and pedometers, however, other more sophisticated techniques such as doubly labeled water have been also used to determine total energy expenditure, but due to its cost these techniques are not used for large epidemiological cohorts (I-Min Lee et al. 2009). From all these techniques accelerometers are the most used objective tool to measure physical activity. Accelerometers provide objective and accurate information about frequency, duration, intensity and patterns of physical activities including children and older adults. However, information regarding to specific types of physical activity (*i.e.* washing dishes, gardening, or upper body movements) are not always captured by these instruments (I-Min Lee et al. 2009).

### 1.12.4 Income, Socio-economic and Educational Status as Potential Contributors of Insulin Resistance and Type 2 Diabetes

Inequalities in health associated with socioeconomic status are large and they are growing. Most theories that explain these inequalities use indicators of socioeconomic status associated with the individual such as income, educational attainment, or occupation (Smith, Shipley, and Rose 1990; Harper, Lynch, and Davey 2010). Socioeconomic environment influences occupation, lifestyle, and nutrition of social classes, which in turn would influence the prevalence of obesity, diabetes and CVDs. A number of studies have addressed this issue in developed and developing countries, suggesting that the risk of diabetes is higher in those socioeconomically disadvantaged populations in developed countries (Evans et al. 2000c; Robbins et al. 2001; Connolly et al. 2000b; Dasgupta, Khan, and Ross 2010; Maty et al. 2005; Connolly and Kesson 1996a). In contrast, studies in developing countries such as India (Ramachandran et al. 2002c), Bangladesh (Abu et al. 1997), diabetes was more common among those of higher socio-economic status. However, this relationship was not the same for every developing country, for example, China and Chile which show a similar relationship as developed societies (Ko et al. 2001; MINSAL 2010). A potential explanation to this opposing effect of SES on obesity and T2D, could be the distribution of the population in different categories of social classes, considering that Chilean population is mainly distributed a middle class. The situation of an individual classified as middle socio-economic class and the impact of this on their lifestyle (physical activity, diet patterns) could be similar to an individual with higher socio-economic class in India, but at the same time, it could be similar to a lower socio-economic status and lifestyle in a developed country like UK. These differences in the classification of socio-economic status in the different countries, could lead in an opposing effect of SES on obesity and T2D (Ramachandran et al. 2002b). Evans and collaborators reported that UK people of the most deprived categories were 1.6 times more likely to have T2D than the least deprived (Evans et al. 2000b). This could be related to a higher prevalence of obesity in the socioeconomically deprived population in UK (Evans et al. 2000a; Connolly et al. 2000a; Connolly and Kesson 1996b). Similarly, an indigenous Australian population showed a higher prevalence of T2D among those of lower socio-

economic status (Cunningham et al. 2008). In contrast, a socioeconomically deprived population from urban southern India showed lower prevalence of T2D compared to those with higher income levels (12.6% versus 25.5%), which could be associated with the higher levels of physical activity reported in this population (Ramachandran et al. 2002a). Chile, a developing country showed a similar association between lower socio-economic status and higher risk of T2D than those developed economies while the recent National Health Survey (2010) revealed that those more socio economically deprived showed a 57% higher risk to have T2D compared to the higher socio-economic groups in the Chilean population. This higher risk of T2D on the socioeconomically deprived Chilean population could be related to the link between socio-economic status and obesity, showing that those more socio-economically deprived are 78% more likely to have obesity than population in higher socio-economic groups. Considering the previous data, the nature of the relationship between socio-economic status and diabetes could differ by country. However, more data are required to elucidate the real contribution of socio-economic status in different populations.

## 1.13 Aetiology of Insulin Resistance and Type 2 Diabetes: a Matter of Genetics?

The previous sections of this chapter have outlined a number of lifestyle factors which clearly contribute to the development of insulin resistance and subsequently increase the risk of T2D. In addition, groups with traditional lifestyles who migrate to a more 'Westernised' environment and lifestyle suffer increased diabetes prevalence (Pavkov et al. 2007). Environmental and lifestyle factors, however, do not seem to explain all of the variance in T2D increase. Offspring of patients with T2D have about a three-fold higher risk of developing the disease than those with no diabetes family history (Ohlson et al. 1988; Kobberling and Tillil 1982). Additionally, diabetes prevalence also differs between ethnic groups and within countries. South Asian populations living in the UK and US have approximately 4-6 times the risk of developing diabetes compared to those of European descent (McKeigue, Shah, and Marmot 1991a). Further evidence of the high heritability of type 2 diabetes come from the higher concordance of type 2 diabetes in monozygotic twins (50–70%) compared with dizygotic twins (20–37%), and this provides

evidence of a genetic contribution to this condition (Vimaleswaran and Loos 2010). Recent genetic association studies are beginning to explain some of this heritability, having identified at least 40 independent, single nucleotide polymorphisms (SNPs) associated with an increased risk of type 2 diabetes. Genetic risk scores (GRS), consisting of the weighted sums of SNP risk alleles, may predict the risk of type 2 diabetes even after adjusting for parental diabetes, however these GRS still only account for about ~10% of the heritability of type 2 diabetes (McCarthy 2008).

### 1.13.1      *Progress in the Genetic of Type 2 Diabetes*

Although recent success of genome-wide association studies in the identification of several genes involved in complex diseases such as obesity and T2D, gene identification for the last 15 years has been based on two broad genetic approaches, *i.e.* candidate genes and genome-wide linkage studies. Candidate genes studies are hypothesis-driven and rely on the current understanding of the biology and pathophysiology that underlies the susceptibility to complex disease. However, this approach is an expensive and tedious task; candidate genes studies typically examined only one or a few variants per gene. Over time genotyping costs have been reduced substantially and different data sets have been created, such as dbSNP and the International Hap Map, have provided deep insight into genetic variation of genes. This knowledge has led to more comprehensive studies that systematically examine the association of all common variants in a gene of interest. Candidate genes for T2D are chosen based on the role of their monogenic form on diabetes, pancreatic beta-cell function, insulin action and glucose metabolism, or other metabolic conditions that increase the risk of T2D. So far more than 60 candidate genes for T2D have been studied in various populations worldwide. However, many of the studies have not been replicated and only detected weak associations. Some of the candidate genes identified, for which the results are more convincing and sample size used was large, are *PPARG, KCNJ11* (potassium inwardly rectifying channel subfamily J), *WFS1* (Wolfram syndrome 1), *HNF1B* (HNF1 homeobox B) and the *IRS1* (insulin receptor substrate 1) (Bonnefond, Froguel, and Vaxillaire 2010a; Prokopenko, McCarthy, and Lindgren 2008a).

The second common genetic approach is genome-wide linkage; a hypothesis-generating approach that examines the whole genome to identify the approximate location of new genes for a disease or traits of interest. So far more than 20 genome-wide linkage scans for T2D have provided suggestive evidence for many loci across the whole genome. However, only one study has successfully identified the gene underlying linkage with T2D, which led to the discovery of the *TCF7L2* (transcription factor 7-like 2) on chromosome 10 (Grant et al. 2006a). Of all genetic variants associated with T2D so far, genetic variation in the *TCF7L2* gene has still the largest effect on T2D susceptibility (Bonnefond, Froguel, and Vaxillaire 2010b; Prokopenko, McCarthy, and Lindgren 2008b).

The latest gene-finding tools, that have led to a rapid understanding in our knowledge of the genetic components of complex disease is the genome-wide association approach (GWAS). Similar to the genome-wide linkage, GWA is a hypothesis-generating approach that through screening of the whole genome aims to identify new, unanticipated genetic variants associated to complex disease. This approach has already resulted in an unprecedented chain of discoveries in the genomics of common diseases such as obesity and T2D. Two major advances have set the stage for GWAS. First, the completion of the reference sequence of the human genome (McPherson et al. 2001) and, more recently, the international HapMap (Frazer et al. 2007) have considerably increased our knowledge of common genetic variation and the underlying linkage disequilibrium structure. Second, substantial progress in genotyping technologies has made it possible to genotype more than one million genetic variants in a single analysis. Additionally, the information provided for this previous advances have led to the design of single nucleotide polymorphism (SNPs) chips that can capture more than 80% of the common genetic variation reported in the HapMap. All this progress in technologies and capacity has facilitated substantial progress in the gene-hunting strategy, with further potential contribution to the genetic architecture of complex disease such as obesity and T2D. During the past few years GWAS studies have identified several novel loci showing robust association with T2D, providing new insight into the aetiological mechanisms of T2D (Dupuis et al. 2010; Sladek et al. 2007; McCarthy and Zeggini 2009b). The loci most consistently associated with T2D risk include variants within or near *SLC30A8, HHEX, CDKAL1, CDKN2A/2B* and *IGF2BP2*

(McCarthy and Zeggini 2009a). A meta-analysis of the GWAS followed by large-scale replication study identified additional susceptibility loci for T2D, with an odd ratio ranging between 1.09 and 1.15 for SNPs near six genes: *JAZF1, CDC123-CAMK1D, TSPAN8-LGR5, THADA, ADAMTS9* and *NOTCH2*. However, further replication studies did not confirm a significant association with T2D and metabolic traits (Schleinitz et al. 2010). So far, *TCF7L2* remains the strongest gene associated with risk of T2D, with each additional risk allele increasing the odds of T2D 1.5-fold (Grant et al. 2006b). Another strong gene found by a GWAS for T2D, was the fat mass and obesity-related gene (*FTO*); this gene was related to an increased risk of T2D mediated by BMI (Dina et al. 2007c; Frayling 2007f). Although, the first studies indicated that the relationship between *FTO* and T2D was mediated by BMI, recent studies reported that *FTO* is associated to an increased risk of T2D in several populations of non white-European backgrounds (Li et al. 2010d; Liu et al. 2010f; Sanghera et al. 2008; Shimaoka et al. 2010e; Bressler et al. 2010).

## 1.13.2 *Progress in the Genetics of Common Obesity*

Obesity is a common, multifactorial condition for which susceptibility is determined by the joint actions of genetic and environmental factors. The dramatic increase in the prevalence of obesity over the past two decades is most likely due to changes in diet and physical activity (Hill et al. 2003b). However, it is well recognised that hereditary influences also contribute significantly to the susceptibility to obesity (Loos 2009c).

The genetic contribution to obesity has been established through family, twin and adoption studies (Maes, Neale, and Eaves 1997; Stunkard, Foch, and Hrubec 1986). Twin studies have shown that genetic factors explain 40–80% of the variance in body mass index (BMI) and in risk of obesity (Herskind et al. 1996; Loos and Bouchard 2008c), while lower heritabilities have been reported for family (20–50%) (Luke et al. 2001; Rice et al. 1999) and adoption (20–60%) studies (Stunkard et al. 1986). As part of the recently GWAS discoveries, individual genome-wide association studies were combined through collaborative efforts in order to increase sample size and power to identify more common variants associated to obesity. The GIANT (Genomic Investigation of Anthropometric Traits) consortium is an international

collaborative initiative that brings together research groups focusing on anthropometric traits from across Europe and the USA. Data from seven genome-wide association scans for BMI ($n = 16,876$) were combined in their first meta-analysis (Loos et al. 2008). Despite a quadrupling increase in sample size compared with previous studies, only *FTO* and one new locus (188 kb downstream of *MC4R, "near- MC4R")*, out of ten loci that were taken forward for replication, were unequivocally confirmed. The near-*MC4R* locus was identified in another study in 2,684 Asian Indians, and confirmed in 11,955 individuals of Asian Indian and European ancestry (Chambers et al. 2008). The effect size was the same in both ethnic groups but the frequency of the risk allele in Asian Indians (36%) was greater than in white Europeans (27%), which might explain why this locus could be identified with a relatively small sample of Asian Indians in the discovery stage.
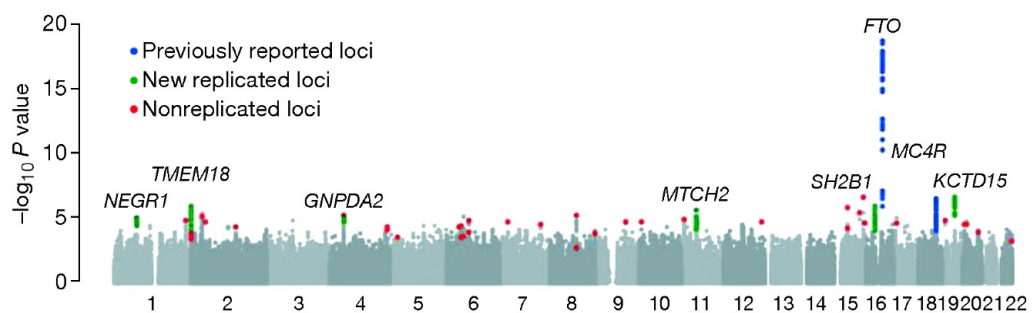


**Figure 1.1 Manhattan plot showing the significance association of all SNPs in the stage 1 meta-analysis with BMI (GIANT consortium).**
Single nucleotide polymorphisms (SNPs) are plotted in the x-axis according to their position in each chromosome, and association with BMI is indicated on the y-axis. SNPs previously reported to show association with BMI are shown in blue, signal examined but not confirmed are shown in red, and the new regions are highlighted in green (Willer et al. 2009b).

A third effort to identify obesity genetics variants, involved an increased sample size of 32,387 adults of European ancestry from 15 cohorts GIANT consortium (Willer et al. 2009a) (Figure 1.1). Of the 35 loci identified in the first stage of the genome-wide scan, eight loci were firmly replicated in an independent series of 59,082 individuals. These included the previously established *FTO* and near-*MC4R* loci and six new loci: near-*NEGR1* (neuronal growth regulator 1), near-*TMEM18* (transmembrane protein 18), in *SH2B1* (SH2B adaptor protein 1), near-*KCTD15* (potassium channel

tetramerisation domain containing 15), near-*GNPDA2* (glucosamine-6-phosphate deaminase 2), and in *MTCH2* (mitochondrial carrier homologue 2). In parallel to these analyses, deCODE Genetics performed a meta-analysis of four genome-wide association studies for BMI, including 34,416 individuals comprising Europeans and African Americans (Thorleifsson et al. 2009). A total of 43 SNPs in 19 chromosomal regions were taken forward for replication in 5,586 Danish individuals and for confirmation in discovery stage data of the GIANT consortium. Besides the *FTO* and near-*MC4R* loci, eight additional loci reached genome-wide significance. Of these, four loci (near-*NEGR1*, near-*TMEM18*, in*SH2B1*, near-*KCTD15)* had also been identified by the GIANT consortium, whereas four loci were novel: *SEC16B* (SEC16 homologue B), between *ETV5* (Ets variant gene 5) and *DGKG* (diacylglycerol kinase), in *BDNF*, and between *BCDIN3D* (BCDIN3-domain-containing) and *FAIM2* (FAS apoptotic inhibitory molecule 2). Despite the discovery of all the novel genetic variants associated to obesity, a recent study that genotyped the 12 obesity-susceptibility variants identified by the GIANT consortium and deCODE Genetics group in 20,431 individuals of a population-based study of white Europeans, showed that these genetic variants had a cumulative effect on BMI, with each additional risk-allele increasing BMI by 0.149 units, or weight by 444 g (Li et al. 2010a) (Figure 1.3). Nevertheless, together these 12 obesity-susceptibility loci explained less than 1% of the variation in BMI and had only limited predictive value of obesity. So far, the single effect of the genetic variant identified through GWAS for BMI are associated to a small effect size on BMI (lying between 0.06 and 0.66 kg.m$^{-2}$ per each copy of the risk allele) and a risk of obesity ranging between 1.03 to 1.32 odds. These recently discovered genetic variants bring in mind the substantial amount of missing hereditability that still remain unexplained in obesity (40–80%), if we consider that all genetic variants so far explain less than 1% of the BMI variation.
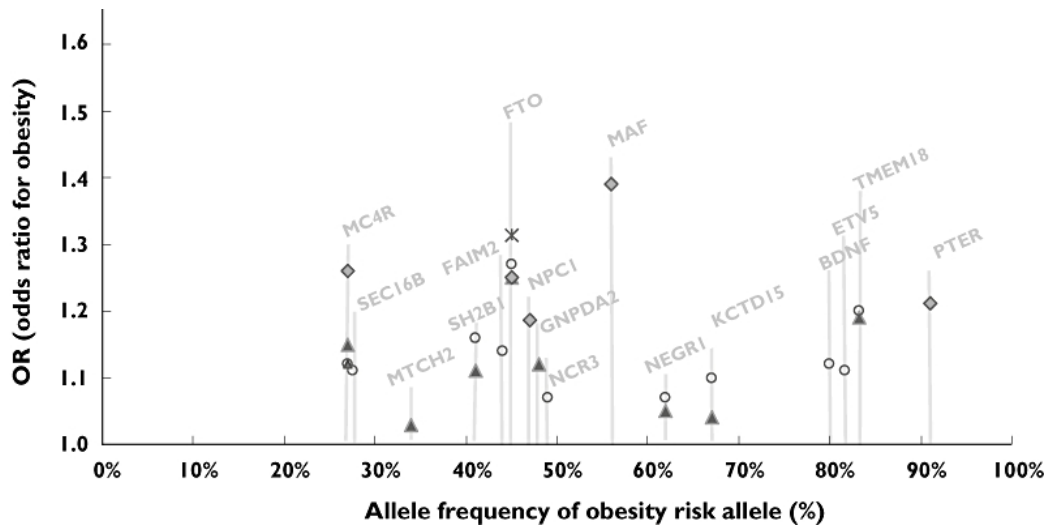
**Figure 1.2 Effect sizes for risk of obesity reported for the established obesity-susceptibility loci.**

Effect sizes represent the increased odds of obesity for each additional risk-allele. ORs for PTER and NPC1 were inferred from the OR reported for the dominant model. Frayling *et al*. (2007) (✖); Loos *et al*. (2008) (▲); Willer *et al*. (2009) (▲); Thorleifsson *et al*. (2009) ( ); Meyre *et al*. (2009) (◆)
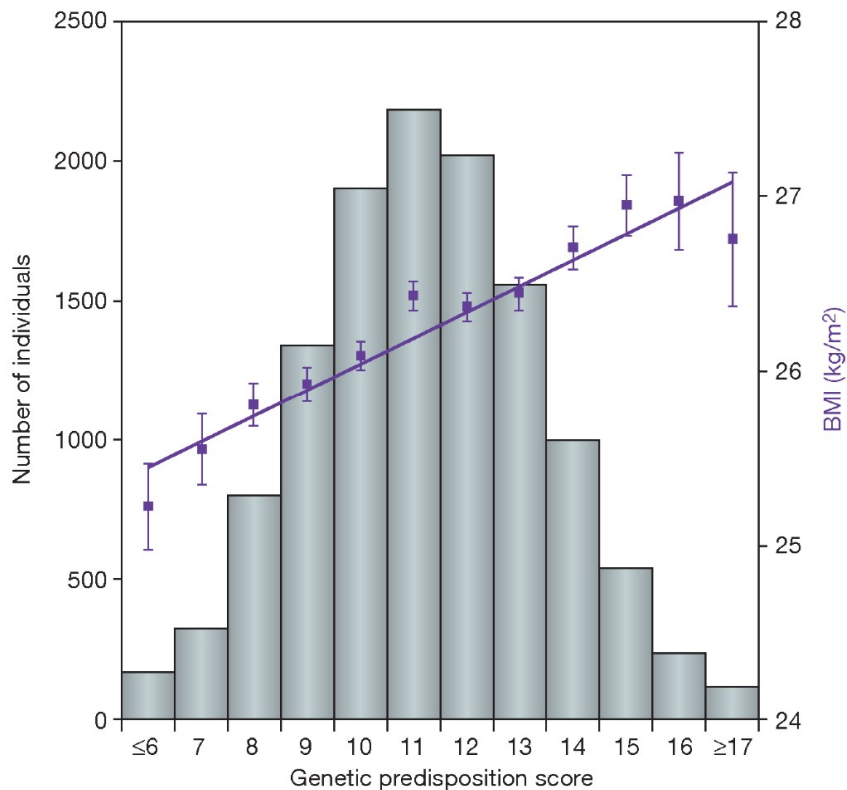


**Figure 1.3 Cumulative effect on BMI of obesity-susceptibility variants**

The distribution of the genetic predisposition score and cumulative effects of the risk alleles of the 12 variants on BMI (mean ± SE) are shown (n = 12,201). Each additional allele is associated with an increase in 0.149 BMI units, or 444g in weight (Li et al. 2010b).

### 1.13.3 Fat Mass and Obesity-associated Gene (FTO): Linked to Obesity, Insulin Resistance and Type 2 Diabetes in Risk Populations.

Fat mass and obesity associated gene, also known as *FTO*, is a gene found on human chromosome 16. Several SNPs of *FTO* - which is part of a large cluster of > 40 single nucleotide polymorphisms (SNPs), that lie within a 47 kilobase linkage disequilibrium (LD), block encompassing part of the first two introns as well as two exons (Loos and Bouchard 2008d; The International HapMap Consortium 2003), have been strongly associated to obesity-related traits and recently to an increased risk of developing T2D risk in populations of non white European backgrounds (Rees et al. 2011f; Ramya et al. 2011; Sanghera et al. 2008).

It is not surprising that initially the association between *FTO* and increased risk of T2D in white population were abolished after correction for BMI. An early section of this thesis presented evidence that obesity is clearly one of the driving risk factors behind developing T2D. However, not all subjects with diabetes are obese and not all obese develop diabetes. With this scenario in mind it is important to consider whether a possible association between a gene and T2D is in fact due to a gene associated with obesity or vice versa. We know that *FTO*, the strongest identified obesity gene so far, increases the risk of T2D (Dina et al. 2007f; Frayling 2007d). It is therefore not surprising that *FTO* was not associated with T2D in the GWAS when BMI was taken into account. Although insulin resistance is an early detectable defect in subjects at increased risk of developing T2D, obesity, and particularly abdominal obesity, it usually precedes insulin resistance in these individuals. It has been reported that about 40% of the variation in body fat can be attributed to genetic factors (Bouchard et al. 1988). The genetic influence is even more impressive for abdominal obesity. For example, genes are considered to explain 60% of the variance in abdominal fat in postmenopausal women (Samaras et al. 1997; Carey et al. 1996).

As mentioned above, the *FTO* gene variants predisposes to diabetes through an effect on BMI (Frayling et al. 2007k). Up to now, genetic variation in *FTO* has the largest effect on BMI, on individuals of European descent. Each risk allele increases BMI by 0.26-0.66 kg.m$^{-2}$ (Loos 2009b). Accordingly, the risk of obesity increases by 25-32%, for each additional risk allele. Although association results were initially

inconsistent in some populations with different ethnic background (Asians), there is increasing evidence that effect sizes are similar to those observed for European populations (Hotta et al. 2008; Cha et al. 2008b; Chang et al. 2008a; Al Attar et al. 2008; Villalobos-Comparan et al. 2008a; Wei et al. 1999h). Although, the relationship between *FTO* and T2D was initially mediated by obesity-related phenotypes in white Europeans populations (Frayling 2007a; Frayling et al. 2007m), recent studies suggest that *FTO* is related to an increased T2D risk (12 - 42%) independent of adiposity factors in other ethnic groups such as Hispanics, South Asians, Japanese and Chinese populations (Shimaoka et al. 2010d; Sanghera et al. 2008; Li et al. 2010c; Liu et al. 2010e; Bressler et al. 2010). Similar results have been found in children, where *FTO* variants were associated to measures of insulin resistance in obese children and adolescents independent of BMI (Jacobsson et al. 2008). Interestingly, all this studies that have reported a modest association between FTO and T2D/obesity, have made a considerable effort to increase the power of the study recruiting several thousands of participants. However, most of these studies have not improved the phenotypic characterisation of the cohort. Taken into account the important role of environemtn in the aetiology of T2D and obesity, it is important to determinate social, lifestyle and other environmental factors that could have a counfinding effect on the association between genes and complex diseases. This will help us to estimate more precise the real effect of single genetic variants on complex disease such as T2D and obesity.

### 1.13.4 Potential Role of the Fat Mass and Obesity-associated Gene (FTO)

The discovery of *FTO* as an obesity susceptibility gene has launched a series of analyses and experiments to unravel the physiological mechanism by which *FTO* variants influence obesity and thus lead to T2D. Despite recent progress, the mechanism by which SNPs in *FTO* influence human body mass remains elusive. Multiple processes could plausibly contribute to the risk of obesity, including neurological circuits governing appetite and whole-body energy expenditure, as well as peripheral pathways involved in energy expenditure. Loss of *FTO* function appears to reduce fat mass in mice, at least in part, through increased energy expenditure but not decreased energy intake (Fischer et al. 2009; Church et al.

2009a). Additionally, Gerken *et al.* have shown that *FTO* expression is increased by ~ 60% in the hypothalamus of mice in the fed state compared with mice in the fasting state (Gerken et al. 2007). However, the study of intermediate phenotypes in humans showed that *FTO* SNPs are associated with appetite and food intake but not energy expenditure (Fawcett and Barroso 2010c). Interestingly, data from rodents suggested that *FTO* might affect neuropeptide Y expression in the hypothalamus, which in turn is known to impact feeding behaviour. An investigation of the association between *FTO* SNPs/expression and neuropeptide levels in human hypothalamus might therefore provide a mechanism for the modulatory effect of *FTO* SNPs on appetite (Fawcett and Barroso 2010d). Others have also supported a central role of *FTO* through an effect on cerobrocortical insulin sensitivity (Tschritter et al. 2007). Tschritter and collaborators recently described a cerebrocortical insulin resistance in obese human (Tschritter et al. 2006). In lean humans, spontaneous cortical activity (beta and theta activity) is increased by insulin, while in obese individuals this effect was absent. In the brain, insulin acts as an adiposity and satiety signal and is critical for normal body weight regulation (Bruning et al. 2000b). Tschirts *et al.* found that the obesity risk variant was associated with a reduced insulin effect on beta activity, which implicates a lower cerebrocortical response to insulin. The effect of being overweight or obese on the insulin effect on beta activity was similar to that of the *FTO* risk variant. This implies that even though participants were matched with respect to BMI, the overall genotype effect on cerebrocortical insulin sensitivity was similar to the effect of increased weight. At least in non humans, insulin resistance in the brain has been shown to cause obesity (Bruning et al. 2000a). It is therefore conceivable that the decreased cerebrocortical insulin effect in humans describes a mechanism by which variation in *FTO* contributes to the pathogenesis of obesity. However, and despite the large amount of work focused on understanding the physiological mechanism of the *FTO*, the function of the gene and how it modulates obesity and other related disease is still not elucidated.

### 1.13.5    FTO and Environment Interaction on Obesity, Insulin Resistance and Type 2 Diabetes

The rapid increase in obesity and T2D during the past five decades must be ascribed to changes in environment and lifestyle factors rather than genes, as the genetic

background has not changed during this period. However, the genetic background determines how we respond to a specific environment. Evidence of gene-lifestyle interaction in the development of obesity and T2D was originally provided by descriptive epidemiological studies such as migration studies that compared the risk of disease between genetically related populations who have adopted different lifestyles. The best and clearest illustration of gene-environment interaction is the comparison of risk in obesity and T2D in Pima Indian native America populations. Pima Indians used to live across North America and Mexico, however due to colonization process and political conflict between the indigenous communities and the new westernised government most of natives Pimas where extinguish in North America or forced to migrate towards Mexico. Nowadays the largest numbers of Pima Indians are located in Arizona. Several studies conducted on those Pimas living in Arizona, showed a prevalence of 69% for obesity and 55% for T2D, while those, with the same ethnic background, living in traditional environments of the remote Mexican Cierra Madre Mountains, reported a prevalence of 13% of obesity with only 6% having T2D. This finding shows that despite a similar genetic background and predisposition, different lifestyle may result in a different prevalence of obesity and T2D. White Americans living in a similar obesogenic environment but who have a different genetic background are much less susceptible to developing obesity (~32%) and T2D (~8%) compared with the Pima Indians living in Arizona (Ravussin et al. 1994a; Ravussin 1993a; Esparza et al. 2000a).

In addition to descriptive epidemiological studies, recent genetic association studies have also identified gene-lifestyle interactions, as can be seen for *FTO*. Recent gene-environment interaction studies in white populations have show that the association between *FTO* variants and obesity-related phenotypes is more pronounced in individuals that are more physically inactive, whereas the association is diminished in those who have physically active lifestyles (Andreasen et al. 2008e; Vimaleswaran et al. 2009d; Lee et al. 2010a; Rampersaud et al. 2008f). These observations suggest that genetic susceptibility towards obesity induced by variation in *FTO* can be overcome by adopting a physically active lifestyle. Yet, lifestyle intervention studies in genetically susceptible individuals could not confirm interaction with *FTO* in relation to weight loss (Haupt et al. 2008b; Franks et al. 2008b; Lappalainen et al. 2009a). So, far no interaction studies have reported an *FTO*\*environment interaction

despite the increasing evidence that relates the *FTO* genetic variant to increased risk of T2D.

The gene-environment relationship is a key issue not only in understanding the pathogenesis of multifactorial diseases, but also in designing appropriate treatments for affected populations. As previously discussed some populations such as South Asians, Pima Indians and Mapuches present higher obesity rates and an increased T2D susceptibility when they adopt a westernised lifestyle. Despite the substantial evidence of an increased susceptibility in some ethnic minorities, there is a lack of gene-environment interaction studies on these populations. Understanding how genes and lifestyle factors interplay may help to understand how these lifestyle factors could modulate genetic contribution to risk of complex diseases and may explain why certain populations appear to be at increased risk of obesity and T2D.

## 1.14 Summary

In summary, the literature reviewed detailed in this chapter has explored the increasing prevalence of obesity and insulin resistance, with the subsequent risk of T2D and cardiovascular disease, as well as the aetiology of insulin resistance and potential factors that could explain the progression of this chronic disease. The pathophysiology of insulin resistance is complex and multifactorial and in many individuals is likely to be a consequence of the interplay between lifestyle and genetic factors, such as a family history of diabetes or ethnicity. Evidence was described that supports the susceptibility to obesity and T2D of certain ethnic groups. These genetic factors could play an important role when risk populations are exposed to an obesogenic environment, which promotes substantial changes in traditional lifestyle patterns, such as increasing or promoting sedentary lifestyle in conjunction with a 'westernised' diet.

Considering the migration and urbanization tendency during the last decades, not only developed countries are experiences an increased frequency of obesity and T2D, but also developing countries have been experiencing this rapid transition from infectious disease to a non-communicable disease, such as obesity and T2D. In this overview attention was focused on South America, specifically Chile, where the

prevalence of T2D is rapidly increasing. A major contributor to this increase is likely to be the transition from rural to urban environments for large portions of the population. However, the increase in diabetes risk associated with adopting an urban lifestyle may not be the same for all American populations, as exemplified by research on the Pima Indians. Pimas living a traditional rural lifestyle in Mexico are lean, active and have low diabetes incidence, whereas Arizonan Pima have a prevalence of diabetes in the adult population of 50%. This effect may also be evident in other Native American populations such as the Mapuches in Chile. Mapuche populations living a traditional rural lifestyle appear to be relatively protected, with limited data existing, against diabetes risk (~1%) which increases markedly in the urban environment (~8%). In comparison, a Chilean European population showed lower prevalence of T2D in the urban environment. Thus, the hypothesis of this study is the following: Mapuches, in common with other ethnic groups such as South Asians and Pima Indians, may have a genetic background that makes them more susceptible to the adverse metabolic consequences of living an urbanized lifestyle. Understanding differences in the physiological responses of Mapuche and European populations in a common, westernized urban and rural environment may help to explain why certain populations appear to be at increased risk of cardio-metabolic diseases when they adopt a westernised lifestyle.

## 1.15 Aims and Objective of the Study

The overall **aim** of this study is to investigate the interplay between genetic and environmental influences on predisposition to T2D and on related physiological traits in the Chilean population. The focus will be particularly on indigenous populations that show increased risk of obesity, diabetes and related cardio-metabolic phenotypes when exposed to a 'westernised' environment, and to characterise the relative contributions of individual genetic variants, and environment and gene-environment interactions to this risk. In doing so, this investigation will also allow comparison between indigenous and immigrant components of the population that display different risks in different environments. These findings will hopefully add to existing efforts aiming at finding appropriate treatments and effective preventative

programmes, with concomitant improvements in public health in Chile. To achieve this overall aim, the study will address the following **specific objectives**:

➢ To characterize anthropometric, dietary, physical activity and physiological profiles in Mapuche and European Chilean populations living in traditional rural environments and westernized urban environments. (Chapter 4)

➢ To determine whether the influences of dietary intake, adiposity, physical activity and fitness, on insulin resistance differ between Chilean populations with Mapuche and European ethnic backgrounds. (Chapter 5)

➢ To determine whether the effects of dietary intake, physical activity and fitness, on adiposity-related traits differ between Chilean populations with Mapuche and European ethnic backgrounds. (Chapter 5)

➢ To investigate the association between the *FTO* gene and obesity-related phenotypes in Chilean populations of European and Mapuche origin. (Chapter 6)

➢ To assess the influence of *FTO* genotypes on insulin resistance, as a marker of type 2 diabetes risk, in Chilean populations of European and Mapuche origin. (Chapter 6)

➢ To assess the influence of *FTO* genotypes on total energy intake, fat and carbohydrate intake in Chilean populations of European and Mapuche origin. (Chapter 6)

➢ To investigate how physical activity levels and cardio-respiratory fitness modulate the association between genetic variation in *FTO* and obesity-related phenotypes as well as insulin resistance in Chilean populations of European and Mapuche origin. (Chapter 6)

➢ To determine the strength of relationship and agreement between objectively (Actigraph) and subjectively (IPAQ) measured physical activity and sedentary behaviours.  (Chapter 3)

➢ To determine whether the relationships between physical activity/sedentary time and risk factors for vascular and metabolic disease differ between objective and subjective measures of physical activity and sedentary behaviours. (Chapter 3)

# 2  Materials and Methods

This chapter is divided into three main parts. In the first part the overall study design is described in detail and the field work stage (including methods of recruitment and sample collection) is discussed. The second part describes the collection of data on body composition, nutritional patterns, physical activity behaviours and socio-economic circumstances. Finally, the third part describes the biological sample collection and the analysis involved in the determination of metabolic markers and genotyping.

## 2.1  Study Design, Collection and Recruitment Methods

In order to fully understand the study design and implementation, it is important to appreciate the social, cultural and demographic characteristics of Chile, and how these factors were taken into account when designing the study.

### 2.1.1 Chilean Population Background and Geographical Shape: General Considerations for the Study Design and Recruitment Methods

The first consideration for the study design was the ethnic background of the Chilean population. Chile's 2002 census reported a population of 15,116,435 people. Approximately 85% of the country's population lives in urban areas, with 60% living in Santiago, the capital of the country (INE 2002a). Chile is a multiethnic society; the Chilean population comprises of a number of Amerindian native groups, migrant populations of European origin, and an increasing number of individuals of mixed origin resulting from interbreeding between these two contingents.

Consistent with the 2002 Census, people of European origin made up 52.7% of the population and Mestizos (people of mixed background) made up 44% of the population (Figure 2.1). The European portion of Chile's population consists mainly

of people descended from Spanish settlers (predominantly Castilian, Andalusian and Basque), with minorities having German, Italian, Irish, French, British, Swiss, and Croatian ancestry, singly or combined. The Mestizo population derives the European part of their ethnicity from colonial Spanish settlers, while their indigenous component originates from various tribes of Native American backgrounds.

According to the 2002 Census, 4.6% of the Chilean population is indigenous (Figure 2.1). These indigenous groups are almost certainly common descendants of a single wave of migration southwards throughout southern South America in the Holocene period (Rocco et al. 2002). The largest indigenous group comprises the Mapuches; which combine several tribal sects living mainly in the central and south portion of the country. In recent times, the Mapuche population has undergone geographical redistribution and individuals now live in both rural areas and in the main urban centres such as Santiago, Concepcion, Temuco and Valdivia. From the last Chilean census (2002), the total Mapuche population numbered >600,000 inhabitants (comprising 4.6% of the population of Chile). Nearly 40% of the Mapuche live in urban environments and have a more-or-less westernized lifestyle (INE 2002b). The remaining portion of the Mapuche population (~60%) has conserved their traditional and ancestral lifestyle (Figure 2.2).

The second criterion for designing the study was the particular geographical characteristics of the country. Chile is a country located in South America occupying a long, narrow coastal strip between the Andes mountains to the east and the Pacific Ocean to the west. It borders Peru to the north, Bolivia to the northeast, Argentina to the east, and the Drake Passage in the far south. The Pacific coastline of Chile is 6,435 km long while the shape of Chile is a distinctive ribbon of land 4,300 km long and on average 255 km wide; therefore Chile is a country with a wide range of landscapes and weather conditions.

Finally, due to the geographical shape of Chile, the climate was deemed as an important aspect and was accounted for when designing the study protocol. Chile extends across 38 degrees of latitude resulting in a wide range of weather conditions across a large geographic scale; therefore generalisations are difficult. According to the Köppen system, there are at least seven major climatic subtypes within Chile's

borders, ranging from desert in the north, to alpine tundra and glaciers in the east and south east, humid subtropical in Easter Island, Oceanic in the south and Mediterranean climate in central Chile. There are four seasons in most of the country: summer (December to February), autumn (March to May), winter (June to August), and spring (September to November).
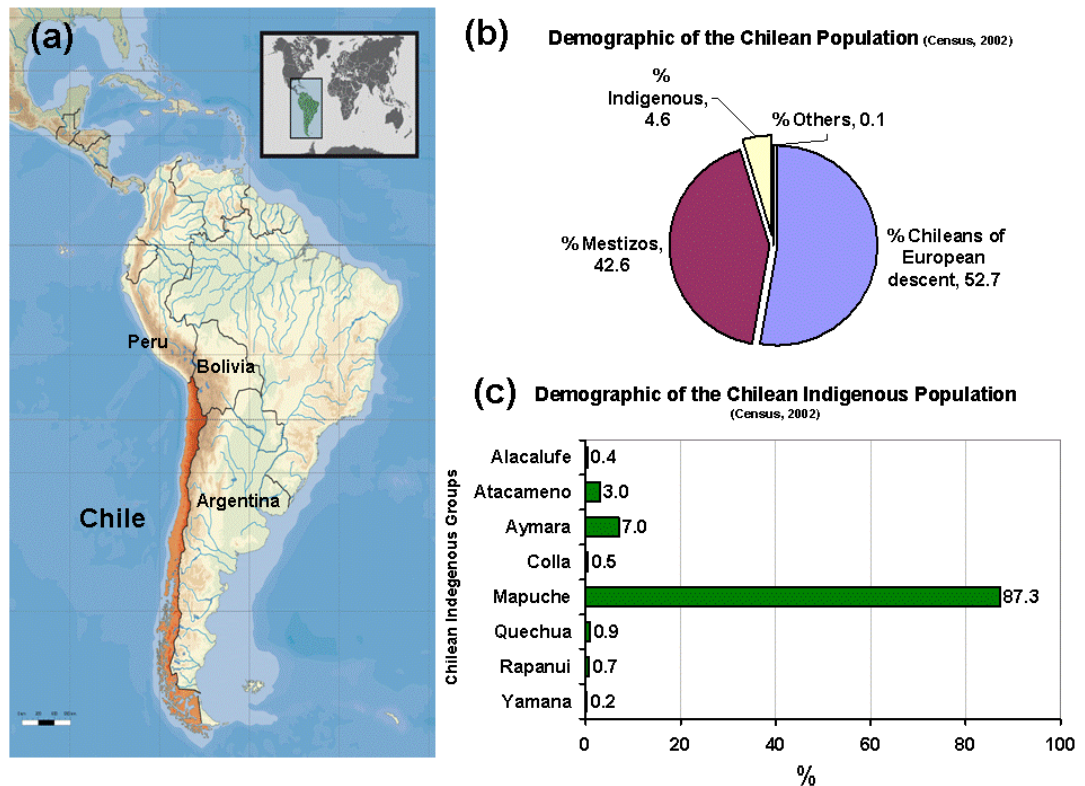


**Figure 2.1. (a) Geographic location of Chile in South America. (b) Demographic distribution of the Chilean population. (c) Demographic distribution of the indigenous population of Chile (INE 2002a).**

## 2.1.2 Chile Population Distribution: A Key Point for Recruitment Design

The Chilean population has a particular ethnic distribution across the country. The indigenous population comprises a number of American natives groups that together make up around 4.6% of the total Chilean population. Mapuche natives are the largest ethnic group in the country, representing around 87.3% of the total Chilean

indigenous population. The second largest ethnic minority is the Aymaras native groups, which comprise up to 7% of the total indigenous population.

The Mapuches are indigenous inhabitants of south-central Chile and southwestern Argentina. They comprise various groups with wide-ranging ethnicities who share common social, religious and economic backgrounds, as well as a common linguistic heritage called "Mapudungun" (from mapu 'earth, land' and dungun 'speak, speech'); this is a language spoken solely in south-central Chile and west central Argentina by the Mapuches.

The 2002 Chilean Census reported that many Mapuche descendants now live across southern Chile; some maintain their traditions and continue to make a living from agriculture, but the majority has migrated to cities in search of better economic opportunities. Many of the Mapuches are concentrated around larges urban settings in the central and south part of Chile, such as Santiago in the Metropolitana region and Concepcion in the Biobio Region. Chile's region IX, the former Araucanía, has a rural population that is 80% Mapuches; there are also substantial Mapuche populations in Los Rios Region (XIV), Los Lagos Region (XIV), Biobio Region, and the XI, XII Regions (Figure 2.2).

## (a)

### Distribution of the Chilean Population By Ethnicity and Enviroment (Census, 2002)



## (b)

### Distribution of Mapuches by Region in Chile (Census, 2002)
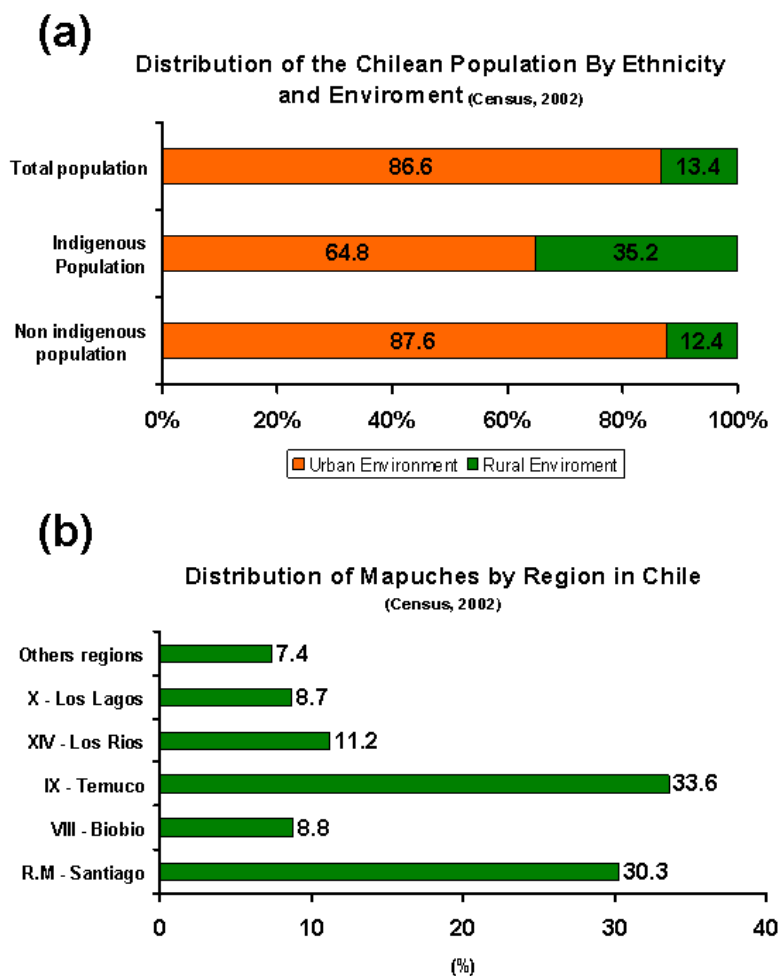


**Figure 2.2. (a) Distribution of Chilean population by residence environment. (b) Demographic distribution of the Chilean indigenous population by region of residence (INE 2002a).**

## 2.1.3 Study Design

To address the aims of our study, and taking into account the information detailed in the previous section regarding the geographic, human demographic and cultural aspect of Chile, we undertook detailed measures of lifestyle factor and other physiological risk factors for type 2 diabetes and cardiovascular disease in Mapuche populations living in rural and urban environments and Chileans of European descent living in rural and urban environments.

For logistic and recruitment purposes, the present study was coded and known as the "GENADIO Study" (from GEN 'genes', A 'ambiente / environment' and DIO

'diabetes and obesity'). This name and the logo shown in Figure 2.3 was used throughout the field work and data collection.



**Figure 2.3. Logo of the study used during the fieldwork.**

## 2.1.4 GENADIO Study: Sample Size Calculation

Data on which to base a sample size calculation for this study were limited as there have been few previous investigations of insulin resistance in Mapuches. The available data, based on small samples, indicated that the population SD for $HOMA_{IR}$ was ~3-4 units in Mapuches (Perez-Bravo et al. 2006d), and <1 unit in the non-obese urban Chilean general population (Acosta et al. 2002). Based on this, we estimated an overall population SD for $HOMA_{IR}$ of ~3 units, and predicted that the study would need power to detect mean group differences of ~2 $HOMA_{IR}$ units. Power calculations using Minitab (version 14, Minitab Inc. Pennsylvania) indicated that 30 participants of each sex in each of the four study groups (*i.e.* Rural Mapuches, Urban Mapuches, Rural Europeans and Urban Europeans) would enable 80% power to detect such differences in $HOMA_{IR}$ between groups at the $p < 0.05$ level. This was therefore set as our minimum recruitment target. The upper limit for participant numbers was determined by participant availability within the data collection time-frame.

## 2.1.5 GENADIO Study: Recruitment Methods

The sampling strategy was cross-sectional in design and involved the planned collection of data from four groups of participants: Mapuches living in rural (MR)

and urban environments (MU) and Chileans of European descent living in rural (ER) and urban environments (EU). Participants recruited for this study, aged between 20 to 60 years. The young age selection criteria was set up at 20 years because approximately from this age onwards, individuals have completed their maturation process, while the old age was set up at 60 years due to the high prevalence of T2D observed on individuals above 60 years. All the recruitment were conducted between February and June 2008, through open invitations to residents in a number of villages and towns/cities, via local radio advertisements, and by invitations to members of local community organisations in rural and urban areas in three regions of Chile: Los Ríos, Bio-Bio and Metropolitana. These 'open' 'general' methods for advertising the study were employed in an attempt to ensure that all groups within each society had an approximately equal chance of seeing the study and of signing up to it, thereby minimising potential recruitment bias. The recruitment methods applied for each group are fully described below:

**Rural Mapuches (MR)**: A total of 247 individuals responded to our call for volunteers. Participants were recruited from population living a traditional, rural lifestyle outside the city of Concepcion, Temuco, Valdivia and Osorno in Chile's VIII, IX, X and XIV regions, respectively (Figure 2.4). Mapuche participants living in rural settings were recruited through two principal forms:

➢ An open invitation to rural residents through the leader or chief in each indigenous community (rural Mapuches live in isolated communities named "reservaciones" which are ruled by a "political chief" and a "spiritual leader"). Prior to recruiting participants from any community, both the Chief and the Spiritual leader had to agree with the study purpose. If either leader disagreed with the study, no recruitment was allowed at that village or community.

➢ An open invitation by invitations to members of local community organisations, as well as primary health care centres, or social-leisure activity establishments located near their "reservaciones".

**Urban Mapuches (MU):** A total of 187 individuals responded to our call for volunteers. Participants were recruited from the main urban cities of Santiago, Valdivia, Temuco and Concepcion in Chile's R.M, XIV, IX, and VIII regions, respectively (Figure 2.4). These cities have adopted a clear westernised lifestyle with an urbanisation level higher than 85%. Mapuche participants living in urban setting were recruited through two principal forms:

➢ Using available database information about Mapuches living in Santiago, Valdivia, Temuco and Concepcion cities.

➢ An open invitation via local radio advertisements, and by invitations to members of local community organisations, as well as primary health care centres, educational centres (such as universities) or social-leisure activity establishments.

**Rural and Urban European:** A total of 439 individuals responded to our call for volunteers, out of which 216 were rural individuals. Urban participants of Chilean European descent (EU) were recruited from the main urban cities of Santiago, Valdivia, Temuco and Concepcion in Chile's R.M, XIV, IX, and VIII regions, respectively. Rural individuals were recruited from rural settings outside the cities of Concepcion and Valdivia in Chile's VIII, and XIV regions, respectively (Figure 2.4). Europeans participants living in urban and rural setting were recruited through two principal forms:

➢ An open invitation via local radio station, or by invitations to members of local community organisations, as well as primary health care centres, educational centres (such as universities), or social-leisure activity establishments.

➢ Using available access database information about Europeans living in the main urban cities of Chile. These databases are public record of people living in a specific location (village, city, or county) and generally describe the ethnicity rate in each location. After identifying those locations with a similar

proportion of both ethnic groups, the recruitment was planned and executed. This strategy was used to recruit participants from both ethnic groups living in similar environmental conditions.
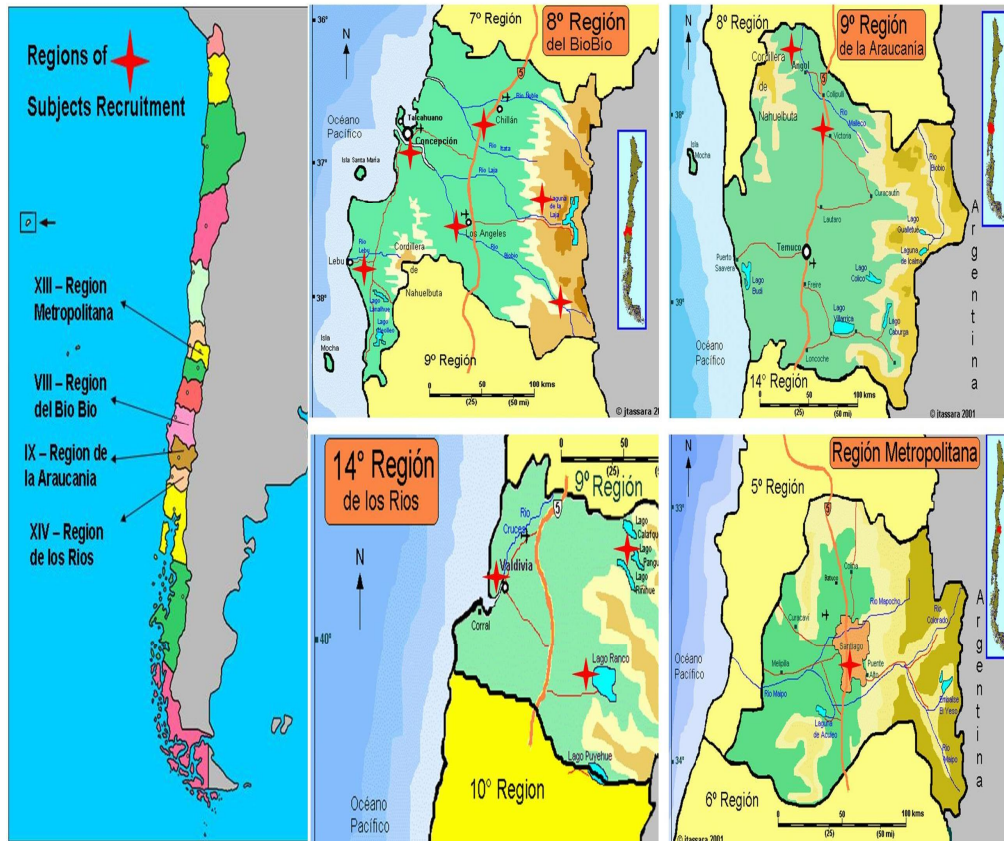


**Figure 2.4. Samples collection and recruitment locations**

## 2.1.6 GENADIO Study: Inclusion and Exclusion Recruitment Criteria

Taking into account that people of European origin made up 52.7% the Chilean population and that Mestizos (population of mixed background) made up 44% of the population, it was essential to ensure that Mapuches and European population groups with as little admixture as possible were studied. The following inclusion criteria were therefore applied:

Mapuches were included if they a) had both maternal and paternal last names of Mapuche origin, these names being identifiably different from European names; b) had both parents of Mapuche origin, c) were of type O blood group in the ABO blood group system (the available data suggest that the Mapuches have a very high frequency (95%) of this blood group (Perez-Bravo et al. 2006c), thus possession of A or B blood groups is likely to indicate mixed ancestry); c) Individuals with a known history of cardiovascular disease or taking anti-hypertensive or diabetes medications, as well those women in pregnancy were excluded from participation. All those participants with unknown diabetes or impaired glucose tolerance condition during the exam period were invited to visit their medical general practitioner (GP).

Europeans were included if they had a) both maternal and paternal last names of European origin; b) both parents of European origin; c) Individuals with a known history of cardiovascular disease or taking anti-hypertensive or diabetes medications, as well those women in pregnancy were excluded from participation.

A total of 873 individuals (247 MR, 187 MU, 216 ER, 223 EU) responded to our call for volunteers. Of these, 472 individuals (123 MR, 124 MU, 91 ER, 134 EU) fulfilled the inclusion/exclusion criteria and agreed to participate in the study. Of the 401 individuals who responded but were not included, 182 subjects showed evidence of mixed ethnic background, 62 subjects were out of the age inclusion range (20 to 60 years), and 47 subjects reported a confirmed diagnosis of metabolic disease (of these 28 were diabetic, 16 were hypertensive and 3 were under hormone therapy). The number of subjects that refused to participate in this study was 110; the main reason was religious belief. All participants gave written informed consent prior to inclusion in this study, which was approved by Research Ethics Committees at the University of Glasgow, University of Chile, and University of Concepcion (Appendix B). All participants were told that personal health-related phenotypic data would be communicated back to them via their general practitioner, but they received no other incentive for participating.
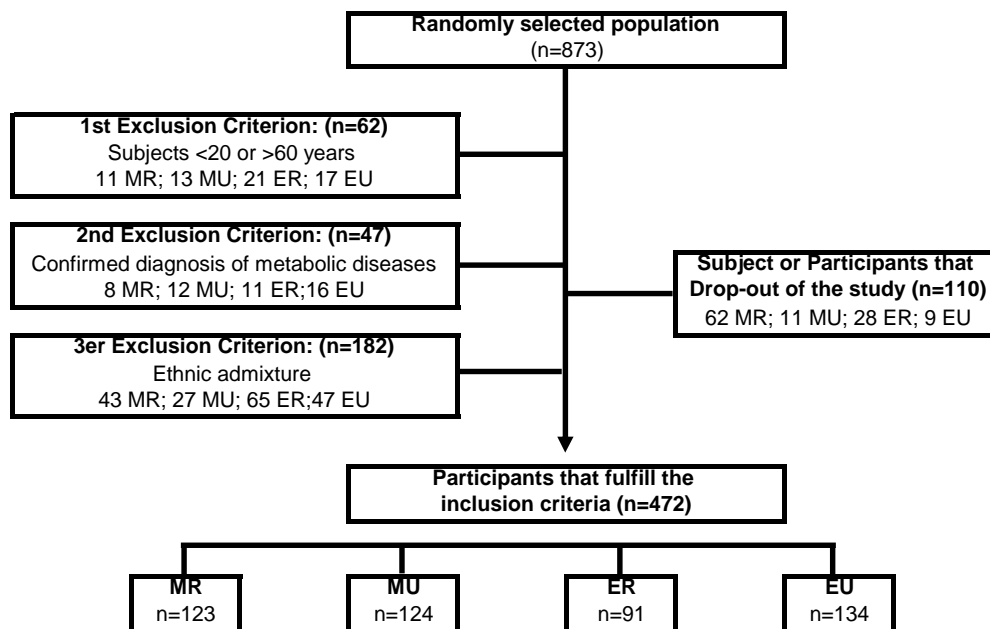
**Figure 2.5. Flow diagram of recruitment of the study population.**

## 2.1.7 Multi-cultural Approach Strategies: the Case of Mapuche Indigenous Population

During the design and planning of the GENADIO study, a lot of unknown but relevant details related to cultural differences between the European and Mapuche populations were not taken into account for the study design. However, all these details were accounted for during the field work.

One of the main challenges was the physical and cultural isolation of the rural Mapuche population. Mapuches are the major ethnic group in Chile; despite the long period of time in which they have been exposed to the Chilean European culture, a large proportion of them still strongly preserve their ancestral beliefs and traditions.

Nowadays, Mapuche living in urban environments are similar to the non-indigenous population in many respects. To date, urban Mapuches have adopted most of the westernized lifestyle, accepting with more ease the Chilean European culture and medicine (western medicine). On the other hand, rural Mapuches strongly protect their belief, traditions and native ancestral medicine based on natural products. For

this reason, rural Mapuches are located in particularly isolated geographical areas, far from any influence of the occidental culture. In some ways they view white European traditions as 'the enemy'. Because of this, our study was not accepted in a straightforward manner by the indigenous living in rural settings, who generally keep a distance from the commonly accepted public health systems and medicine as it is practiced today. Words like diabetes, cardiovascular risk, metabolic disease and obesity were not common expressions for them. Furthermore, Mapuche medicine strongly believes in the role of the machi "shaman". The machi performs ceremonies for curing diseases, warding off evil, influencing weather, harvests, social interactions and dream work. Machis often have extensive knowledge of Chilean medicinal herbs. For them, a traditional blood examination will have a different meaning. For Mapuches whole human blood has a special spiritual value related to deities and/or spirits in Mapuche mythology; for this reason most of the rural communities are not enrolled in preventive medicine programs like the people of European origin living in the same places. This made our recruitment strategies for the rural Mapuches communities more complex and ineffective than Europeans. To improve the level of acceptance we included in our research group the help of an indigenous language translator and also the support of a Mapuche natural medicine leader (know as "Machi or shaman of the tribe"). Once these new strategies were applied to the recruitment of rural Mapuches communities, rural Mapuches were less reluctant to take part in our study.

## 2.1.8 Recruitment Strategies: Logistic and Transportation Issues

A second challenge faced was the transportation and logistic aspects related to sample collection and conservation. As was described previously, Mapuche populations can be found in isolated areas of Los Rios, BioBio and Temuco regions of Chile. Their lifestyle is drastically different to the westernised lifestyle of the urban setting. The rural Mapuches generally live in remote areas without road access or electricity, with some Mapuche villages being a 2-5 hour horseback journey from the nearest road. For this reason, data collection from these villages required transportation of all equipment (including generators to provide electricity,

centrifuges to spin blood, dry ice and a portable and adjustable testing laboratory) by horseback from site to site (Figure 2.6 c-d).

One visit to the rural Mapuches communities involved a trip by horseback from site to site. Once we arrived at each village, an introductory meeting was arranged with the chief and the spiritual leader of the community. The response from each community's leader generally came during the same day or the day after the meeting. If this was the case, we set up temporary base (sleeping bags, portable facilities such as kitchen, storage room and electrical power supply) outside the community perimeter (Figure 2.6 a-b). If the community decided to take part in our study all the recruitment and testing were carried out during 7 consecutive days; on the seventh day, the fasting blood sample testing was carried out. Dry ice was used to ensure that the collected blood samples were kept frozen for future storage at -20 C.



**Figure 2.6. Illustration of the logistic aspect related to transportation and accommodation during the fieldwork.**

### 2.1.9 Adaptation of the Standard Laboratory Methods to the Reality of the Field Work

As data collection from some of these villages involved transportation of all equipment (including generators to provide electricity and centrifuges to spin blood) by horseback from site to site, sophisticated testing methods were not the most appropriate for the reality of the field work (Figure 2.7). For this reason, the simplest but valid and flexible methods were chosen to assess the wide range of metabolic and physical phenotypes as well as dietary and physical activity patterns. All these methods are described in detail in the following paragraphs.



**Figure 2.7. Illustration of the field work data collection in the rural environments.**

## 2.2 GENADIO Study: Data Collection and Sample Analysis

This section describes two main sub-parts; the first part is related to the assessment of anthropometrics, body composition, dietary intake and physical activity measurement. The second part is related to the analytical methods and genotyping analysis carried out in standard university research laboratories. All the field work, data collection, analytical laboratory work and genotyping analysis were carried out by myself and with the collaboration of field work assistants.

### 2.2.1 Field Work Data Collection

It is important to highlight that all anthropometrics, body composition, socio-economic, health screening, dietary and physical activity assessment were carried out in Chile. Moreover, I was leading the fieldwork team and testing all participants of this study. Two Chilean student nurses and one Mapuche language translator assisted the author in the fieldwork. Their main role was the application of the questionnaires; I instructed them previously to the data collection to standardise the interview and questionnaire protocols. I was in charge of the logistic aspect of the field work and the adaptation of data collection which was conducted in two drastically different settings; in the "traditional environment" where no laboratories, hospital or clinical facilities were available, and in the "urban environment" where all the standard laboratories, clinical and research facilities were accessible. Due to differences in lifestyle and political organizations of European and Mapuche populations living in rural and urban environments, the recruitment process could be exposed to recruitment bias. Howeverr, in order to avoid this, and ensur a reduced bias in sample recruitment, socio-economic and demographic data were also collected to verify that recruited participants were similar in both ethnic groups and environments.

## 2.3 Anthropometry and Body Composition

### 2.3.1 Height

Height was measured using a stadiometer (Portable stadiometer, Seca model 214). The volunteer was measured barefoot, with their back positioned against a fixed backboard and their arms relaxed in the lateral position. The head was also positioned against the backboard, with the line of eyesight perpendicular to the backboard. Measurement was performed when the volunteer was positioned and relaxed, and a moveable headboard was lowered on to the top of the head with light pressure allowing hair compression. The investigator applied gentle upwards pressure underneath the angle of the mandible and measurement was made to the nearest 0.01 metre.

### 2.3.2 Body Mass

Body mass was measured using a digital weight scale (Tanita Scale, model BC 351). The same scale was used for all volunteers throughout the study. Subjects were weighed wearing only lightweight clothing (*e.g.* shorts and t-shirt) and no shoes. Extraneous jewellery and clothing was removed prior to weighing. Body mass was measured with both feet flat on the balance and with arms positioned in the lateral position. Measurement was made to the nearest 50 g.

### 2.3.3 Body Mass Index (BMI)

BMI was calculated as the body mass in kilograms divided by the square of the height in metres. Categorisation was done using the World Health Organization criteria; a BMI over 25 ($kg.m^{-2}$) was defined as overweight, and a BMI of over 30 ($kg.m^{-2}$) as obese (WHO 2006c).

### 2.3.4 Skinfold Thickness and Body Composition

All measurements of skinfold thickness were performed in private by a qualified researcher of the same sex as the participant. This method was chosen as its portability and cost-efficiency (in contrast to other methods, such as DXA and underwater weighing) made this method the most suitable for collecting data in different environments. Measurements were made to 0.1 mm using Harpenden skinfold calipers (Cranlea & Company, Birmingham, UK) according to the International Society for the Advancement of Kinanthropometry protocol (ISAK) (Norton & Olds, 1996). The same calipers were used throughout the study. The skinfold was held between the investigator's thumb and index finger, the caliper was applied and the measurement made after pressure had been applied for between 5 and 8 seconds. The left hand side of the body was used for measurement, and measurements were made with the volunteer standing up. Biceps skinfold was marked with the elbow flexed at an angle of 90º, at the midpoint between the most lateral point of the acromion and the inferior border of the olecranon. Once marked, the arm was allowed to hang loosely with the palm facing anteriorly, and the measurement made in the midline of the biceps muscle, at the level of the mark. Triceps skinfold was measured at the same level as the biceps measurement, on the posterior aspect of the arm, in the midline. The subscapular skinfold was measured along the line of natural skin cleavage inferior to the scapula, with both arms hanging loosely by the volunteer's side. The suprailiac fold was measured above the iliac crest, on a diagonal fold beginning at the anterior axillary line.

Body composition of the subjects was estimated using the recommendations of ISAK protocol (Norton & Olds, 1996). In order to determine % bodyfat (%BF) using anthropometry, body density was first calculated using a prediction equation and then converted into %BF values. In the current study, body density was predicted using an equation developed by Durnin and Womersley (1974).

$$\text{Body Density} = 1.1765 - 0.0744 \, (\log_{10} \Sigma X1 \text{ skinfold})$$

Where   X1= triceps + biceps + subscapular + suprailiac skinfolds in millimetres. Body fat was predicted using Siri's %BF prediction equation (Siri 1993).

$$\% \text{ Body Fat} = (495 \text{ / Body density)} - 450$$

### 2.3.5 Waist and Hip Circumference

Circumferences were measured using a non-elastic tape measure, and measurement was made with the abdominal muscles relaxed, at the end of normal expiration according to ISAK protocol. Both hip and waist measurements were taken three times, and an average of the three readings was noted. Waist circumference was measured horizontally around the waist, at a point midway between the superior border of the ilium and the inferior border of the lateral margin of the ribs (costal margin). Hip circumference was measured around the point of maximal width around the hip region, at approximately the level of the pubic symphysis. The same investigator performed the measurements on each volunteer, on every occasion. Waist to hip ratio was determined by dividing the waist circumference by the hip circumference.

### 2.3.6 Assessment of Cardiorespiratory Fitness

Cardiorespiratory Fitness was assessed using the Chester Step Test (CST) (Sykes 1999). The CST is a multistage, sub-maximal test which requires the subject to step on and off a step at a rate set by a metronome or music beat tape (Figure 2.8). CST is an incremental test, commencing at the relatively slow pace of 15 steps per min and increasing every two minutes to 20, 25, 30 and 35 steps per min. The step heights of 6" (15 cm), 8" (20 cm), 10" (25 cm) and 12" (30 cm) have been selected so the results are largely independent of the individual's height and leg length. Mechanical efficiency of the upper and lower limb is maximised by the vast majority of both males and females during the stepping exercise when using these step heights (Sykes 1999). The choice of step height depends essentially on the age and physical ability of the subject. We aimed to select step heights that enable the participant to comfortably reach at least Level III of the test, giving a minimum of 3 points on the

graph. Heart rate was measured every 15 second during the test using a short-range telemetry system, which utilised a chest wall sensor with transmitter and a watch device which received the signal, and displayed the heart rate (Polar s610i Heart Rate Monitor, Polar Electro Oy, Kempele, Finland). Maximum Heart Rate (HR) was calculated by the formula ($HR_{max}$= 220 - Age). Test, were terminated once subjects reached 80% of age-predicted maximum heart rate. The Borg Scale was used to estimate the Rating of Perceived Exertion (RPE) of the participant performance (Borg 1983). RPE has been found to be a reliable and valuable indicator in monitoring a person's exercise tolerance and correlates well with exercise heart rates and oxygen consumption values.

The CST prediction of cardiorespiratory fitness ($VO_{2max}$) is based on the extrapolation of a "line of best fit" assuming a linear relationships between workload, oxygen consumption and exercise heart rates (Astrand and Rodahl 1986). Work rate (WR) is given by the product of step height (h), the mass of the subject (m), the acceleration due to gravity (g), divided by time (t) [$WR = m.g.h.t^{-1}$]. This is proportional to oxygen consumption ($VO_2$). The extrapolation passes through the submaximal heart rate response for each stepping stage, up to a level which equals the participant's age estimated $HR_{max}$. At this point a vertical line is dropped down to the x axis of the graph, which represents the estimated $VO_2$ for box stepping exercise. The assumptions of this of predictive procedure include the following: that $VO_2$ can be estimated from the work rate of stepping, that a linear relationship exists between each stage of the CST with heart rate and $VO_2$; that $HR_{2max}$ and $VO_{2max}$ are coincident; and that maximal heart rate is equal to 220 minus the participant's age.

All cardiorespiratory fitness calculations were computed in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, USA). Cardiorespiratory fitness ($VO_{2max}$) was expressed in milliliters of oxygen per kilogram of body weight per minute (ml.kg.min-1).

**Figure 2.8. Illustration of the Chester Step test to estimate cardiorespiratory fitness.**
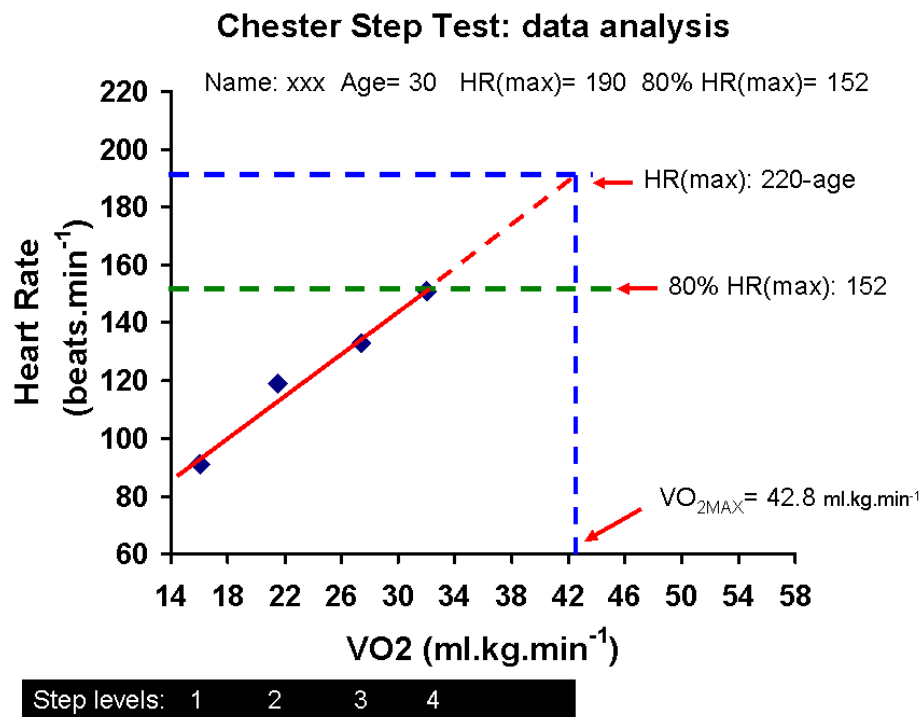


**Figure 2.9. Illustration of the Chester Step test extrapolation analysis to estimate cardiorespiratory fitness (VO$_{2max}$).**

### 2.3.6.1 Chester Step Test as a Surrogate of the Gold Standard Method for Measure of Cardiorespiratory Fitness (VO$_{2max}$)

The objective measurement of CRF, is not always practical in a situation where large cohorts need to be tested or where standard laboratory conditions are not available, make it necessary to count with valid and accurate field test protocols. Cardiorespiratory fitness can be measured by a gold standard protocol using a breath to breath gas analyzer system and Douglas bags. However, these methods are not always available and suitable for fieldwork conditions. For this special condition a range of submaximal protocol tests has been created (I-Min Lee et al. 2009). However, these entire field tests are based on the well known relationship to estimate VO$_2$ and predict VO$_{2max}$. Most of these tests use heart rate (HR) to predict the oxygen consumption that would have occurred during maximal workloads. Submaximal VO$_2$ tests are able to predict the VO$_{2max}$ because of the linear relationship that exists between HR and exercise workload/intensity (as workload/intensity increases, HR increases) (Astrand and Rodahl 1986). Examples of a submaximal and valid test include the Chester Step Test (CST). This test was originally developed by Kevin Sykes at University College Chester to assess aerobic fitness by predicting maximal aerobic power (VO$_{2max}$). The CST is one of the many test designed to provide a safe and practical means of assessing aerobic fitness under submaximal conditions. The limited equipment needed (step, heart rate monitor, portable cassette or compact disk player and perceived exertion scale) make the CST very portable and requirement for space is minimal, which is advantageous compared with similar protocols using treadmills, shuttle walks, or cycle ergometers. These advantages became quite relevant at the moment of planning fieldwork testing under not standard conditions. To date, the validity of the CST has been assessed in term of its ability to predict VO$_{2max}$ compared with a direct VO$_{2max}$ test, with the error in this ranging from 5% to 15% (Stevens and Sykes 1996).

## 2.3.7 Assessment of Physical Activity Patterns by Accelerometer and Self Reported Questionnaires

### 2.3.7.1 Actigraph Accelerometer Methods

The physical activity patterns and sedentary behaviour were objectively measured using the uni-axial ActiTrainer accelerometer (Actigraph, Pensacola; FL, USA). This accelerometer measures acceleration in a vertical axis, within the frequencies of 0.25 and 2.5 Hz. This device uses piezoelectric transducers to convert acceleration into a digital signal knows as counts. These counts can be summed over a user specified time sampling interval, referred to as epoch recorder to internal memory.

Prior to testing, each uni-axial accelerometer was tested and fully charged. Subjects were each given an activity monitor and accelerometer belt and also participants received an explanation on how to use the device. The activity monitor was worn during at least 7-days on the right hip and kept secure against the body at all wearing times using the fitted strap. Participants were also asked to complete a daily diary during the 7-days monitoring period with instructions to record the time the accelerometer belt was attached and removed. Subjects were required to wear the accelerometer from the moment they woke in the morning until it was time for them to go to bed in the evening. This time, as well as any other time of the day that the device was removed and reattached, was recorded in the diary. Subjects were instructed to remove the accelerometer during showering or bathing as the device is not waterproof.

### 2.3.7.2 Accelerometer Data Analysis

Accelerometer data analysis was carried out using a manual method. This manual method involved the data being downloaded using ActiLife software (ActiGraph, Pensacola, FL, USA), and all the outcome variables were computed in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, USA). This involved pasting the raw data into a specifically designed macro. This macro calculated counts per minute and other summary statistics for each subject. Accelerometer readings were summarized in 60-second epochs. Accelerometer wear time was calculated by subtracting non-wear time from 24 hours. Non-wear was defined by intervals of at

least 60 minutes of zero activity counts (Troiano et al. 2008). Physical activity intensity domains were determined using published Freedson cut-points (light $<1,952$ count.min$^{-1}$ or $<3.0$ METs; moderate $1,952$-$5,724$ count.min$^{-1}$ or $3.0$ to $5.9$ METs; vigorous $>5,725$ count.min$^{-1}$ or $>6.0$ METs) (Freedson, Melanson, and Sirard 1998b). Activity count values of $<100$ count.min$^{-1}$ were defined as sedentary behaviour (Hagstromer, Oja, and Sjostrom 2007). Days in which accelerometer were worm for at least 10 hours were considered valid for data analysis. Data from participants with at least 10 hours of daily accelerometer wear time for 4 days were included in the analysis. A total of 472 participants were measured, however only 317 individuals met the inclusion criteria, and a total of 155 subjects were excluded of the analysis (82 participants worm the accelerometer $<10$ hour per day and 68 participants worm the accelerometer $<4$ days a week and 5 participants did not were the accelerometer at all).

### 2.3.7.3  International Physical Activity Questionnaire – IPAQ methods

Physical activity (PA) patterns were also measured subjectively for 7 days, by using the long self-administered version of the International Physical Activity Questionnaire (IPAQ) (Appendix E). IPAQ is an instrument designed primarily for population surveillance of physical activity among adults and has been validated in South American countries including Chile (Hagstromer, Oja, and Sjostrom 2006c). It has been developed and tested for use in adults (age range of 15 - 69 years). The IPAQ questionnaire collects information on time (*i.e.* number of sessions and average time per session) spent walking, in moderate-intensity PA, in vigorous-intensity PA and sitting, on week and weekend days across a range of domains including: (a) leisure time physical activity, (b) domestic and gardening (yard) activities, (c) work-related physical activity and (d) transport-related physical activity. The participants completed the questionnaire at their last visit (after 7-days of accelerometer recording); the questionnaire was self completed on those subjects with a minimum standard education (writing and reading skills). However, for those subjects that did not have sufficient literacy to complete the questionnaire by themselves (n = 24), the questionnaire was completed with the help of in-person interviews (EU = 2; ER = 7; MU = 4; MR = 11).

### 2.3.7.4 IPAQ Data Analysis

Post collection of the IPAQ form the data was analyzed in accordance with the IPAQ protocol; Data from questionnaire where summed within each item (*i.e.* vigorous intensity, moderate intensity, walking and sitting time) to estimate the total amount of time spent in PA per week. Total daily PA (MET.min.day$^{-1}$) was estimated by summing the product of reported time within each item by a MET value specific to each category of PA and expressed as a daily average MET score (where MET is metabolic equivalent; 1 MET = 3.5 ml.kg$^{-1}$.min$^{-1}$ or resting energy expenditure) according to the official IPAQ scoring protocol (www.ipaq.ki.se). Vigorous intensity of PA was assumed to correspond to 8 METs, moderate-intensity activity to 4 METs and walking to 3.3 METs.

## 2.3.8 Assessment of Dietary Intake Patterns

Dietary intake was assessed by 7-day weighed-food intake. Participants were instructed to keep a food diary for seven consecutive days which involved weighing all food and drink on electronic scales. Measurement was made with a precision of 1 gram (Tanita, Kitchen Scale, model kd-404) and time of consumption was also noted in the diary (Appendix F). Instructions were provided in addition to a visual demonstration by the researcher to ensure proper use of the scales and diary. The participants were advised to maintain their normal dietary intake. Volunteers were asked to weigh constituents of each meal separately and record the details of the food type and weight of food used. If a meal was not completely consumed, they were asked to weigh and record the leftovers to allow investigators to calculate accurate consumption. In circumstances where it was not possible to weigh constituent components of a meal (*e.g.* a pre-packed sandwich), the volunteers were asked to record the weight of the total meal and provide a description of the meal. The dietary intake data were analysed by the Chilean Food Composition Database (Software MINUTA, University of Concepcion, Chile). Results were reported for total energy intake (kcal), macronutrients intake (protein, fat, carbohydrate) and alcohol consumption for each subject.

### 2.3.9 Assessment of Income Level, Socio-economic Status, Educational Levels

Socioeconomic, demographic and cultural data (age, attained education, most recent occupation, and ethnicity) were collected during in-person interviews with the ESOMAR questionnaire validated in the Chilean population (Méndez 1999) and the Chilean Socioeconomic Characterisation Questionnaire (CASEN 2006). These are included in appendix D. This information was also used to compare clusters within groups (EU, ER, MU and MR) that could be caused from bias on participants recruitment.

### 2.3.10 Assessment of Health Status and Family History of Type 2 Diabetes

Participants' health history including smoking status, and family health history was determined by questionnaire (Appendix C).

### 2.3.11 Blood Pressure

Blood pressure was measured in a supine position after at least 10 minutes of rest in concordance to the European Society of Hypertension guidelines on blood pressure measurement (O'Brien *et al.* 2003). An automated monitor was used (OMRON M10-IT Healthcare UK Limited, Milton Keynes, UK), and the average value of three readings was taken as the recorded value.

## 2.4 Sample Preparation and Analysis

### 2.4.1 Fasting Blood Sampling

Fasting blood sample was collected by venopuncture between 07:00am and 11:00 after an overnight fast of 10-12 hours. Venous blood was obtained via a butterfly needle, placed in an antecubital vein. Blood samples were collected directly in 3 tubes of 10 ml containing $K_3EDTA$ as an anticoagulant (BD, vacutainer System, Franklin Lakes, NJ USA). Two of the blood sample tubes (20 ml) were then placed

immediately in ice and centrifuged for 15 minutes at 3,000 revolutions per minute (rpm) (Hettich EBA20S Portable Centrifuge, Uttlingen, Germany). Whole blood from the third tube (10 ml) was kept for further DNA extraction.

### 2.4.2 Oral Glucose Tolerance Test

A 75 g oral glucose load was given to the volunteer. This was prepared by using 82.5g of Dextrose Monohydrate (Roche Ltd, Chile) in 340 ml of water, with 10 ml of lemon juice added (Roche Ltd, Chile) to give a total volume of 350 ml. Volunteers were instructed to drink the full volume in a 2-minute period, and a stopwatch was started once the volunteer commenced drinking. Druring fasting and 120 minutes after ingestion of the glucose drink, a 10 ml blood sample was taken into an EDTA tube. Volunteers were supine at all blood collection points. This test was initially planed to be performed for all participants. However, due to lack of interest of participants in the rural areas, the test was excluded.

### 2.4.3 Plasma and DNA Preparation, Storage and

### 2.4.4 Transportation

Blood samples were taken in the field work in drastically different environments (hospital, clinical and portable laboratories that were setup in the mountains and isolated villages). After the blood samples were drawn, two blood tubes were placed immediately on ice and centrifuged for 15 minutes at 3,000 rpm (Hettich EBA20S Portable Centrifuge, Uttlingen, Germany). Plasma was aspirated after centrifugation using a disposable plastic Pasteur pipette. Fasting plasma was dispensed in 0.5 ml aliquots into labelled 2 ml sterilised screw cap tubes (Iberica Laboratories Ltd, Iberica, Chile). Sixteen aliquots were stored from fasting plasma sample for each subject. The whole blood for further DNA extraction was dispensed in 1.5ml aliquots into labelled sterilised 2 ml screw cap tubes (Iberica Laboratories Ltd, Iberica, Chile). Six aliquots samples of whole blood were stored at -20ºC for each subject.

Those samples collected in an urban setting (where hospital and proper laboratories facilities were available) were immediately frozen at -20ºC until the transportation-logistics were planned and coordinated to move the samples in dry-ice to a base laboratory located in Santiago de Chile and frozen at -80 ºC.

Those samples collected in the rural places (mountains and isolated villages, where no laboratories facilities were available), were placed immediately in dry-ice and kept there until the transportation-logistics were planned and coordinated to move the samples back to the closest city or village, once there samples were temporarily stored at -20ºC for one week. Once collection was completed at that specific location, samples were placed in dry-ice and moved to the base-laboratory located in Santiago de Chile and frozen at -80ºC for 1 to 5 month (end of the fieldwork sample collection).

 Once field work was finished and all samples were stored (-80ºC) in our base-laboratory in Santiago de Chile, these sample were transported to Glasgow University, United Kingdom by a logistic transportation courier (BIOCAIR, UK). Samples were placed in a Polystyrene Cold Boxes and covered with dry-ice. The container arrived to Glasgow University 23 hours post collection time and the temperature of samples was monitored the whole time. To back up our samples, 8 of 16 plasma aliquot of each subject were kept in our base-laboratory in Santiago de Chile.

## 2.4.5 Enzyme Colorimetric Methods

Assays were performed in the biochemistry laboratory of the Institute of Cardiovascular and Medical Sciences of Glasgow University, United Kingdom and in the metabolic and clinical molecular laboratory of the Institute of Nutrition and Food technology (INTA) at the University of Chile, Chile.

Total cholesterol, HDL-cholesterol and triglycerides were analysed by enzymatic colorimetric methods using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol concentrations were determined using the Friedewald equation (Friedewald, Levy, & Fredrickson 1972). Concentrations of

liver enzymes Gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) were determined by enzymatic colorimetric methods, using a commercially available kit (Randox Laboratories Ltd., Co. Antrim, Ireland). Glucose was assayed using the hexokinase method, utilising a Roche kit ((Roche Diagnostics GmbH, Mannheim, Germany). High sensitivity C-reactive protein (hsCRP) was measured using an immunoturbidimetric assay (Kamiya Biomedical, Seattle, USA). Glucose and Lipids profile assays were performed on a photometric clinical analyzer 4010 (Roche, Basel, Switzerland). GGT, ALT and hsCRP assays were performed on a Cobas Mira Plus (ABX Diagnostics, France). Analysis was performed by the author at Glasgow University and the INTA research center in Chile. All samples for each participant were analysed in duplicate. The accuracy and precision of the assays was monitored using quality control sera (Roche Diagnostics GmbH, Mannheim, Germany; Randox Laboratories Ltd, Co. Antrim, Ireland; Kamiya Biomedical, Seattle, USA). The coefficients of variation were <3.1% for all colorimetric assays.

### 2.4.5.1 Enzyme-linked Immunoassays

All enzyme-linked immunoassay (ELISA) procedures were carried out in the metabolic and clinical molecular laboratory of the Institute of Nutrition and Food technology, (INTA) at the University of Chile, Chile.

All enzyme-linked immunoassay (ELISA) procedures were based on a 'sandwich' technique. Commercially produced plates were used for all ELISA. The wells of the plates were coated with a monoclonal antibody to the protein of interest. The addition of plasma to the wells bound protein to the antibody, with unbound molecule removed by washing. A second antibody specific to another area of the protein was then added, with unbound molecules removed by a second washing. The second antibody was linked to an enzyme which would catalyse the conversion of a nonfluorescent substrate to a fluorescent product. The intensity of the subsequent fluorescence was directly proportional to the amount of protein in the initial sample (Stryer 1988).

Insulin was measured using a commercially available ELISA (Diagnostic System Labs, TX, USA and Linco Research Inc, St. Louis MO, USA). Leptin was analysed using commercially available kits (Diagnostic System Labs, TX, USA and Linco

Research Inc, St. Louis MO, USA) as previously described and validated (Considine et al. 1996b). All samples for each participant were analysed in duplicate. The accuracy and precision of the assays was monitored using quality control sera (Diagnostic System Labs, TX, USA and Linco Research Inc, St. Louis MO, USA). Coefficients of variation were 5.0% for insulin and 3.1% for leptin.

## 2.4.6 DNA Preparation and Genotyping Methods

### 2.4.6.1 Genomic DNA Extraction and Storage Method

EDTA anticoagulated whole blood 2 ml aliquot samples were stored in -20$^{o}$C prior to DNA extraction. Genomic DNA (gDNA) from the GENADIO cohort was isolated using the QIAamp DNA blood midi kit (QIAGEN, Ltd. UK) as per the manufacturer's instructions. Briefly, 2 ml of whole blood was isolated for each subject, cells were lysed using proteinase K and ethanol was added to the lysate, which was then applied to a spin column with a specialised silica-gel membrane that binds DNA. The contaminants were removed by washing the column with the buffers provided and the DNA was eluted. DNA was eluted twice using 3x300 µl volumes of AE buffer provided. The extracted gDNA was dispensed in 100 µl aliquots into labelled 2 ml sterilised screw cap tubes (STARLAB, Ltda. UK) and stored for long-term at -20°C.

### 2.4.6.2 Measurement and Standardization of DNA Concentration

Genomic DNA concentration was quantified on a ThermoFisher NanoDrop ND-8000 UV-Vis Spectrophotometer (ThermoFisher, Waltham, MA) using 1.5 µl of gDNA sample as per the manufacturer's instructions. All samples were measured in duplicate, when difference within each reading was >10%, an additional reading was made. The average of all reading was used to determine the final gDNA concentration for each sample. Subsequent to obtaining the average gDNA concentration, all samples were diluted and standardised to a concentration of 10 ng.µl$^{-1}$ of gDNA, using the elution buffer AE (10 mM Tris-Cl and 0.5 mM EDTA; pH 9.0) provided by QIAGEN. A re-reading was made to verify the target concentration (10 ng.µl$^{-1}$) for each sample. These samples were dispensed into a

sterilised 2 ml 96 deep well plate (STARLAB, Ltda. UK) and stored temporarily for 2 weeks at 4°C for genotyping. After genotyping the samples were stored at -20°C.

## 2.4.7 Genotyping Methods

### 2.4.7.1 TaqMan SNP Genotyping: Chemistry Overview

All the specific gene variants were genotyped with the ABI 7900-HT Sequence Detection System® (Applied Biosystems, Warrington, UK) using the TaqMan SNP genotyping assay for allelic discrimination. The assay contains the two primers for amplifying the polymorphic sequence of interest and two TaqMan MGB probes for distinguishing between the two alleles. Briefly, the assay takes advantage of the 5' to 3' nuclease activity of AmpliTaq Gold® DNA polymerase. This system uses a forward and reverse primer, and a probe that specifically targets each single nucleotide polymorphism (SNP) allele. The probe is labeled at its 5' end with two fluorescent dyes, VIC® dye is linked to the 5' end of the allele 1 probe whilst FAMTM dye is linked to the 5' end of the allele 2 probe, and at the 3' end with a nonfluorescent quencher. As long as these two are close to each other (*ie*: when they are on the intact probe), the quencher absorbs the fluorescence. During the polymerase chain reaction (PCR), if the probe matches the sequence, the AmpliTaq Gold® polymerase moves along the region to be amplified in a 5' to 3' direction and cleaves the probe, separating the dye and the quencher. The fluorescence is no longer absorbed by the quencher and the fluorescence emissions generated by the PCR amplification are processed by the ABI 7900-HT Sequence Detection System® (SDS) (Applied Biosystems, Warrington, UK) that outputs genotypes with the allelic discrimination end-point analysis mode of the SDS software package, version 2.0 (Applied Biosystems, Warrington, UK).

### 2.4.7.2 Polymerase Chain Reaction (PCR) Conditions and Endpoint Analysis for the ABI Assay

To genotype the specific gene variants a gDNA with a standardised concentration was used as per the manufacturer's instructions (Applied Biosystems, California. USA). To perform the real-time polymerase chain reaction (PCR), a total final volume of 20 μl per well (with 2.0 μl of gDNA, 1.0 μl of TaqMan genotyping assay,

10.0μl of TaqMan universal PCR master mix and 7.0 μl of DNase-free water) as recommended for the ABI SNP genotyping protocol.

Each sample was dispensed into a definite labelled well in a specific PCR 96-well plate (Applied Biosystems, Warrington, UK). Each plate included 94 DNA reactions and two negative controls, where gDNA was replaced for DNase-free water. The genotyping was carried out in the ABI 7900-HT Fast Real-Time PCR device using the manufacturer's recommended PCR thermal cycling, which started with the enzyme activation (10 min at 95°C), following by a denaturing step of 40 cycles at 92°C for 15 seconds. It was then completed by a 1 min extension step at 60°C.

After PCR amplification was performed an endpoint plate read using the Sequence Detection System (SDS) Software, which use the fluorescence measurements made during the plate read to plot the fluorescence (Rn) values based on the signal from each well. The plotted fluorescence signal indicates which alleles are in each sample. The SDS software was set up with the automatic allele call system. All the information generated from the analysis was saved for posterior analysis in a Excel file. Genotyping success rate was 98.7% (n = 406), and no discordant genotypes were observed in 40 random duplicate samples.

## 2.5 Statistical Analysis

Data were analyzed using Statistica (version 8.0; StataSoft, Tulsa, USA). STATA (version 11.0; College Station. Texas. USA) and MedCalc (version 10.0; Broekstraat 9030 Mariakerke, Belgium). Prior to analysis, quantitative data were tested for normality using the Anderson–Darling normality test and transformed as appropriate. Box-Cox plots were used to determine the most appropriate transformation for data that did not follow a normal distribution. Specific statistical analyses used are described in detail in the method section of each chapter.

# 3 A Comparison of Direct (Accelerometer) Versus Self-reported Measures (IPAQ) for Assessing Physical Activity and Sedentary Behaviour.

## 3.1 Introduction

Physical activity is an important behaviour related to a number of health outcomes such as hypertension, metabolic syndrome, T2D and cardiovascular diseases (Gill and Malkova 2006f; Gill and Cooper 2008; I-Min Lee et al. 2009; Eyre et al. 2004). Accurate and valid assessment of physical activity levels is important to understand and quantify the relationships between physical activity and health-related outcome, to monitor secular trends in behaviour, and to evaluate the effectiveness of interventions and programs (Ward et al. 2005). However, obtaining valid and appropriate assessment of physical activity is challenging and complicated by the fact that several health-related dimensions of physical activity may need to be considered, such as energy expenditure, intensity, frequency, duration, weight bearing, flexibility, and strength (I-Min Lee et al. 2009; Caspersen, Powell, and Christenson 1985).

Epidemiological studies have typically used subjective measures, such as the questionnaire, to assess physical activity in populations. Physical activity questionnaires are easy to administer, non-reactive (does not alter the behaviour of the individual being surveyed), relatively inexpensive and accepted by study participants (I-Min Lee et al. 2009). Yet, the limitations of the self-reporting of physical activity are also well documented (I-Min Lee et al. 2009). Activity information obtained by self-reporting measures is subject to response bias (*e.g.*, imprecise recall, social desirability) that may influence the precision in measures of physical activity. Bias in self-reported methods appears to be highest for low-

intensity physical activity that are habitual behaviours (*e.g.*, walking, housework) compared to higher intensity structured physical activity such as sport and conditioning activities, which are planned and intentional behaviours (I-Min Lee et al. 2009). The type of information required is dependent on the research question posed, *e.g.* sports activities, leisure time activities, work-related activities and active transportation. In addition, interest can be on 'habitual' or usual physical activity or physical activity in the past day(s), week, month, year or even a lifetime. Hence, many questionnaires have been developed for different purposes. A few questionnaires provide a measure of sedentary behaviour, usually in the form of time spent sitting, TV watching time or screen time (I-Min Lee et al. 2009). The importance of sedentary behaviour in influencing health outcomes is becoming increasingly recognized, with a growing body of recent evidence reporting that sedentary behaviour is associated with an increasing risk of obesity, diabetes and cardiovascular diseases, independent of physical activity levels (Owen et al. 2010c; Tremblay et al. 2010a). Therefore, it is important to assess the amount of time spent on sedentary behaviours as well as on physical activity. However it is unclear the extent to which this self-reported proxy measures provide an accurate assessment of actual time spent in sedentary activities (Gordon-Larsen, McMurray, and Popkin 2000).

Physical activity questionnaires differ in their length and complexity and their ability to provide an accurate and reproducible measure of activity behaviour. One of the methods that has been extensively validated is the International Physical Activity Questionnaire (IPAQ) (Craig et al. 2003e). This questionnaire was developed to provide a comprehensive measure of physical activity in a variety of contexts in order to provide comparable assessments of activity, both cross-culturally and across research studies. From a research perspective, the IPAQ is appealing as it specifically prompts recall of physical activity in a variety of contexts, potentially providing a more accurate and reliable measure of activity, particularly for individuals for whom activity occurs outside of leisure time (Craig et al. 2003d).

The validity and reliability of the IPAQ as a population surveillance tool was initially examined and reported in an international investigation involving 12 countries, each of which examined either the short or long form of the questionnaire (Craig et al.

2003c). Questionnaires were either self-administered or administered via telephone, and the authors reported up to eight-day test-retest reliability Spearman coefficients, ranging from r = 0.67 (*p*<0.0004) to r = 0.91 (*p*<0.0001) for total activity, across the different countries and forms of applications. Accuracy was determined by comparison between IPAQ and objectively obtained accelerometry data, there was fair agreement between methods (r = 0.30, *p*<0.001) (Craig et al. 2003b). However, as only total physical activity reliability and validity coefficients were reported, the measurement characteristics of specific intensities and settings of activity data, which are of interest in research contexts, remain unclear.

A large body of evidence derived from questionnaire-based measures has demonstrated a clear relationship between physical activity and health-related outcomes, and risk factors for chronic diseases (I-Min Lee et al. 2009). A smaller, but growing, body of evidence has made similar observations for indices of sedentary behaviour (I-Min Lee et al. 2009). However, it is unclear whether the relationships between questionnaire-based measures of physical activity/sedentary time and health markers reflect the actual relationships between these variables when true objectively-determined measures of physical activity and sedentary time are used. The purpose of this study was therefore to determine, in a large sample of South Americans, living in a rural and urban environments with a diverse range of demographic characteristics:

(a) The strength of relationship and agreement between objectively (Actigraph) and subjectively (IPAQ) measured physical activity and sedentary behaviours.

(b) Whether the relationships between physical activity/sedentary time and risk factors for vascular and metabolic disease differ between objective and subjective measures of physical activity and sedentary behaviours.

## 3.2 Methods

### 3.2.1 Participants

Volunteers were recruited and screened as detailed in section 2.1.5. Specific inclusion criteria are defined in section 2.1.6. Further information detailing recruitment response and excluded volunteers can be found in Figure 2.5. Of the 472 individuals who were assessed in the study, 317 participants fulfilled the Actigraph wearing time inclusion criteria as described in section 2.3.7; of these 140 were male (31 MR, 48 MU, 29 ER, 32 EU) and 177 were female (50 MR, 25 MU, 44 ER, 58 EU).

### 3.2.2 Assessment of Physical Activity Patterns by Accelerometer

Participants wore accelerometers (ActiTrainer, ActiGraph, LLC, Pensacola, FL, USA) on the right hip at all times, except when showering, swimming and sleeping, for seven consecutive days to objectively assess physical activity levels as described in section 2.3.7. Accelerometer readings were summarized in 60-second epochs and Freedson cut-points were used to define intensity domains (light $<1,952$ count.min$^{-1}$; moderate 1,952-5,724 count.min$^{-1}$; vigorous $>5,725$ count.min$^{-1}$) (Freedson, Melanson, and Sirard 1998a). Activity count values of $<100$ count.min$^{-1}$ were defined as sedentary behaviour (Pate, O'Neill, and Lobelo 2008). Accelerometer wear time was calculated by subtracting non-wear time from 24 hours. Non-wear was defined by intervals of at least 60 minutes of zero activity counts. Valid days were defined as having at least 10 hours of wear time and volunteers with four or more valid days of accelerometer data were included in the analysis, as detailed in section 2.3.7.2.

### 3.2.3 Assessment of Physical Activity Patterns by Self-reported Questionnaire

Physical activity (PA) patterns were measured on the last 7 days, by a long, self-administered version of the International Physical Activity Questionnaire (IPAQ) as

detailed in section 2.3.7.3. The participants completed the questionnaire at their last visit (after 7-days of accelerometer recording); the questionnaire was self reported on those subjects with a minimum standard education (writing and reading skills). However, for those subjects that didn't achieve the minimum standard to complete the questionnaire by themselves (illiteracy), the questionnaire was completed during in-person interviews by the author or a trained assistant. Data were analyzed in accordance with the IPAQ protocol as described in section 2.3.7.4. The activity domains collected from the IPAQ were walking, moderate and vigorous physical activity. Sitting time was used as a measure of sedentary behaviour.

### 3.2.4 Statistical Analysis

Five measures ('intensity domains') were derived from the Actigraph data (sedentary, light, moderate, vigorous and moderate to vigorous physical activity (MVPA), and five from the IPAQ data (sitting, walking, moderate, vigorous and MVPA). The ethnic-specific mean and standard deviation (SD) of each of these was calculated, and the significance of ethnic differences tested using t-tests. Moderate to vigorous physical activity (MVPA) was calculated by merging moderate + vigorous physical activity for both objective and self-reported measures. In addition, two other intensity domains moderate activity plus walking and MVPA plus walking were calculated for IPAQ. This was done in an attempt to provide a more comparable measure for the Actigraph-derived moderate and MVPA measures as walking typically falls into the 'moderate' intensity range but is considered in a separate category by the IPAQ.

Only three of the outcomes were measured by both the IPAQ and Actigraph; moderate physical activity, vigorous physical activity (min.day$^{-1}$) and sedentary behaviour (min.day$^{-1}$). First a t-test was performed to examine whether accelerometer and IPAQ measures of sedentary time, moderate, vigorous and MVPA differ significantly. In addition, moderate + walking (IPAQ) with moderate (Acti) and MVPA+walking (IPAQ) with MVPA (Acti), were compared, as often self-reported walking is classified as moderate physical activity. The extent and direction of the relationship between Actigraph and IPAQ on these variables was calculated using the Pearson (r) and Spearman ($r_s$) correlation analysis. To assess the degree of agreement

between the two measures of each of these outcomes (MVPA and sedentary behaviour) a bias analysis was performed (Bland-Altman approach); with calculation of the percentage mean difference and SD of the differences. The range of ±1.96 SD of the differences estimates the 95% level of agreement between the two techniques (Petrie and Sabin 2009).

In addition, the Concordance Correlation Coefficient (Pc) method was used to assess the significance of agreement (Petrie and Sabin 2009). This provides a measure of precision using the Pearson correlation coefficient (r); this measures how far each observation deviates from the best-fit line. It also includes a measure of accuracy (Cb), which is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin. The Concordance Correlation Coefficient ranges from zero (no agreement) to one (perfect agreement). The "Pc" is the product of Pearson correlation coefficient (r) by a bias correction factor (Cb).

The relationships between Actigraph- and IPAQ-derived measures of MVPA and sedentary/sitting time and risk factors for vascular and metabolic disease were compared by dividing the cohort into tertiles for MVPA and sedentary/sitting time by each measure and comparing differences in risk factors across tertiles by GLM. A significant measurement method x tertile interaction would reveal a difference in the relationship between Actigraph- and IPAQ-derived measures and risk factors. All models were adjusted for age and further models adjusted for potential confounding variables (ethnicity, sex, environment, educational level and BMI) were undertaken as appropriate. For all analyses significance was accepted at $p < 0.05$.

## 3.3 Results

### 3.3.1 Differences in the Physical Activity Measures

There were significant differences for all matched subcomponents of physical activity measured by accelerometer and IPAQ (Table 3.1). Additionally, we examined whether objective measures of moderate intensity physical activity was significant different to self-reported "moderate+walking" time and whether objective

MVPA was different to self-reported "MVPA+walking". Significant differences were found in both comparisons (Table 3.1).

**Table 3.1. Descriptive of objective (Actigraph) and self-reported (IPAQ) physical activity measures in European and Mapuche population.**

| Objective (Actigraph) vs. Self-reported (IPAQ) | Actigraph | IPAQ | *p*-value |
|---|---|---|---|
| Sedentary vs. sitting time (min.day$^{-1}$) | $523.0 \pm 90.9$ | $454.2 \pm 103.1$ | <0.0001 |
| Moderate vs. moderate (min.day$^{-1}$) | $33.7 \pm 23.7$ | $31.5 \pm 16.7$ | <0.0001 |
| Vigorous vs. vigorous (min.day$^{-1}$) | $2.61 \pm 4.83$ | $10.4 \pm 9.42$ | <0.0001 |
| MVPA vs. MVPA (MET.min.day$^{-1}$) | $173.2 \pm 129.5$ | $209.9 \pm 110.4$ | <0.0002 |
| Moderate vs "Moderate+walking" (MET.min.day$^{-1}$) | $151.9 \pm 106.8$ | $314.2 \pm 214.6$ | <0.0001 |
| MVPA vs "MVPA+walking" (MET.min.day$^{-1}$) | $173.2 \pm 129.5$ | $397.8 \pm 248.3$ | <0.0001 |

Data presented as mean ± SD. Significance was accepted at <0.05.

### *3.3.2 Validity of IPAQ*

The correlations between self-reported (IPAQ) and objective measures (Actigraph) of sedentary and MVPA were assessed by parametric Pearson (r) and non parametric Spearman ($r_s$) correlation tests. Self-reported time spent in sitting activities was strongly and positively correlated with Actigraph measured time spent in sedentary behaviour (*i.e.* <100 count.min$^{-1}$), with a similar correlation coefficient for both tests (r = 0.654, *p*<0.0001; $r_s$ = 0.681, *p*<0.0001). The correlation between the two measures of moderate physical activity intensity was similarly high (r = 0.612, *p*<0.0001; $r_s$ = 0.733, *p*<0.0001). However, vigorous intensity physical activity showed a poor correlation between methods (r = 0.253, *p*<0.0001; $r_s$ = 0.161, *p*<0.005). Self-reported MVPA (MET.min.day$^{-1}$) showed a strong and positive correlation with accelerometry-measured time spent in MVPA in both correlation tests, however the extent of the correlation was slightly higher for the Pearson than the Spearman test (r = 0.728, *p*<0.0001; $r_s$ = 0.588, *p*<0.0001). Further analyses revealed a moderate relationship between objectively measured MVPA and self-

reported "MVPA+walking" ($r = 0.477$, $p<0.0001$; $r_s = 0.482$, $p<0.0001$) and a similar correlation between objectively measured moderate physical activity and self-reported "moderate+walking" ($r = 0.484$, $p<0.0001$; $r_s = 0.481$, $p<0.0001$).

The concordance and bias between objectively measured physical activity (Actigraph) and self-reported physical activity (IPAQ) were assessed. The percentage difference between the two methods was plotted against the mean of both methods for time spent in sedentary behaviour and MVPA. The Bland-Altman analysis revealed (Figure 3.1) an underestimated bias of 15.1% for IPAQ, with 95% limits of agreement ranging from 48.9% to -18.6%, compared to objective-measures of time spent in sedentary behaviour. In addition, the Concordance Correlation coefficient analysis (Figure 3.1) between both methods in sedentary behaviour showed a strong precision ($r = 0.654$) and accuracy ($Cb = 0.792$), with a significant concordance coefficient between techniques ($Pc = 0.518$; $p<0.0001$). Agreement between methods in time spent on MVPA is shown in Figure 3.2, we found an overestimated bias of -26.7% for IPAQ compare to accelerometer, with 95% limits of agreement ranging from 87.5% to -141.0%, while the precision between methods was moderate ($r = 0.615$) and accuracy was higher ($Cb = 0.943$), when correcting for a bias factor with the concordance correlation coefficient remaining significant ($Pc = 0.511$; $p<0.0001$). Additionally, whether the agreement between methods increased if we compared objective measures of MVPA against a combine self-reported measurements of "walking + MVPA" (Figure 3.3) was examined. After analysis, we found a higher underestimated bias (mean: -78.1%; 95%CI: 23.5% to -179.8%) for IPAQ compared to accelerometer. The precision ($r = 0.510$), accuracy ($Cb = 0.498$) and concordance correlation coefficient ($Pc = 0.254$; $p<0.0001$) were poor but still significant. Similarly, poor agreement and weak relationship were found when we compared objective "light + MVPA" activities against "MVPA +walk" self-reported activities measurements (figure not shown). An underestimated bias was observed between methods (mean: 57.7%; 95%CI: 150.9% to -35.4%), with a poor precision ($r = 0.403$), accuracy ($Cb = 0.255$) and concordance correlation coefficient ($Pc = 0.239$; $p<0.0001$). Furthermore and despite the higher correlation in sedentary behaviour and MVPA time between methods, the Cusum linearity test indicated that the observed slope was significantly different ($p<0.05$) from the perfect fit line for all intensity domains.
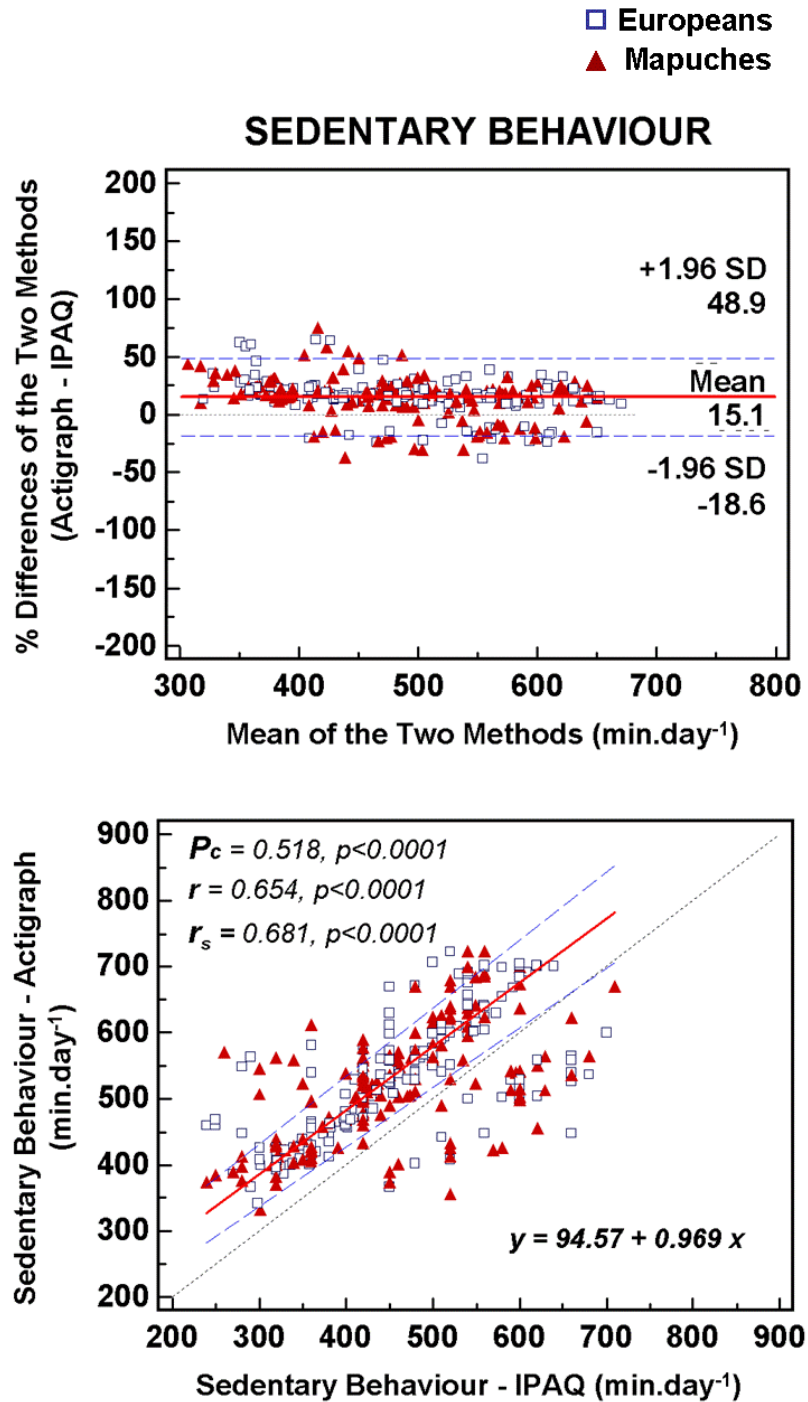
**Figure 3.1. Bland-Altman plot (top panel) and Concordance Correlation Coefficient (bottom panel) of objective and self-reported measured of sedentary behaviours.**
Figure on the top panel show the Bland-Altman Plot analysis, with data presented as % differences of the mean and ±1.96 SD. Bottom figure showed concordance correlation coefficient (Pc), Pearson correlation (r) and the Spearman correlation ($r_S$), with their respectively *p*-values for sedentary time measured by Actigraph and self-reported questionnaire.
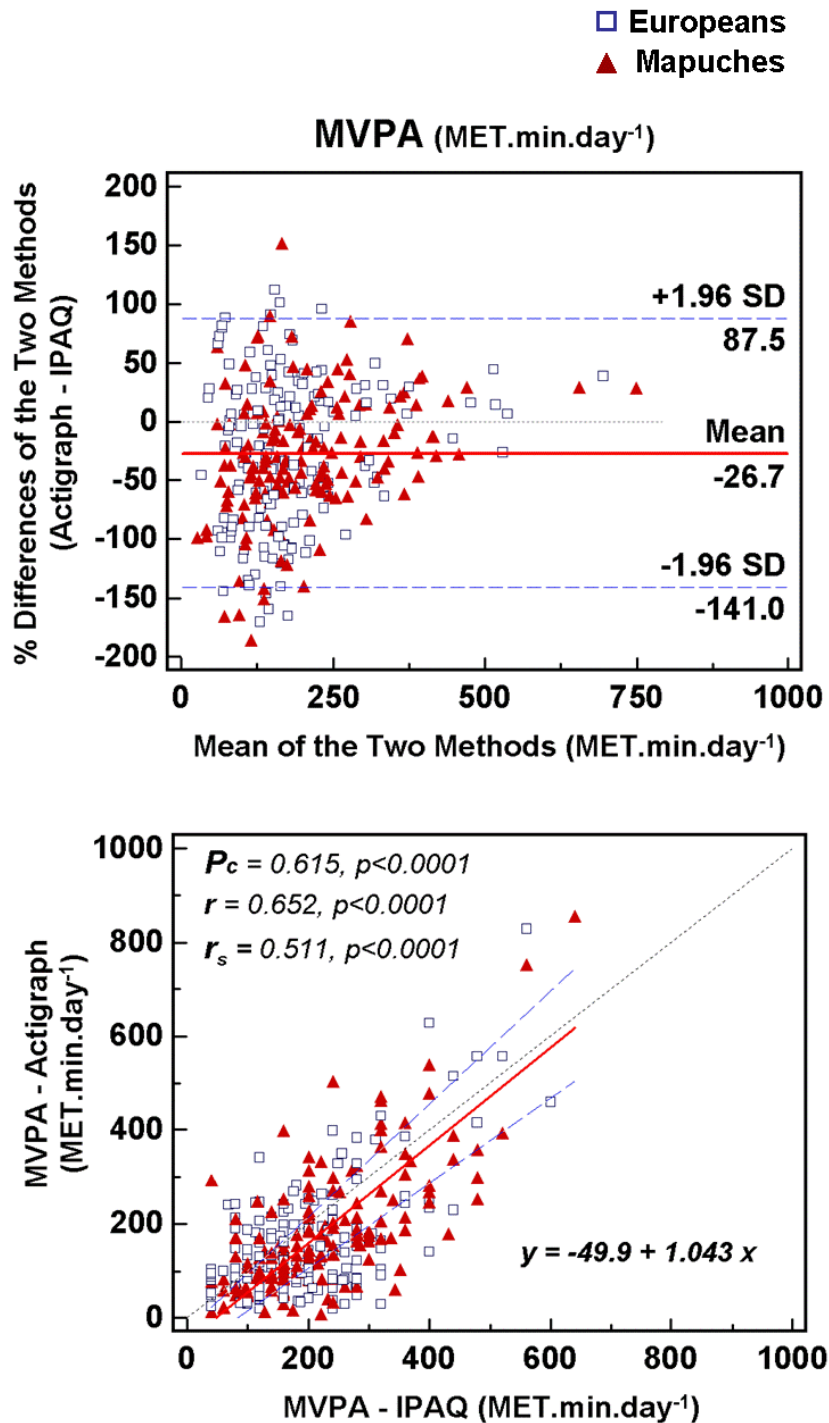
**Figure 3.2. Bland-Altman plot (top panel) and Concordance Correlation Coefficient (bottom panel) of objective and self-reported measured of time spent in MVPA.**
Figure on the top panel show the Bland-Altman Plot analysis, with data presented as % differences of the mean and ±1.96 SD.  Bottom figure showed concordance correlation coefficient (Pc), Pearson correlation (r) and the Spearman correlation ($r_S$), with their respectively *p*-values for MVPA time measured by Actigraph and self-reported questionnaire.
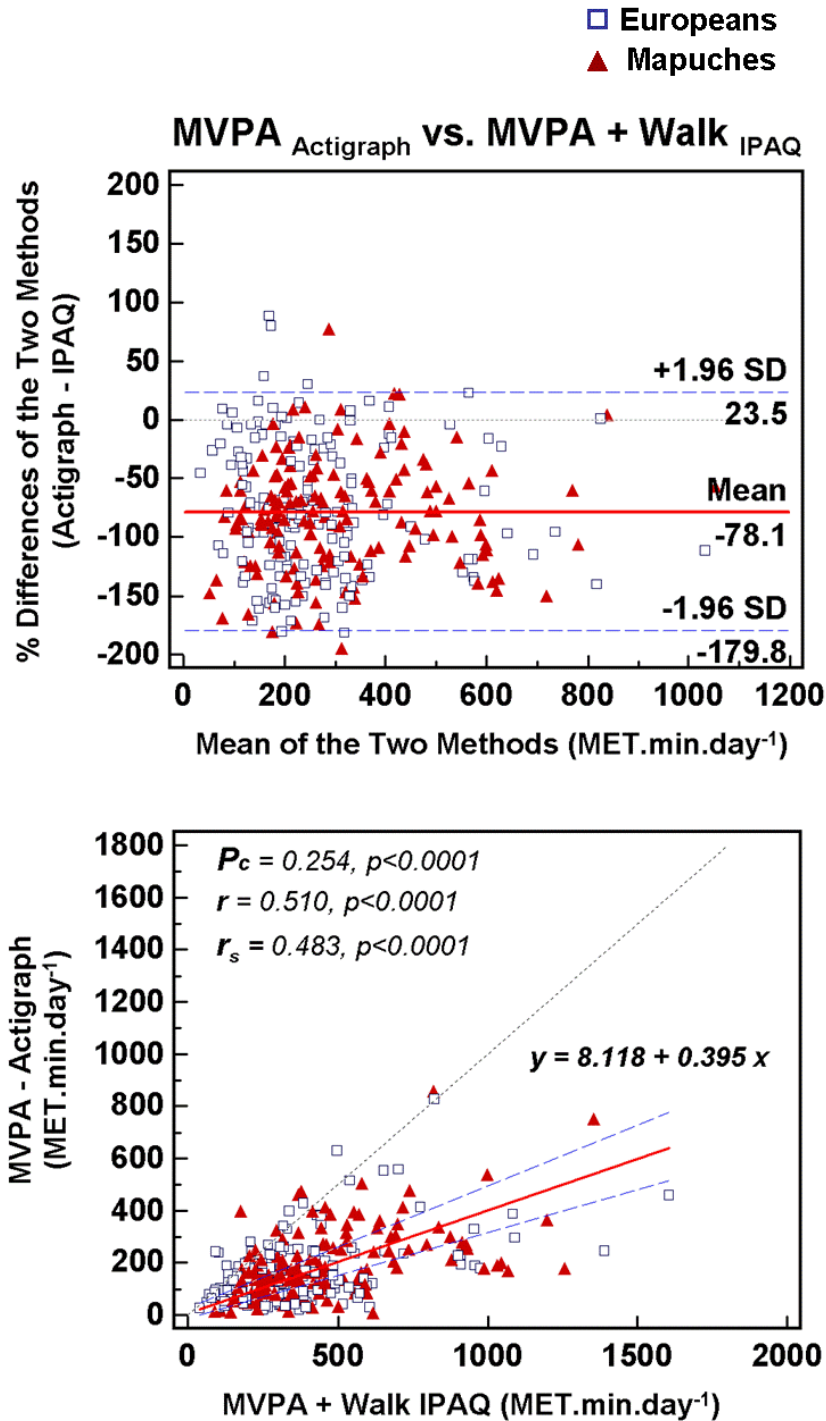
**Figure 3.3. Bland-Altman plot (top panel) and Concordance Correlation Coefficient (bottom panel) of objectively measured MVPA and self-reported "MVPA + walk" measurement.**
Figure on the top panel show the Bland-Altman Plot analysis, with data presented as % differences of the mean and ±1.96 SD. Bottom figure showed concordance correlation coefficient (Pc), Pearson correlation (r) and the Spearman correlation ($r_S$), with their respectively *p*-values for MVPA time measured by Actigraph and self-reported "MVPA + walk".

Figure 3.4 shows that for all of the vascular and metabolic risk factors considered decreasing sedentary time was associated with a change in the risk factor in a favourable direction. There were no significant measurement methods x sedentary/sitting time tertile interactions for any of the measured risk factors. Figure 5 shows similar relationships for MVPA, except the differences in total and LDL cholesterol with increasing MVPA as measured by IPAQ. Again no significant measurement method x interactions were observed. Further adjustment for potential confounding factors (sex, environment, ethnicity, socio-economic level and BMI) did not alter these any of results.
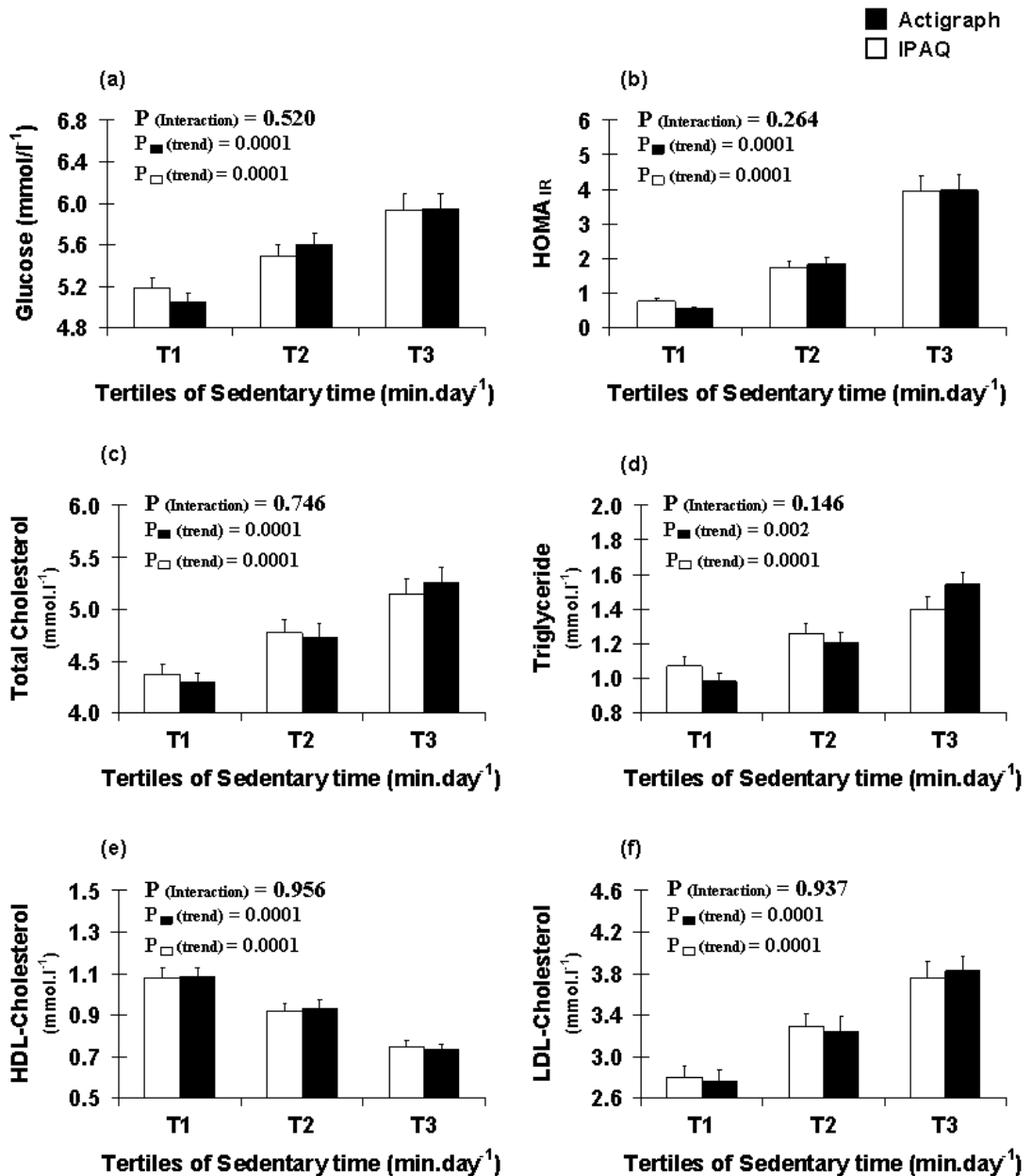
**Figure 3.4. Effect of tertiles of sedentary time measured by self-reported (IPAQ) and objective (Actigraph) methods on metabolic markers.**
Bars show mean ± SEM for all tertile. Values defining IPAQ tertile were (T1 <411.0 min.day$^{-1}$ and T3 >510.1 min.day$^{-1}$), while for Actigraph tertiles values were (T1 <471.8 min.day$^{-1}$ and T3 >559.6 min.day$^{-1}$).
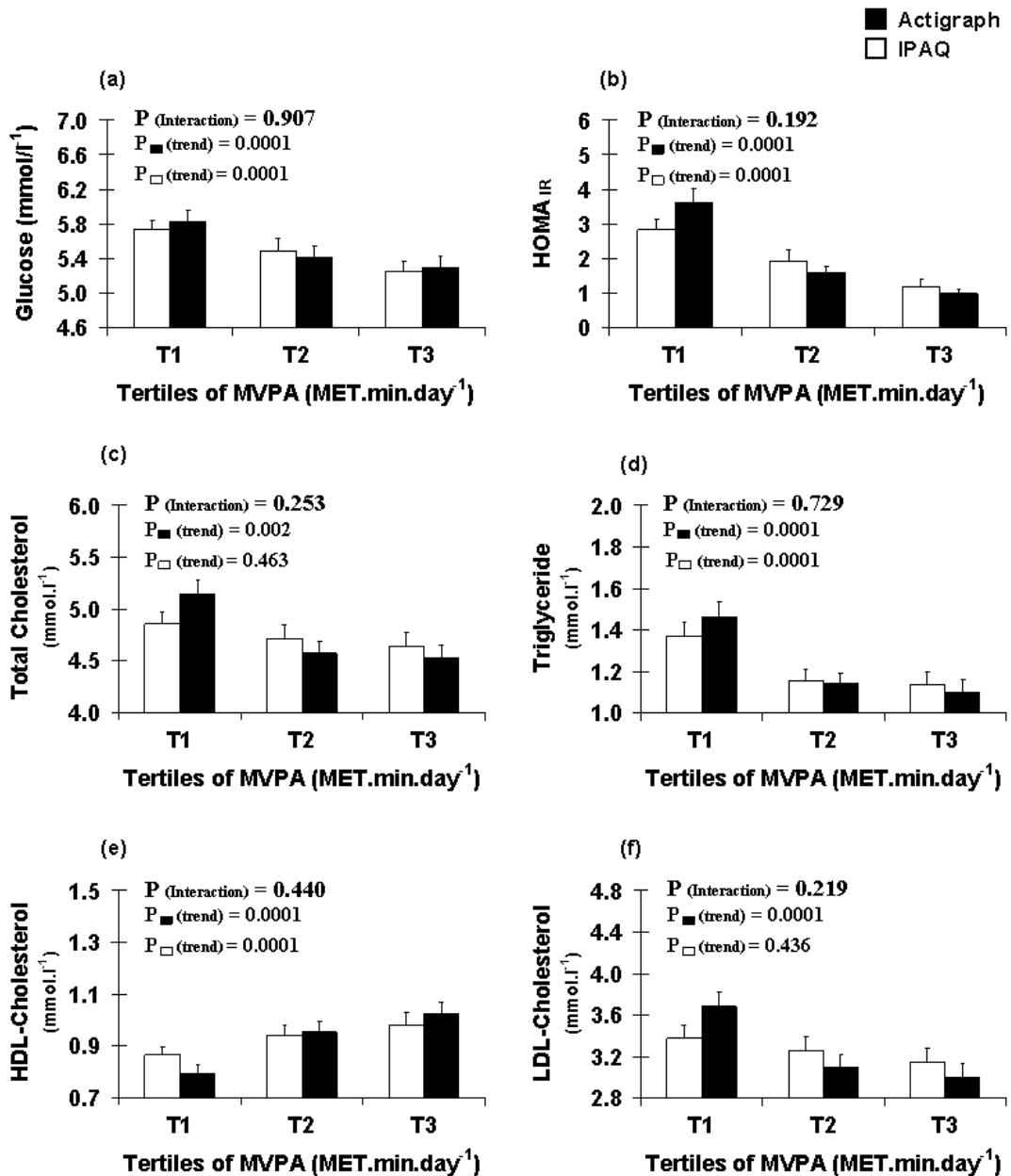
**Figure 3.5. Effect of tertiles of MVPA time measured by self-reported (IPAQ) and objective (Actigraph) methods on metabolic markers.**
Bars show mean ± SEM for all tertile. Values defining IPAQ tertile were (T1 <160 MET.min.day$^{-1}$ and T3 >240 MET.min.day$^{-1}$), while for Actigraph tertiles values were (T1 <98.1 MET.min.day$^{-1}$ and T3 >187.2 MET.min.day$^{-1}$)

## 3.4 Discussion

The main results in this chapter are: (a) the strong agreement and validity of self-reported measurements of sitting time, as a proxy of sedentary behaviours compared to the objective criterion measure of time spent in sedentary activities; (b) additionally, other important subcomponent of physical activity such as time spent in moderate to vigorous physical activity also revealed a strong agreement and validity between the self-reported time captured by IPAQ, compared to the Actigraph as an objective criterion method.

Due to the physiological link between physical activity/inactivity and many chronic diseases (I-Min Lee et al. 2009; Healy et al. 2008b; Owen et al. 2010b; Tremblay et al. 2010c), and the need of appropriate guidelines of physical activity patterns to maintain good health, many assessment tools have been created to determine physical activity levels of certain populations. However, few of them have focus on sedentary behaviours. To examine the relationship between physical activity and specific disease or conditions, it is important to focus on the dimension of physical activity (sedentary time, MVPA) rather than just total amount of physical activity. One of the instruments that have been widely used for the measurement of physical activity is the International Physical Activity Questionnaire (IPAQ). Despite the IPAQ multicultural reliability and validity for measuring total physical activity, there is limited published evidence in the current literature regarding the reliability and validity of IPAQ as an instrument that could be used to accurately assess sedentary behaviours and time spent in MVPA activities.

Our study extended the evidence of IPAQ as a valid instrument to measure different physical activity intensity domains in a South America population. Our results showed a strong correlation of MVPA and sedentary time derived from self-reported (IPAQ) and the objective measure (Actigraph). The findings in this chapter showed higher correlation between methods (objectively and self-reported than the Craig and colleagues study, this 12-countries reliability and validity study reported a moderate correlation of total self-reported physical activity against the accelerometer measurement ($r = 0.33$), while self-reported sitting time showed a moderate correlation ($r = 0.30$) with time spent in sedentary behaviour derived from the

Actigraph. This agrees with  Rosenberg and colleagues that also reported a significant and moderate correlation (r = 0.33) between sitting time derived from IPAQ and objectively measured sedentary time using Actigraph in an adult population (Rosenberg et al. 2008). Additionally, a previous small scale study in adults (46 participants), reported a significant moderate correlation in time spent in MVPA measured by IPAQ and accelerometer ($r_s$ = 0.36). However, this study found a weak and not significant correlation between self-reported time spent sitting and objectively measured sedentary time ($r_s$ =0.17) (Hagstromer, Oja, and Sjostrom 2006b). Many previous studies have tested the relationship between self-reported and direct measures by using correlation coefficients , but this is limited as a correlation is only able to measure the strength of the relationship between two variables and cannot determine the level of agreement between them, as well as ignoring any bias in the data. A more useful approach, the Concordance Correlation Coefficient (Pc), provides bias corrected correlation coefficients for assessing the level of agreement between self-reported and direct measures. This analysis reveals moderate to high criterion validity for sedentary time (Pc= 0.518) and MVPA (Pc= 0.689), but a weaker criterion validity for total physical activity (Pc= 0.239). These correlation coefficients are higher for sedentary time but lower for total physical activity than those correlations reported for the 12-country reliability and validity study of IPAQ (Craig et al. 2003a). The lower validity between methods in estimating overall physical activity, could be explained due to intensity reason. For example many questionnaires do not take account of activities that are less intense than brisk walking, or that have a duration of less than ten minutes (Blair et al. 1985). These kinds of activities are less likely to be accurately reported and could be one of the driving factors for bias in measurement of physical activity (Shephard 2003d).

Bland-Altman analysis revealed a constant underestimated bias (~15%) of the self-reported time spent sitting compared to the Actigraph measure. However, the variability of the data shows that those people spending more time in sedentary activities show a slightly tendency to overestimate their self-reported sedentary time, while those subject spending less time in this risk behaviours show an underestimation tendency. Moderate to vigorous physical activity showed an opposite bias in the data, with an apparent overall tendency to overestimate the time spent in MVPA activities (~27%). The Bland-Altman plots illustrate that the

variation on self-reported MVPA depends strongly on the magnitude of the measurement (time spent in that specific intensity domain), those participants that spent less time in MVPA activities show a proportional and higher bidirectional variability (overestimate and underestimate) in self-reported time spent in MVPA, while those that spent more time in this intensity domain were more accurate with the self- reported time.

This pattern on the reported MVPA data has been previously explained. Those individuals that spent more time in MVPA activities are generally part of a structured training system, or systematic sport practice, so they will be more able to remember with a higher accuracy that time spent in this specific intensity of physical activity (I-Min Lee et al. 2009). Total physical activity measured by IPAQ (MVPA + walk) showed a clear and more strong bias to overestimate the time spent in this intensity domains (~78%) compared to objectively measured MVPA, a clear tendency to overestimate was observed across all groups. As was explained before, light activities such as walking or home duties, are more likely to be under or over estimated by the population compared to those activities that are structured (training or sport practice) (I-Min Lee et al. 2009; Shephard 2003b). Similarly, when total self-reported physical activity was compared to objective measures of total physical activity (MVPA + light intensity) a similar tendency to overestimate was observed for the self-reported method. Our results are in agreement with precious studies that reported a similar bias. Macfarlane and colleagues assessed the validity of the IPAQ on a small sample (n = 49) of Chinese adults, they reported a higher overestimate bias for MVPA (~97%) and total physical activity (~102%). However, this study did not report the validity and agreement for sedentary time (Macfarlane et al. 2007). Hagstromer and colleges reported a relative higher agreement of total activity derived from IPAQ compared to the Actigraph, with a 15 min.day$^{-1}$ underestimated bias between methods. However, this study did not report a bias for MVPA or sitting time (Hagstromer, Oja, and Sjostrom 2006a). Another study in Swedish adults reported an underestimate bias (3.4 min.day$^{-1}$) when self-reported MVPA was compared to Actigraph MVPA. When they plotted self reported MVPA + walking against MVPA objectively measured, they found an overestimated bias (~25 min.day$^{-1}$) between methods (Ekelund et al. 2006).

The strong link between physical activity levels and several diseases, and the need to define physical activity and health doses-response relationships in large epidemiological studies, justified the validation of subjective methods to measure physical activity. Questionnaires such as IPAQ offer several advantages compared to objective methods, the application of questionnaires involves a lower cost and could be used in large epidemiological cohorts; these methods also provide a more detailed description of the different types of physical activity. While objective methods, give us a more valid measurement of physical activity intensities, the cost and complexity make them not always the best option for large sample studies. These are some of the reasons that justified the validation of subjective methods that will allow us to determine with more accuracy the levels of physical activity in the population. To assess the construct validity of IPAQ, we tested whether the physical activity /inactivity dose-relationship with several metabolic risk factors, using tertiles of MVPA and sitting time derived from this subjective method, give us a similar answer to the objectively measured physical activity using Actigraph. We found that self-reported sitting time shows a strong validity compared to the criterion methods in the relationship with all markers of metabolic risk across tertiles. On the other hand, self-reported MVPA showed a similar relationship with some of the risk markers (glucose, $HOMA_{IR}$ and HDL) but not all. Total and LDL-cholesterol was not associated to the same extent to IPAQ compared to their relationship with Actigraph. Despite this difference in the relationship for some metabolic markers, self-reported sitting time and MVPA did not revealed significant different across tertiles. These suggest that IPAQ is a reliable and valid method, for measuring physical activity and inactivity levels.

Although the strong agreement and validity between method to quantify sedentary behaviours and MVPA, comparison between accelerometry and self-reported physical activity needs to be interpreted with care. When deriving the time estimated from accelerometer data, *i.e.* time spent in MVPA, all minutes spent above the predetermined threshold for MVPA were included whereas the questionnaire prompted for 10-min blocks of physical activity. As a result, the observed agreement between self-reported and objectively measured MVPA needs to be interpreted while keeping this in mind. Furthermore, accelerometry has limitations as it does not accurately record body movements during specific activities such as cycling, and

climbing stairs, and cannot be used during water activities. A further consideration is that individuals may find it difficult to accurately quantify the amount of time spent walking. This could explain the higher bias of total physical activity between self-reported and objective measures (57.7% of differences between methods) compared to the low bias between methods in MVPA (-26.7%) and sitting time compared to sedentary behaviours (15.5%).

In summary, the results of this chapter indicate that IPAQ have reasonable validity properties for assessing intensities of physical activity in Chilean adults. Additionally, the analysis on this chapter showed that IPAQ is a valid instrument to estimate time spent in MVPA intensities, and also is a good method to estimate time spent sitting, as a proxy of sedentary behaviours, it is important to highlight that not too many questionnaires have been focused to estimated sedentary behaviours indicating its usefulness for estimating this risk behaviours in this population.

# 4 Physical, Metabolic and Lifestyle Characteristics of Mapuche and European Populations, Living in Urban and Rural Environments

## 4.1 Introduction

Prevalence of diabetes is increasing worldwide, but there are large differences in prevalence between regions (IDF 2009), which can, in part, be attributed to differences in urbanization and obesity (Zimmet 2000). However, changes or differences in environment alone do not appear to tell the whole story, particularly in elucidating why certain populations and ethnic groups experience a disproportionately high prevalence of T2D when they adopt a western lifestyle. The classic example of this is the Pima Indians, who when living a traditional rural lifestyle in Mexico are lean, active and have low diabetes incidence, but when living in the US, are generally obese and have a diabetes prevalence in the adult population of ~40% (Schulz et al. 2006a). This pattern is also evident in a number of other aboriginal populations throughout the Americas and Australasia (Yu and Zinman 2007c).

Mapuches – an indigenous Native American population from Chile – appear to follow the same pattern seen in other aboriginal populations of a disproportionate increase in risk of diabetes (compared to Chileans of white European descent) when they move from a traditional rural lifestyle to an urban one. Limited data, based on observations from small sample groups, suggest that diabetes prevalence for Mapuches living in a traditional rural environment is low, at between 1% and 4% of the adult population (Perez-Bravo et al. 2001c; Larenas et al. 1985b) and that prevalence rises to 6.3-8.2% amongst urban-dwelling Mapuches (Perez-Bravo et al. 2006b; Carrasco et al. 2004d). In contrast, Chileans of European descent (who

comprise ~92% of the Chilean population) have higher rates of diabetes than Mapuches in Rural areas (4.5%) and lower rates in Urban (5.8%) settings (Baechler et al. 2002b), the differential between the two environments thus being much smaller in this group. Interestingly, differences in BMI do not explain the difference in diabetes prevalence between rural and urban Mapuches (Perez-Bravo et al. 2001b; Perez-Bravo et al. 2006a), which contrasts with observations from other aboriginal groups where the transition to an urban environment is associated to a substantial increase in obesity prevalence (Schulz et al. 2006e; Collins et al. 1994; King et al. 1984). It is likely that, in common with other aboriginal groups, Mapuches have innate factors that predispose them to increased insulin resistance and diabetes risk and whose effects are revealed on adoption of an urbanised lifestyle. However, it is also possible that Mapuches have a greater lifestyle shift compared to European Chileans when moving from rural to urban settings, which could explain the larger apparent effect of environment on diabetes risk in this group. For example, physical activity or cardiorespiratory fitness levels both predict diabetes risk independently of obesity (Gill and Cooper 2008; Wei et al. 1999c). Larger differences in these factors or in aspects of body composition not reflected in BMI measurements may be evident between urban and rural Mapuches compared to Europeans living between these two environments. Thus, in order to determine whether Mapuches are indeed more susceptible to the adverse metabolic effects of an urban environment than European Chileans, detailed and objective measures of physical and lifestyle variables of both Mapuches and European Chileans living in rural and urban environments are required.

Therefore, the aims of the present chapter were: to characterize anthropometric, dietary, physical activity and physiological profiles in European and Mapuche population living in traditional and westernised environment.

## 4.2 Research Design and Methods

### 4.2.1 Participants

Four groups of participants were recruited to this study: Mapuches (M) living in rural (MR) and urban environments (MU) and Chileans of European descent (E) living in rural (ER) and urban environments (EU). Volunteers were recruited and screened as detailed in section 2.1.5. Specific inclusion criteria were defined in section 2.1.6. Further information detailing recruitment response and excluded volunteers can be found in Figure 2-4. A total of 472 individuals (123 MR, 124 MU, 91 ER, 134 EU) fulfilled the inclusion/exclusion criteria and agreed to participate in the study. All participants gave written informed consent prior to inclusion in this study, which was approved by Research Ethics Committees at Glasgow University, UK, University of Chile, Santiago, Chile and University of Concepcion, BioBio, Chile.

### 4.2.2 Anthropometric Assessment

All phenotype measurements were conducted 'in the field', at the locations where the study populations resided. Facilities for centrifuging blood samples and for cooling/freezing blood and plasma were transported to all locations including remote rural communities. Height, body mass, waist and hip circumferences and skinfolds at four sites (biceps, triceps, subscapular, suprailiac) were measured using standard protocols as detailed in sections 2.3. Body composition was calculated from skinfold measure according to the equations of Durnin and Wormesley (see section 2.3.4). Blood pressure was measured as described in section 2.3.11.

### 4.2.3 Fitness, Physical Activity and Nutritional Assessment

The Chester step-test, a validated 3-stage incremental stepping test, was used to estimate maximal oxygen uptake ($VO_{2max}$) as a measure of fitness. Participants wore accelerometers (ActiTrainer, ActiGraph, LLC, Pensacola, FL, USA), except when showering, swimming and sleeping, for seven consecutive days to objectively assess physical activity levels (details in section 2.3.7). Dietary intake was assessed by 7-

days weighed food record and analyzed using the Chilean Food Composition Database as detailed in section 2.3.8 (Software MINUTA, University of Concepcion. Chile).

## 4.2.4 Metabolic Testing

Venous blood samples were drawn and stored as described in section 2.4. Glucose, triglyceride (TG), total cholesterol, HDL cholesterol, LDL cholesterol, -glutamyl transferase (GGT), alanine aminotransferase (ALT), C-reactive protein (hsCRP), insulin and leptin concentrations were determined using commercially available kits described in section 2.3. Insulin resistance was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA$_{IR}$). More specific details were described in section 2.4.

## 4.2.5 Socio-economic, Health and Cultural Screening

Participants' health history, including smoking status and family health history, was determined by questionnaire. Socioeconomic status was determined with the ESOMAR questionnaire validated in the Chilean population. The original six ESOMAR socioeconomic classes were re-grouped for analysis into three classes: lower, middle and higher socioeconomic status. Demographic and cultural data (age, attained education, most recent occupation, and ethnicity) were determined using the Chilean Socioeconomic Characterisation Questionnaire. All questionnaire data were collected during in-person interviews. (For details see section 2.3.9)

## 4.2.6 Data and Statistical Analysis

Data were analyzed using Statistica (version 8.0; StataSoft, Tulsa, USA) and STATA (version 11.0; College Station. Texas. USA). Prior to analysis, quantitative data were tested for normality using the Anderson–Darling normality test and transformed as appropriate. All continues variables were analysed using a sensitivity identification analysis for outliers, and were standardised concidering values above or bellow ±3SD as extreme or outliers. These extreme values were excluded from the data set

and analysis. Fasting insulin, $HOMA_{IR}$, GGT, ALT and hsCRP were logarithmically transformed (ln) in order to normalize skewed distributions (Dot plot graph for insulin and $HOMA_{IR}$, are shown in Appendix G). Means were compared using General Linear Models. All models were adjusted for age and further models adjusted for potential confounding variables were undertaken as appropriate. For all analyses significance was accepted at $p < 0.05$.

## 4.3 Results

Table 4.1 shows demographic data, by sub-group (MR, MU, ER and EU) for men and women participating in the study. In the sample taken for this study, the four sub-groups differed slightly in age distribution. In the men, urban participants were significantly younger than rural participants, but there was no significant difference in age between Mapuche and European groups. In the women, Mapuche participants were significantly younger than European participants. Because of these small differences, all subsequent statistical analyses were performed on age-adjusted data.

Table 4.2 displays anthropometric variables for both men and women. Rural men were shorter than urban men, and there was a tendency for Mapuche men to be shorter than European men ($p = 0.083$). For BMI, there was a significant 'ethnicity x environment' interaction (Figure 4.1), such that BMI was considerably higher in MR than MU, but there was a much smaller difference between ER and EU. In addition, there was a residual main effect of environment, with higher BMI in rural than urban participants. In contrast, body fat percentage was significantly lower in the rural compared to urban men, despite their higher BMI, and did not differ between Mapuche and European groups. For women, Mapuches were shorter and had a higher BMI than Europeans and rural women had larger hips but lower percentages of body fat than their urban counterparts.
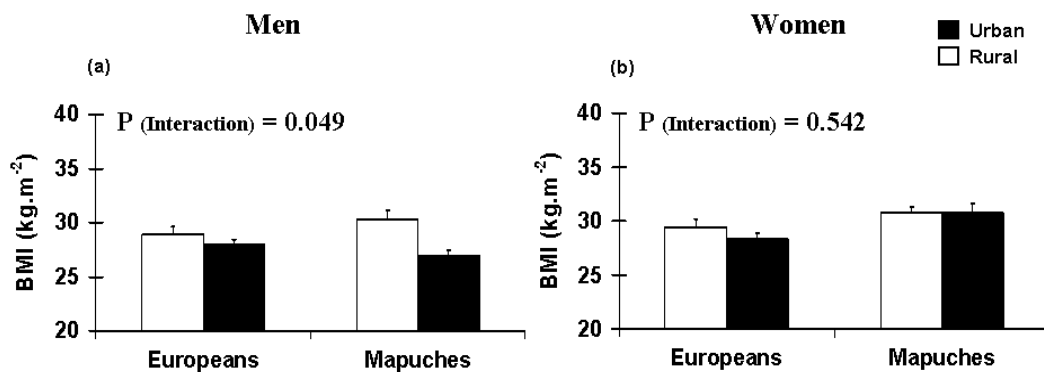
**Figure 4.1. Effect of ethnicity and environment on BMI in European and Mapuche participants.** Bars show age-adjusted mean ± SEM for all groups.

Table **4.4** show metabolic variables for men and women, respectively. In both men and women, fasting insulin concentrations and HOMA$_{IR}$ differed markedly between subgroups (Figure 4.2). An 'ethnicity x environment' interaction was observed in both sexes (more strongly in women), whereby fasting insulin and HOMA$_{IR}$ were found to be higher in Mapuches than Europeans and higher in urban than rural groups, the difference between the urban and rural groups being significantly greater in the Mapuches than the Europeans (Figure 4.2). Fasting glucose levels also differed between urban and rural participants in women.

**Table 4.1. Demographic variables in men and women by ethnic group and environment**

| | | Mapuche | | European | | p value | | |
|---|---|---|---|---|---|---|---|---|
| | | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
| *Men* | n | 54 | 45 | 36 | 42 | | | |
| | Age | 38.4 ± 12.6 | 32.1 ± 12.9 | 41.1 ± 12.7 | 34.5 ± 14.5 | 0.172 | **0.001** | 0.948 |
| | Smoking Status (Never / Ex / Current) | 37 / 6 / 11 | 30 / 14 / 35 | 23 / 6 / 7 | 17 / 7 / 18 | - | - | - |
| | Socio economic Level (Lower / Middle / Higher) | 43 / 8 / 3 | 17 / 39 / 23 | 18 / 10 / 8 | 9 / 12 / 21 | - | - | - |
| | Education Level (Primary / Secondary / Tertiary) | 44 / 9 / 1 | 4 / 53 / 22 | 13 / 13 / 10 | 1 / 20 / 21 | - | - | - |
| *Women* | n | 69 | 79 | 55 | 92 | | | |
| | Age | 35.4 ± 11.2 | 37.6 ± 11.1 | 40.7 ± 14.3 | 38.9 ± 11.3 | **0.030** | 0.981 | 0.186 |
| | Smoking Status (Never / Ex / Current) | 43 / 10 / 16 | 20 / 11 / 14 | 27 / 10 / 18 | 27 / 29 / 36 | - | - | - |
| | Socio economic Level (Lower / Middle / Higher) | 52 / 13 / 4 | 7 / 26 / 12 | 20 / 23 / 12 | 23 / 30 / 39 | - | - | - |
| | Education Level (Primary / Secondary / Tertiary) | 50 / 9 / 10 | 6 / 30 / 9 | 17 / 27 / 11 | 6 / 32 / 54 | - | - | - |

Data are presented as mean ± SD for untransformed, unadjusted data or counts. *p* values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Ethn x Env) interaction effect.

**Table 4.2. Anthropometric variables in men and women by ethnic group and environment**

| | | Mapuche | | European | | p value | | |
| | | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
|---|---|---|---|---|---|---|---|---|
| *Men* | Normal / Overweight / Obese [a] | 5 / 20 / 29 | 23 / 38 / 18 | 7 / 17 / 12 | 16 / 13 / 13 | | | |
| | Body mass (kg) | 78.9 ± 9.9 | 74.3 ± 11.9 | 78.4 ± 13.4 | 76.7 ± 13.5 | 0.606 | 0.104 | 0.393 |
| | Height (m) | 161.3 ± 6.0 | 165.8 ± 6.1 | 164.7 ± 9.8 | 166.1 ± 8.3 | 0.083 | **0.006** | 0.139 |
| | BMI (kg.m$^{-2}$) | 30.3 ± 3.5 | 26.9 ± 3.9 | 28.9 ± 3.5 | 27.9 ± 3.5 | (0.524) | **(0.005)** | **0.049** |
| | Waist (cm) | 107.1 ± 10.4 | 101.4 ± 16.7 | 105.3 ± 12.6 | 102.2 ± 17.4 | 0.548 | 0.234 | 0.524 |
| | Hip (cm) | 115.1 ± 8.1 | 111.4 ± 10.2 | 113.4 ± 7.1 | 111.0 ± 11.5 | 0.295 | 0.102 | 0.600 |
| | Body fat (%) | 27.3 ± 4.6 | 30.1 ± 5.1 | 25.7 ± 6.7 | 30.5 ± 6.8 | 0.304 | **0.0001** | 0.270 |
| *Women* | Normal / Overweight / Obese [a] | 13 / 31 / 25 | 16 / 17 / 12 | 10 / 24 / 21 | 28 / 34 / 26 | | | |
| | Body mass (kg) | 73.4 ± 12.6 | 71.7 ± 13.3 | 70.9 ± 12.5 | 68.7 ± 12.2 | 0.080 | 0.217 | 0.921 |
| | Height (m) | 154.5 ± 6.1 | 152.6 ± 5.5 | 155.4 ± 6.6 | 156.2 ± 6.5 | **0.0002** | 0.607 | 0.206 |
| | BMI (kg.m$^{-2}$) | 30.7 ± 4.8 | 30.7 ± 5.2 | 29.4 ± 5.2 | 28.3 ± 5.5 | **0.002** | 0.354 | 0.542 |
| | Waist (cm) | 106.3 ± 11.6 | 103.6 ± 13.3 | 103.1 ± 15.3 | 102.4 ± 15.7 | 0.129 | 0.314 | 0.438 |
| | Hip (cm) | 118 ± 11.1 | 115.4 ± 12.6 | 115.9 ± 13.1 | 112.9 ± 10.5 | 0.061 | **0.037** | 0.944 |
| | Body fat (%) | 31.2 ± 5.2 | 33.7 ± 4.8 | 30.5 ± 4.9 | 32.1 ± 5.5 | 0.074 | **0.009** | 0.787 |

Data are presented as mean ± SD for untransformed, unadjusted data. Statistical analysis was undertaken on age-adjusted data (except for age comparisons). *p* values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Ethn x Env) interaction effect: for the main effects, those in parentheses indicate residual main effects in a model with a significant interaction term, while those not in parentheses indicate main effects in a model without the interaction term. Significant *p* values are shown in bold. [a] Participants were classified using BMI as normal weight (<25.0 kg.m$^{-2}$), Overweight (25.0–29.9 kg.m$^{-2}$) or Obese (>30.0 kg.m$^{-2}$).
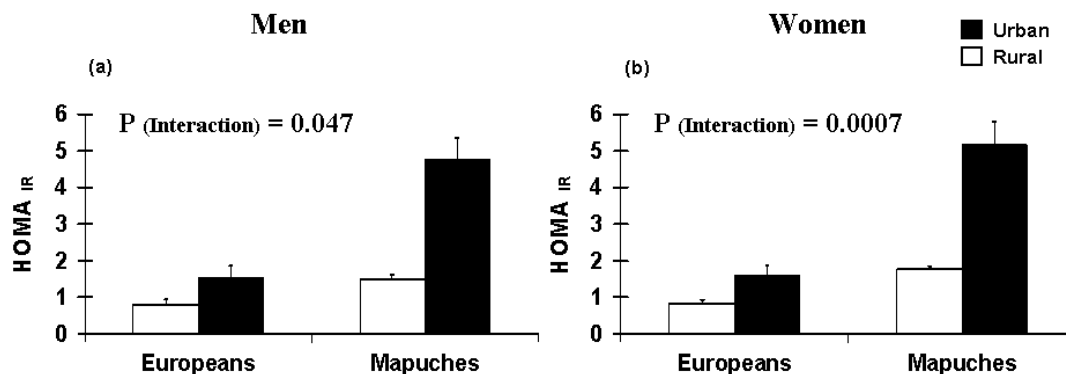
**Figure 4.2. Effect of ethnicity and environment on HOMA$_{IR}$ in European and Mapuche participants.**
Bars show age-adjusted mean ± SEM for all groups.

In both sexes, triglycerides and hsCRP were higher, and HDL cholesterol was lower, in urban than rural participants, but there was no difference between Mapuches and Europeans for these variables. Differences between urban and rural participants total and LDL cholesterol concentrations approached significance in both sexes. Circulating GGT concentrations were higher in urban than rural participants and higher in Mapuches than Europeans in both men and women. The effects of ethnicity and urbanisation on leptin concentrations differed between men and women, although the trend was the same in both groups. In men, urban participants had higher leptin concentrations than rural participants, but there was no significant difference between Mapuches and Europeans. In women, there was a significant 'ethnicity x environment' interaction – urban women had higher leptin concentrations than rural women, Mapuche women had higher concentrations than European women and the difference between rural and urban women was greater in Mapuches than in Europeans.

**Table 4.3. Metabolic variables in men by ethnic group and environment**

| | Mapuche | | European | | *p* value | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
| Systolic Blood Pressure (mmHg) | 121.7 ± 14.1 | 125.3 ± 17.1 | 122.7 ± 16.3 | 124.2 ± 16.9 | 0.551 | **0.010** | 0.661 |
| Diastolic Blood Pressure (mmHg) | 78.6 ± 12.5 | 75.5 ± 12.1 | 77.6 ± 12.2 | 72.3 ± 12.3 | 0.099 | 0.149 | 0.592 |
| Glucose (mmol.l$^{-1}$) | 5.42 ± 1.32 | 5.34 ± 1.31 | 5.35 ± 0.80 | 5.92 ± 0.83 | 0.111 | 0.151 | 0.099 |
| Insulin (pmol.l$^{-1}$)[b] | 43.0 ± 61.7 | 141.8 ± 102.2 | 22.2 ± 17.1 | 39.7 ± 28.6 | **(0.001)** | **(0.0005)** | **0.041** |
| HOMA$_{IR}$[b] | 1.47 ± 1.97 | 4.76 ± 5.51 | 0.79 ± 0.67 | 1.52 ± 1.19 | **(0.001)** | **(0.0001)** | **0.047** |
| Triglyceride (mmol.l$^{-1}$) | 1.18 ± 0.51 | 1.36 ± 0.67 | 1.27 ± 0.63 | 1.35 ± 0.78 | 0.965 | **0.022** | 0.639 |
| Total cholesterol (mmol.l$^{-1}$) | 4.44 ± 0.91 | 4.58 ± 1.34 | 4.77 ± 1.28 | 4.69 ± 1.25 | 0.414 | 0.105 | 0.580 |
| HDL cholesterol (mmol.l$^{-1}$) | 1.14 ± 0.45 | 0.91 ± 0.37 | 1.01 ± 0.46 | 0.93 ± 0.42 | 0.738 | **0.002** | 0.263 |
| LDL cholesterol (mmol.l$^{-1}$) | 2.76 ± 0.95 | 3.05 ± 1.19 | 3.18 ± 1.41 | 3.15 ± 1.33 | 0.377 | **0.044** | 0.430 |
| Leptin (ng.ml$^{-1}$) | 8.98 ± 7.53 | 17.3 ± 14.87 | 7.11 ± 6.11 | 13.3 ± 11.38 | 0.083 | **0.0001** | 0.590 |
| GGT (U.L$^{-1}$)[b] | 34.6 ± 18.9 | 51.2 ± 44.3 | 32.3 ± 19.2 | 31.9 ± 25.6 | **0.014** | **0.011** | 0.134 |
| ALT (U.L$^{-1}$)[b] | 43.7 ± 20.8 | 34.4 ± 24.5 | 37.2 ± 24.1 | 38.6 ± 25.7 | 0.901 | 0.499 | 0.175 |
| HsCRP (mg.l$^{-1}$)[b] | 0.81 ± 0.69 | 1.21 ± 1.61 | 0.84 ± 0.68 | 1.43 ± 1.57 | 0.647 | **0.002** | 0.660 |

Data are presented as mean ± SD for untransformed, unadjusted data. Statistical analysis was undertaken on age-adjusted data (except for age comparisons). *p* values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Ethn x Env) interaction effect: for the main effects, those in parentheses indicate residual main effects in a model with a significant interaction term, while those not in parentheses indicate main effects in a model without the interaction term. Significant *p* values are shown in bold. [b]statistical analyses performed on log-transformed data.

**Table 4.4. Metabolic variables in women by ethnic group and environment**

| | Mapuche | | European | | p value | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
| Systolic Blood Pressure (mmHg) | 119.2 ± 16.1 | 122.7 ± 14.3 | 119.4 ± 17.1 | 123.5 ± 17.0 | 0.546 | 0.087 | 0.503 |
| Diastolic Blood Pressure (mmHg) | 75.1 ± 11.1 | 76.1 ± 13.5 | 74.4 ± 11.7 | 77.4 ± 11.5 | 0.808 | 0.135 | 0.325 |
| Glucose (mmol.l$^{-1}$) | 5.19 ± 1.14 | 5.58 ± 1.55 | 5.20 ± 1.01 | 5.73 ± 1.13 | 0.923 | **0.005** | 0.272 |
| Insulin (pmol.l$^{-1}$)[b] | 48.9 ± 57.9 | 149.1 ± 106.4 | 24.8 ± 15.7 | 41.8 ± 34.1 | **(0.0001)** | **(0.0001)** | **0.0004** |
| HOMA$_{IR}$[b] | 1.76 ± 2.09 | 5.15 ± 4.16 | 0.84 ± 0.58 | 1.57 ± 1.43 | **(0.0001)** | **(0.0001)** | **0.0006** |
| Triglyceride (mmol.l$^{-1}$) | 0.97 ± 0.39 | 1.35 ± 0.71 | 1.17 ± 0.49 | 1.18 ± 0.74 | 0.457 | **0.014** | 0.169 |
| Total cholesterol (mmol.l$^{-1}$) | 4.56 ± 1.06 | 5.09 ± 1.45 | 4.72 ± 1.38 | 4.84 ± 1.16 | 0.412 | 0.054 | 0.546 |
| HDL cholesterol (mmol.l$^{-1}$) | 1.04 ± 0.36 | 0.89 ± 0.49 | 0.92 ± 0.36 | 0.88 ± 0.33 | 0.615 | **0.029** | 0.835 |
| LDL cholesterol (mmol.l$^{-1}$) | 3.07 ± 1.16 | 3.58 ± 1.59 | 3.26 ± 1.44 | 3.42 ± 1.29 | 0.629 | 0.065 | 0.735 |
| Leptin (ng.ml$^{-1}$) | 10.3 ± 8.47 | 23.2 ± 11.63 | 9.98 ± 7.39 | 13.2 ± 15.32 | **(0.001)** | **(0.0001)** | **0.009** |
| GGT (U.L$^{-1}$)[b] | 29.2 ± 19.1 | 49.1 ± 37.2 | 24.1 ± 15.7 | 30.1 ± 24.1 | **0.0001** | **0.0003** | 0.163 |
| ALT (U.L$^{-1}$)[b] | 41.9 ± 19.4 | 38.6 ± 26.9 | 36.6 ± 19.3 | 34.6 ± 22.8 | 0.096 | 0.400 | 0.534 |
| HsCRP (mg.l$^{-1}$)[b] | 1.21 ± 1.29 | 1.84 ± 1.40 | 1.45 ± 1.41 | 1.64 ± 1.44 | 0.855 | **0.040** | 0.390 |

Data are presented as mean ± SD for untransformed, unadjusted data. Statistical analysis was undertaken on age -adjusted data (except for age comparisons). p values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Et hn x Env) interaction effect: for the main effects, those in parentheses indicate residual main effects in a model with a significant interaction term, while those not in parentheses indicate main effects in a model without the interaction term. Significan t p values are shown in bold. [b]statistical analyses performed on log -transformed data.

Fitness and activity measures also showed differences between subgroups (Table 4.5). In both men and women, $VO_{2max}$ was higher in rural than urban participants (Figure 4.3). In men, Mapuches had significantly higher $VO_{2max}$ values than Europeans and there was a borderline 'ethnicity x environment' interaction ($p = 0.077$). In women, there was a significant 'ethnicity x environment' interaction, with a greater difference in $VO_{2max}$ between Urban and Rural groups in Mapuches than Europeans. In men, urban participants spent more time sedentary and less time in moderate-to-vigorous activity than rural participants, but there were no differences between Mapuches and Europeans. There were no differences in time spent in light activity between groups in men. The effects of environment and ethnicity on activity were different in women. Urban women spent more time sedentary and less time in light activities than rural women. In addition, European women spent more time in light activities than Mapuche women. However, there was no effect of environment or ethnicity on moderate-to-vigorous activity in women. Thus, in this cohort, urbanisation appears to be characterised by an increase in sedentary time at the expense of time in moderate-to-vigorous activity in men, but an increase in sedentary time at the expense of light activity in women.
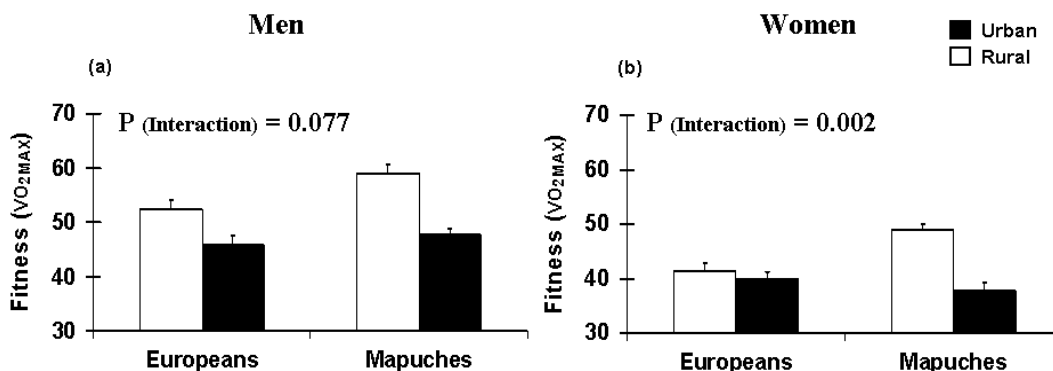


**Figure 4.3. Effect of ethnicity and environment on fitness in European and Mapuche participants.**
Bars show age-adjusted mean ± SEM for all groups.

**Table 4.5. Fitness and physical activity variables in men and women by ethnic group and environment**

| | | Mapuche | | European | | *p* value | | |
|---|---|---|---|---|---|---|---|---|
| | | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
| *Men* | $VO_{2max}$ (ml.kg.min$^{-1}$) | 59.1 ± 12.3 | 47.6 ± 9.4 | 52.4 ± 10.9 | 45.7 ± 9.8 | **0.044** | **0.0001** | 0.077 |
| | Sedentary time (min.day$^{-1}$) | 495.5 ± 77.3 | 537.9 ± 99.0 | 482.6 ± 91.9 | 547.6 ± 109.8 | 0.771 | **0.0004** | 0.595 |
| | Light activity (min.day$^{-1}$) | 244.1 ± 64.1 | 242.3 ± 88.2 | 217.8 ± 90.5 | 241.8 ± 95.8 | 0.475 | 0.611 | 0.361 |
| | Moderate-vigorous activity (min.day$^{-1}$) | 46.3 ± 36.5 | 32.6 ± 25.8 | 55.1 ± 39.6 | 22.8 ± 16.8 | 0.532 | **0.0001** | 0.087 |
| *Women* | $VO_{2max}$ (ml.kg.min$^{-1}$) | 48.9 ± 10.4 | 37.7 ± 7.8 | 41.3 ± 10.3 | 39.9 ± 9.4 | (0.072) | **(0.0001)** | **0.002** |
| | Sedentary time (min.day$^{-1}$) | 501.7 ± 72.9 | 534.9 ± 98.1 | 521.3 ± 96.4 | 546.3 ± 79.6 | 0.285 | **0.031** | 0.947 |
| | Light activity (min.day$^{-1}$) | 257.6± 68.3 | 223.8 ± 69.5 | 287.8 ± 88.6 | 249.6 ± 90.9 | **0.025** | **0.004** | 0.810 |
| | Moderate-vigorous activity (min.day$^{-1}$) | 36.8 ± 24.8 | 29.6 ± 20.2 | 29.5 ± 15.8 | 29.1 ± 16.3 | 0.194 | 0.303 | 0.434 |

Data are presented as mean ± SD for untransformed, unadjusted data. S tatistical analysis was undertaken on age-adjusted data (except for age comparisons). *p* values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Ethn x Env) interaction effect: for the main effects, tho se in parentheses indicate residual main effects in a model with a significant interaction term, while those not in parentheses indicate main effects in a model without the interaction term. Significant *p* values are shown in bold.

Dietary factors also differed noticeably between subgroups (Table 4.6). In women, there were significant 'ethnicity x environment' interactions for all dietary components except carbohydrate intake; in men, significant interactions were evident for all components except energy, carbohydrate and fat intakes. In addition to any interactions, higher intakes of all dietary components (in women), or all except protein intake (in men) were observed in Mapuches. Furthermore, rural participants had higher intakes than urban participants for all dietary components. The interactions suggest that ethnic differences exist for the effects of urbanisation on diet, for almost all dietary components the differences between rural and urban populations were greater in Mapuches than Europeans. This pattern was reversed only for protein intake in men, where the difference between urban and rural participants was greater in Europeans than in Mapuches. For alcohol intake, the interaction was largely mediated by a considerably higher intake in rural Mapuches, in whom the alcohol intake was largely from a locally fermented wine consumed regularly with meals.

**Table 4.6. Dietary variables in men and women by ethnic group and environment**

|  |  | Mapuche | | European | | *p* value | | |
|---|---|---|---|---|---|---|---|---|
|  |  | Rural | Urban | Rural | Urban | Ethn | Env | Ethn x Env Interaction |
| *Men* | Energy intake (kcal.day$^{-1}$) | 3424.7 ± 805.1 | 2862.5 ± 772.1 | 2663.4 ± 638.2 | 2330.3 ± 664.4 | **0.0001** | **0.0001** | 0.323 |
|  | Carbohydrate intake (g.day$^{-1}$) | 480.3 ± 135.1 | 424.9 ± 127.8 | 375.4 ± 114.1 | 360.3 ± 110.7 | **0.001** | **0.042** | 0.310 |
|  | Fat intake (g.day$^{-1}$) | 104.1 ± 37.1 | 80.6 ± 31.4 | 70.7 ± 33.7 | 57.8 ± 25.9 | **0.0001** | **0.0001** | 0.301 |
|  | Protein intake (g.day$^{-1}$) | 109.7 ± 58.6 | 97.6 ± 24.3 | 121.1 ± 32.3 | 84.8 ± 31.8 | 0.736 | **0.001** | **0.050** |
|  | Dietary fibre intake (g.day$^{-1}$) | 8.7 ± 3.7 | 5.9 ± 3.6 | 4.5 ± 2.4 | 4.2 ± 1.4 | **(0.0001)** | **(0.001)** | **0.017** |
|  | Alcohol intake (g.day$^{-1}$) | 18.1 ± 13.1 | 6.6 ± 5.5 | 5.7 ± 8.4 | 4.1 ± 2.6 | **(0.0001)** | **(0.0001)** | **0.0001** |
|  |  |  |  |  |  |  |  |  |
| *Women* | Energy intake (kcal.day$^{-1}$) | 3317.1 ± 856.9 | 2375.7 ± 373.5 | 2217.1 ± 504.5 | 2180.1 ± 601.8 | **(0.0001)** | **(0.0001)** | **0.0001** |
|  | Carbohydrate intake (g.day$^{-1}$) | 448.3 ± 131.1 | 365.3 ± 131.1 | 333.6 ± 89.2 | 298.6 ± 102.4 | **0.0001** | **0.0008** | 0.151 |
|  | Fat intake (g.day$^{-1}$) | 110.6 ± 45.3 | 60.2 ± 23.4 | 56.2 ± 29.5 | 67.3 ± 28.4 | **(0.0001)** | **(0.0001)** | **0.0001** |
|  | Protein intake (g.day$^{-1}$) | 108.3 ± 43.7 | 84.1 ± 27.1 | 84.3 ± 30.1 | 83.6 ± 32.3 | **(0.020)** | **(0.017)** | **0.023** |
|  | Dietary fibre intake (g.day$^{-1}$) | 7.4 ± 4.8 | 4.1 ± 1.1 | 4.4 ± 2.7 | 3.9 ± 1.9 | **(0.001)** | **(0.0001)** | **0.002** |
|  | Alcohol intake (g.day$^{-1}$) | 13.4 ± 10.1 | 5.3 ± 2.6 | 5.6 ± 7.3 | 6.4 ± 13.1 | **(0.033)** | **(0.017)** | **0.005** |

Data are presented as mean ± SD for untransformed, unadjusted data. Statistical analysis was undertaken on age -adjusted data (except for age comparisons). *p* values shown are main effects for ethnicity (Ethn) and for environment (Env), and the Ethnicity x environment (Ethn x Env) interaction effect: for the main effects, those in parentheses indicate residual main effects in a model with a significant interaction term, while those not in parentheses indicate main effects in a mode l without the interaction term. Significant *p* values are shown in bold.

## 4.4 Discussion

The main findings of this chapter were: (a) that Mapuche men and women are more insulin resistant (as assessed by $HOMA_{IR}$) than Chilean men and women of European descent; (b) urbanisation appears to have a greater effect on insulin resistance in Mapuches than European Chileans – there was a three-fold difference in $HOMA_{IR}$ between urban and rural Mapuches, compared with a two-fold difference in European Chileans, with a significant ethnicity x environment interaction for this effect in both men and women; (c) the differences between urban and rural populations for dietary intake, physical activity and sedentary time, and percentage body fat were similar between the two ethnic groups, suggesting that the greater difference in $HOMA_{IR}$ between urban and rural Mapuches compared to Europeans is not simply the consequence of a larger lifestyle shift between rural and urban environments in the former group.

The finding that the Mapuches were more insulin resistant than the Chileans of European descent and showed greater differences in insulin resistance between rural and urban settings is consistent with the pattern observed in Pima Indians. It has recently been reported that Pima Indians in the USA with normal glucose tolerance have four-fold higher $HOMA_{IR}$ values than Mexican Pimas (Esparza-Romero et al. 2010a). This difference in $HOMA_{IR}$ was attenuated, but remained significant, after adjustment for differences in BMI between the two groups, but was also evident in a subgroup matched for BMI (Esparza-Romero et al. 2010b). In addition, US Pima Indians have been shown to be more insulin resistant than age- and adiposity-matched Americans of European descent (Stefan et al. 2004a). In the present study the greater insulin resistance in the urban, compared to rural, Mapuche occurred in the absence of a higher BMI, although percentage body fat was greater in the urban Mapuche group. However, although percentage body fat was greater in the urban compared to rural groups in the present study, the extent of the difference did not differ between the Mapuche and Chileans of European descent, indicating that increasing adiposity, in itself, could not explain the disproportionately large increase in insulin resistance associated with urbanisation in the Mapuche population. Similar observations have been made when considering other groups at increased risk of

T2D. For example, adults (Razak et al. 2007a) and children (Whincup et al. 2002a) of South Asian origin exhibit larger increases in insulin resistance with increasing adiposity than White Europeans.

It is well established that high levels of physical activity (Gill and Cooper 2008) and cardiorespiratory fitness (Wei et al. 1999g) are protective against T2D and are associated with a favourable metabolic risk profile (Gill and Malkova 2006e). Recently, it has also become evident that increasing sedentary time is associated with increased diabetes risk and an adverse metabolic risk profile (Healy et al. 2008f; Gill et al. 2011d). Reports have shown that low levels of self-reported physical activity, or low cardiorespiratory fitness are associated with insulin resistance in other aboriginal groups (Kriska et al. 2001c). However, the findings in the present study showed that Mapuches are more insulin resistant than Chileans of European descent when they live in urban environments, despite the similar amount of time spent in sedentary activities or moderate to vigorous physical activity in both sexes, indicating that increasing time spent in sedentary behaviours or reducing time spent in MVPA, in itself, could not explain the disproportionately large increase in insulin resistance associated with urbanisation in the Mapuche population.

In conclusion, the present chapter reveals that urbanisation influences insulin resistance to a greater extent in Chilean Mapuches than in Chileans of European descent. The cross-sectional nature of these data does not allow firm conclusions to be drawn about potential causality of insulin resistance, but the findings: 1) highlight the fact that environmental and lifestyle effects on metabolic risk differ between ethnic groups and 2) suggest that further studies into the mechanisms underpinning this effect are needed. This has potential implications both for the design and implementation of lifestyle strategies to reduce metabolic risk in different ethnic groups, and for advancing the basic understanding of the mechanisms underpinning human insulin resistance.

# 5 Association of Obesity and Physical Activity Lifestyle Patterns with Insulin Resistance in Mapuche and European Chilean Population

## 5.1 Introduction

In the previous chapter, it was found that Mapuches were more insulin resistant (IR) than Chileans of European descent, and had greater increases in insulin resistance with urbanisation compared to European Chileans. However, this occurred in the absence of a higher level of adiposity, or a clear difference in physical activity lifestyle patterns between the Mapuches and Europeans, suggesting that, differences in insulin resistance between the ethnic groups might not be explained in a straight forward manner by adiposity or physical activity. These results are intriguing given that physical activity and diet, and their associated consequences, cardiorespiratory fitness and obesity; appear to play an important role in the aetiology of T2D.

Increasing levels of physical activity (Gill and Cooper 2008) or increasing cardiorespiratory fitness (Wei et al. 1999b) has been shown to be protective against T2D and are associated with a favourable metabolic risk profile (Gill and Malkova 2006d). However, sedentary behaviour (*i.e.* excessive sitting, as distinct from insufficient PA) has increasingly gained interest as a potential determinant of ill health (Dunstan et al. 2004c). Cross-sectional and prospective evidence support the adverse association between sedentary behaviours and several intermediate cardiovascular risk factors, including metabolic syndrome and related metabolic markers, obesity, abnormal glucose metabolism and T2D (Hill and Peters 1998; Astrup 2001; Gill et al. 2011c; Hu et al. 2001b). Interesting, these associations were independent of physical activity and an adverse effect of sedentary activities has been observed in physically active individuals (Healy et al. 2008e).

The other key factor that has been associated with increased increase risk diabetes risk is adiposity (WHO 2000). Increased adiposity is the consequence of chronic positive energy balance and increased energy intake is likely to play a central role in this. A higher dietary energy intake has been linked to rising obesity rates and other metabolic disorders (Mendoza, Drewnowski, and Christakis 2007a; Howarth et al. 2006c). Considering that T2D is fundamentally a condition of disordered glucose metabolism strongly influenced by adiposity, it is not unreasonable to suggest that if increased energy intake leads to increased carbohydrate intake (starches and added sugars); they may contribute to the development of insulin resistance by virtue of their increased glycemic load (Hu, van Dam, and Liu 2001; Salmeron et al. 1997). Additionally, increased energy intake may contribute to insulin resistance by their higher levels of fats, which have been shown to be related to impaired insulin sensitivity (Vessby et al. 2001).

In South America, the prevalence of obesity and T2D has rapidly increased. This could be related to prominent changes in the lifestyle factors mentioned before, such as lack of physical activity and diets with high energy density (Barcelo and Rajpathak 2001c; Avezum et al. 2009). These, together with the high prevalence of physical inactivity 88% of Chileans do not meet current physical activity guidelines) are likely to be the major driving forces for the high prevalence of overweight (66.7% of the Chilean population have a BMI over 25 $kg.m^{-2}$), and the increasing prevalence of diabetes (from 4.2% in 2003 to 9.4% in 2010) in the Chilean population (MINSAL 2010).

It is important to note that these National Health Survey diabetes prevalence figures do not tell the full story, as the prevalence of diabetes differs widely by ethnicity (Yu and Zinman 2007e). The information provided by this survey should be interpreted with caution since Chile's population is comprised of an important number of Amerindian populations as well as a large proportion of people with white European backgrounds. As reported in the previous chapter of this thesis, Mapuches appear to follow a pattern of a disproportionate increase in insulin resistance (compared to Chileans of white European population descent) when they move from a traditional rural lifestyle to an urban one. Thus, it seems likely that, in common with other ethnic groups, Mapuches have innate factors that predispose them to increased

insulin resistance and diabetes risk. However, it is also possible that Mapuches respond differently to European when they change their lifestyles. Thus, in order to determine whether Mapuches are indeed more susceptible to the adverse metabolic effects of low physical activity and obesity than European Chileans, the aims of the present chapter are:

(a) To examine the effects of dietary intake, physical activity and fitness, on adiposity-related traits in Mapuches and Europeans; and

(b) To determine whether the influences of dietary intake, adiposity, physical activity and fitness, on insulin resistance differ between populations with different ethnic backgrounds.

## 5.2 Methods

### 5.2.1 Participants and Data Collection

The sample was stratified as described in chapter 2. Specific details of anthropometric assessment, fitness, physical activity, dietary intake patterns, socio-economic, educational and metabolic testing were previously described in chapter 2.

### 5.2.2 Data and Statistical Analysis

Data were analyzed using Statistica (version 8.0; StataSoft, Tulsa, USA) and STATA (version 11.0; College Station. Texas. USA). Quantitative data were tested for normality using the Anderson–Darling normality test, subjected to Box-Cox analysis, and transformed as appropriate. $HOMA_{IR}$ were logarithmically transformed (natural log) in order to normalized skewed distributions. Data analyses were performed with transformed data when appropriate.

Due to women possessing higher BMIs and body fat percentages than men, sex-stratified analyses were performed. Men and women were divided into tertiles for

indices of adiposity (BMI, waist circumference and body fat), physical activity (time spent in sedentary behaviours and MVPA), fitness ($VO_{2max}$) and dietary intake (total energy, carbohydrates, starch, sugar, total fat, monounsaturated fat, polyunsaturated fat, saturate fat and protein intake). To determine whether the effect of increasing physical activity, fitness, adiposity and diet factors on insulin resistance ($HOMA_{IR}$) differed between the European and Mapuche populations, general linear models (GLM) were performed, with $HOMA_{IR}$ as the outcome variable. Since GLM analysis required the use of categorical factors for modelling an interaction, continuous variables such as PA variables were transformed into tertiles. This analysis was repeated (*i.e.* considering tertile and environment main effects and the tertile x ethnicity interaction) with the obesity-related phenotypes (BMI, waist circumference and body fat) and tertiles of dietary intake and physical activity as explanatory variables. All models were adjusted for age and further models adjusted for potential confounding variables were undertaken as appropriate. A trend analysis was performed using a multiple regression analysis to assess whether increasing tertiles of the explanatory factors showed a linear effect. Lower tertiles were coded as "0", middle tertiles as "1" and upper tertiles as "2" in each case. For all analyses significance was accepted at $p < 0.05$.

## 5.3 Results

Figure 5.1 shows the effect of increases in BMI, waist circumference and percentage body fat on $HOMA_{IR}$ for European and Mapuche men and women, in age-adjusted analyses. Multiple regression analyses confirmed the visible trend in both men and women for $HOMA_{IR}$ to increase with increasing BMI, waist circumference and percentage body fat; statistically significant trends were observed for all the terms except waist circumference in female Mapuche participants ($p = 0.092$). Interestingly, there was a significant 'ethnicity x BMI tertile' interaction for $HOMA_{IR}$ in women ($p = 0.018$) but borderline significant interaction in men ($p = 0.078$), with Mapuches experiencing significantly greater increases in $HOMA_{IR}$ with increasing BMI than Europeans. This interaction was also evident in men for waist circumference ($p = 0.014$). Further adjustments for smoking status, environment (rural or urban), socio-economic level and education level not did alter the statistical

significance of some of these findings, such as the 'ethnicity x BMI tertile" interaction in men and 'ethnicity x Body Fat tertile" interaction in women, which became significant after adjustment ($p = 0.004$ and $p = 0.023$, respectively) and the 'ethnicity x waist tertile' interaction in men, which became not significant after adjustment ($p = 0.091$).
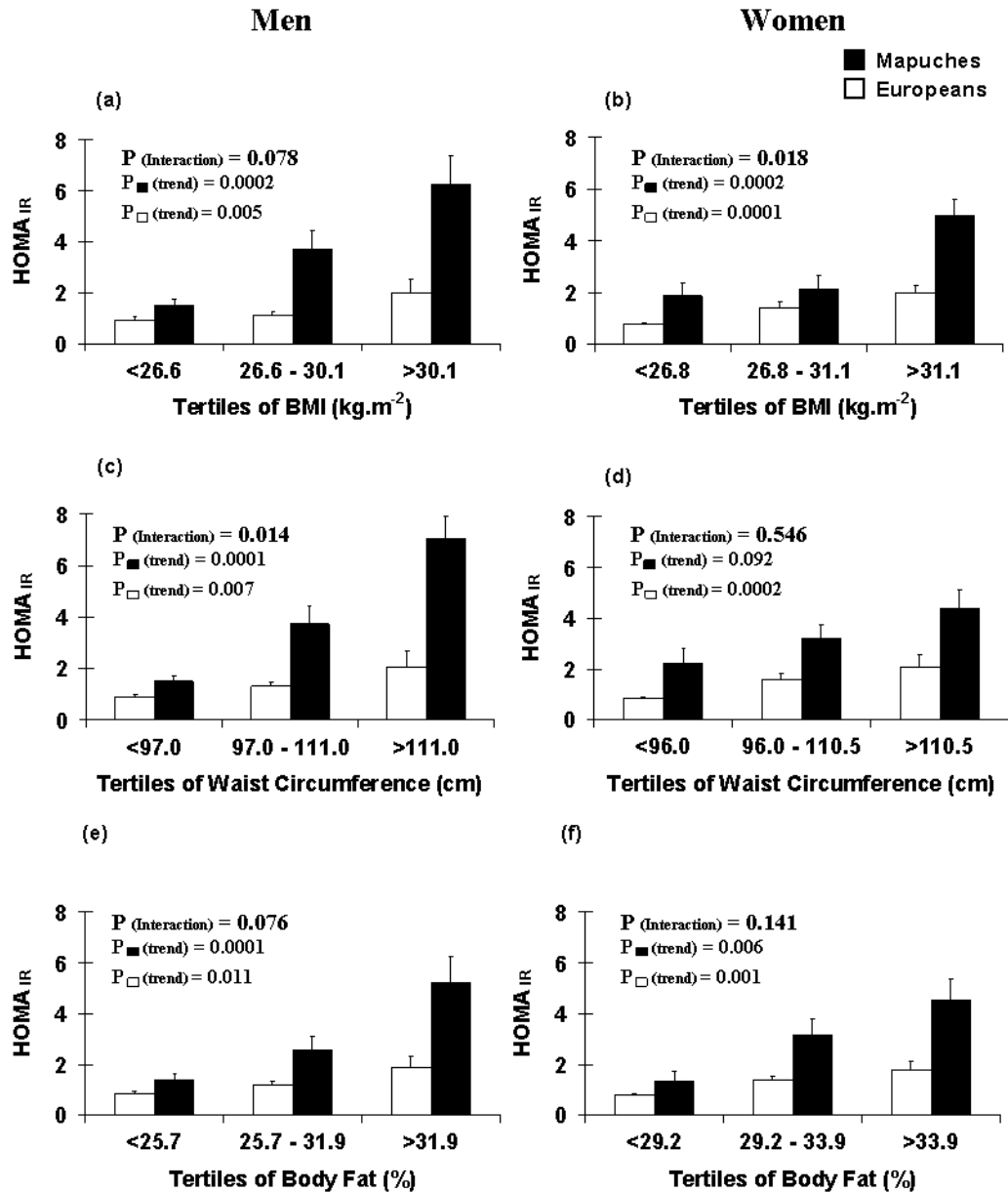


**Figure 5.1. Effects of BMI, waist circumference and percentage of body fat on HOMA$_{IR}$ in European and Mapuche participants.**
Bars show mean ± SEM for all groups. Values defining each tertile are given for each of the three distributions. Statistical tests were carried out in males and females separately.

Figure 5.2 shows the effects of increasing amounts of sedentary time, moderate-to-vigorous physical activity (MVPA) and fitness (as measured using $VO_{2max}$) on $HOMA_{IR}$ in European and Mapuche men and women, in age-adjusted analyses. As for the anthropometric variables described above, in the multiple regression analysis there were significant trends for $HOMA_{IR}$ to increase with increasing tertiles of sedentary time, and with decreasing tertiles of MVPA and decreasing tertiles of $VO_{2max}$ in both men and women. In both sexes there were significant 'ethnicity x sedentary time tertile' ($p = 0.0002$ in men and $p = 0.0001$ in women) and 'ethnicity x MVPA tertile' interactions ($p = 0.0001$ in men and $p = 0.044$ in women), such that Mapuches experienced greater increases in $HOMA_{IR}$ with increasing sedentary time and decreasing MVPA than Europeans (Figure 5.2). In women there was a significant 'ethnicity x $VO_{2max}$ tertile' interaction, such that low $VO_{2max}$ was associated with greater increases in $HOMA_{IR}$ in Mapuches than Europeans ($p = 0.021$). The 'ethnicity x sedentary time tertile' ($p = 0.0003$ in men and $p = 0.0001$ in women) and 'ethnicity x MVPA tertile' ($p = 0.0001$ in men and $p = 0.012$ in women) interactions with $HOMA_{IR}$ remained significant for both men and women after further adjustment for: accelerometer wear time, $VO_{2max}$, BMI, waist circumference, percentage body fat, energy intake, smoking status, environment, socio-economic level, education level and MVPA (for the sedentary time model) or sedentary time (for the MVPA model). After further adjustment for BMI, waist circumference, percentage body fat, energy intake, smoking status, environment, socio-economic level, education level, MVPA, sedentary time and accelerometer wear time significant 'ethnicity x fitness tertile' interactions were observed in both men ($p = 0.023$) and women ($p = 0.042$).
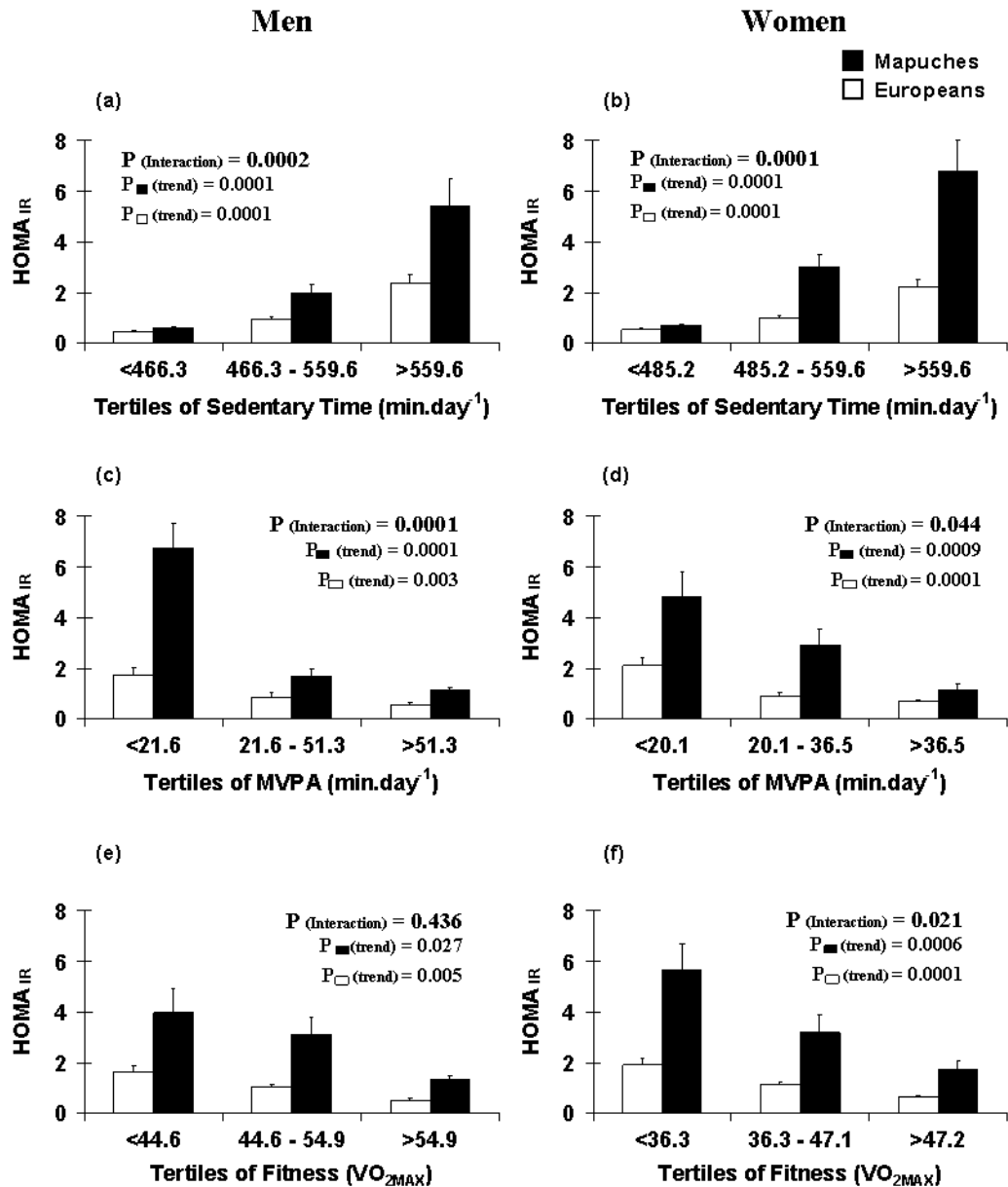
**Figure 5.2. Effects of sedentary time, moderate-to-vigorous physical activity and fitness on HOMA$_{IR}$ in European and Mapuche participants.**
Bars show mean ± SEM for all groups. Values defining each tertile are given for each of the three distributions. Statistical tests were carried out in males and females separately.

Table 5.1 and Table 5.2 shows the effect of increasing diet intake (total energy, proteins, fat and CHO) on $HOMA_{IR}$ in European and Mapuche men and women, in age-adjusted analyses. There was no evidence of statistically significant trend or ethnicity*dietary factors interaction effect on $HOMA_{IR}$. Further adjustment for age, environment, smoking status, education and socio-economic status did not alter the previous finding. In addition, we examined the influences of sugar, starch and alcohol intake on $HOMA_{IR}$ in European and Mapuche men and women (Table 5.3). Similarly to previous diet factors no trend or ethnicity*diet interaction was found.

**Table 5.1. Summary of the effects of dietary intake on HOMA $_{IR}$ in European and Mapuches men participants**

| Model Factors | | Tertiles of dietary factors | | | p-values | | |
|---|---|---|---|---|---|---|---|
| **Men** | | **T1 (<2,480)** | **T2 (2,480-3,112)** | **T3 (>3,112)** | **p-Ethnicity** | **p-Main effect** | **p-Interaction** |
| Ethnicity | | | | | | | |
| Energy Intake tertiles (Kcal.day$^{-1}$) | Europeans | 1.13 ± 0.11 | 0.92 ± 0.15 | 1.69 ± 0.40 | 0.020 * | 0.575 * | 0.204 * |
| Covariates † | Mapuches | 1.40 ± 0.35 | 3.19 ± 0.80 | 2.77 ± 0.61 | 0.009 † | 0.063 † | 0.532 † |
| | | T1 (<82.3) | T2 (82.3 – 107.1) | T3 (>107.1) | | | |
| Ethnicity | | | | | | | |
| Protein tertiles (g.day$^{-1}$) | Europeans | 1.21 ± 0.13 | 1.46 ± 0.29 | 1.10 ± 0.40 | 0.004 * | 0.101 * | 0.177 * |
| Covariates † | Mapuches | 2.08 ± 0.69 | 4.21 ± 0.83 | 1.39 ± 0.26 | 0.001 † | 0.082 † | 0.641 † |
| | | T1 (<60.3) | T2 (60.3 – 89.6) | T3 (>89.6) | | | |
| Ethnicity | | | | | | | |
| Fat tertiles (g.day$^{-1}$) | Europeans | 1.16 ± 0.11 | 1.30 ± 0.35 | 1.38 ± 1.45 | 0.006 * | 0.855 * | 0.834 * |
| Covariates † | Mapuches | 2.64 ± 0.83 | 3.26 ± 0.79 | 2.09 ± 0.45 | 0.002 † | 0.849 † | 0.459 † |
| | | T1 (<331.9) | T2 (331.9 – 472.0) | T3 (>472.0) | | | |
| Ethnicity | | | | | | | |
| CHO tertiles (g.day$^{-1}$) | Europeans | 1.02 ± 0.12 | 1.01 ± 0.16 | 1.82 ± 0.40 | 0.032 * | 0.110 * | 0.254 * |
| Covariates † | Mapuches | 1.37 ± 0.36 | 3.03 ± 0.76 | 2.91 ± 0.63 | 0.004 † | 0.086 † | 0.145 † |

Data are presented as mean ± SEM for untransformed and age -adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (total energy intake, proteins, fat and CHO ) and ethnicity x diet interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. Tertiles unit are presented in kca.day$^{-1}$ for energy intake and in g.day$^{-1}$ for proteins, fat and CHO intake. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

**Table 5.2. Summary of the effects of dietary intake on HOMA$_{IR}$ in European and Mapuches women participants**

| Model Factors | | Tertiles of dietary factors | | | *p*-values | | |
|---|---|---|---|---|---|---|---|
| **Women** | | **T1 (<2,089)** | **T2 (2,089-2,752)** | **T3 (>2,752)** | ***p*-Ethnicity** | ***p*-Main effect** | ***p*-Interaction** |
| Ethnicity | | | | | | | |
| Energy Intake tertiles (Kcal.day$^{-1}$) | Europeans | 1.35 ± 0.15 | 1.22 ± 0.29 | 1.13 ± 0.66 | 0.0001 * | 0.284 * | 0.603 * |
| Covariates † | Mapuches | 4.38 ± 1.21 | 3.11 ± 0.85 | 1.96 ± 0.31 | 0.0001 † | 0.621 † | 0.954 † |
| | | T1 (<69.8) | T2 (69.8 – 97.7) | T3 (>97.7) | | | |
| Ethnicity | | | | | | | |
| Protein tertiles (g.day$^{-1}$) | Europeans | 1.18 ± 0.15 | 1.36 ± 0.23 | 1.42 ± 0.29 | 0.0001 * | 0.664 * | 0.533 * |
| Covariates † | Mapuches | 3.47 ± 0.88 | 2.69 ± 0.68 | 2.36 ± 0.52 | 0.0001 † | 0.651 † | 0.336 † |
| | | T1 (<55.7) | T2 (55.7 – 86.9) | T3 (>86.9) | | | |
| Ethnicity | | | | | | | |
| Fat tertiles (g.day$^{-1}$) | Europeans | 1.12 ± 0.16 | 1.67 ± 0.25 | 1.08 ± 0.20 | 0.0001 * | 0.064 * | 0.103 * |
| Covariates † | Mapuches | 5.42 ± 1.21 | 2.44 ± 0.61 | 1.83 ± 0.34 | 0.0001 † | 0.375 † | 0.065 † |
| | | T1 (<290.9) | T2 (290.9 – 385.6) | T3 (>385.6) | | | |
| Ethnicity | | | | | | | |
| CHO tertiles (g.day$^{-1}$) | Europeans | 1.38 ± 0.16 | 1.33 ± 0.29 | 0.90 ± 0.14 | 0.0001 * | 0.623 * | 0.582 * |
| Covariates † | Mapuches | 2.77 ± 1.04 | 2.89 ± 0.62 | 2.57 ± 0.54 | 0.0001 † | 0.099 † | 0.126 † |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (total energy intake, proteins, fat an d CHO) and ethnicity x diet interaction effect in an age-adjusted **(\*)** and fully adjusted model **(†)**. Tertiles unit are presented in kca.day$^{-1}$ for energy intake and in g.day$^{-1}$ for proteins, fat and CHO intake. **(\*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

**Table 5.3. Summary of the effects of sugar, starch and alcohol intake on HOMA $_{IR}$ in European and Mapuches men and women par ticipants**

| Model Factors | | Tertiles of dietary factors (g.day$^{-1}$) | | | *p*-values | | |
|---|---|---|---|---|---|---|---|
| **Men** | | **T1 (<97.0)** | **T2 (97.0 – 182.2)** | **T3 (>182.2)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity Sugar tertiles (g.day$^{-1}$) Covariates † | Europeans | 0.93 ± 0.14 | 1.32 ± 0.12 | 1.77 ± 0.60 | 0.024 * | 0.270 * | 0.268 * |
| | Mapuches | 2.75 ± 1.11 | 2.24 ± 0.70 | 2.92 ± 0.56 | 0.00 † | 0.175 † | 0.108 † |
| | | T1 (<188.4) | T2 (188.4 – 297.7) | T3 (>297.7) | | | |
| Ethnicity Starch tertiles (g.day$^{-1}$) Covariates † | Europeans | 1.62 ± 0.35 | 1.14 ± 0.20 | 1.09 ± 0.70 | 0.018 * | 0.472 * | 0.542 * |
| | Mapuches | 2.44 ± 0.64 | 2.68 ± 0.94 | 2.87 ± 0.63 | 0.003 † | 0.705 † | 0.780 † |
| | | T1 (<3.01) | T2 (3.01 – 9.02) | T3 (>9.02) | | | |
| Ethnicity Alcohol tertiles (g.day$^{-1}$) Covariates † | Europeans | 1.32 ± 0.19 | 1.25 ± 0.34 | 1.06 ± 0.21 | 0.011 * | 0.849 * | 0.572 * |
| | Mapuches | 2.57 ± 0.80 | 2.19 ± 0.58 | 2.97 ± 0.66 | 0.003 † | 0.209 † | 0.371 † |
| **Women** | | **T1 (<72.0)** | **T2 (72.0 – 141.1)** | **T3 (>141.1)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity Sugar tertiles (g.day$^{-1}$) Covariates † | Europeans | 1.45 ± 0.19 | 1.25 ± 0.20 | 0.85 ± 0.18 | 0.0001 * | 0.051 * | 0.459 * |
| | Mapuches | 4.07 ± 0.93 | 2.55 ± 0.76 | 2.11 ± 0.41 | 0.0001 † | 0.396 † | 0.643 † |
| | | T1 (<175.8) | T2 (175.8 – 276.1) | T3 (>276.1) | | | |
| Ethnicity Starch tertiles (g.day$^{-1}$) Covariates † | Europeans | 1.36 ± 0.19 | 1.37 ± 0.24 | 1.09 ± 0.18 | 0.0001 * | 0.474 * | 0.572 * |
| | Mapuches | 2.56 ± 0.65 | 3.18 ± 0.87 | 2.59 ± 0.54 | 0.0001 † | 0.298 † | 0.675 † |
| | | T1 (<3.65) | T2 (3.65 – 6.83) | T3 (>6.83) | | | |
| Ethnicity Alcohol tertiles (g.day$^{-1}$) Covariates † | Europeans | 1.52 ± 0.23 | 1.21 ± 0.14 | 0.78 ± 0.14 | 0.0004 * | 0.112 * | 0.260 * |
| | Mapuches | 2.75 ± 1.24 | 3.60 ± 0.64 | 1.76 ± 0.28 | 0.0001 † | 0.423 † | 0.736 † |

Data are presented as mean ± SEM for untransformed and age -adjusted data. P values shown are age -adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (sugar, starch and alcohol) and ethnicity x diet interaction effect in an age -adjusted **(*)** and fully adjusted model **(†)**. Tertiles unit are presented in g.day$^{-1}$ for sugar, starch and alcohol intake. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

The effect of increases in energy, protein, fat and carbohydrates (CHO) intake on BMI (Table 5.4), waist circumference (Table 5.5) and body fat (Table 5.6) in European and Mapuche men and women, in age-adjusted analysis, were examined. A visible trend in both men and women for BMI to increase with increasing total energy intake, fat and CHO intake, but not with increasing protein intake was observed (Table 5.4). When the influence of dietary intake on waist circumference was examined, a statistical significant trend to increase waist circumference with increasing total energy and CHO intake was found for men, while just CHO intake showed a significant trend for women. Protein or fat intake did not show any trend with waist circumference for both men and women (Table 5.5). No statistically significant trends were found for any of the dietary factors on percentage of body fat (Table 5.6). Additionally, an ethnicity*dietary factors interaction was examined, but no significant interaction between ethnicity and macronutrients were found. Further adjustment for age, environment, educational level, socio-economic status and smoking status did not modify the previous results.

**Table 5.4. Summary of the effects of dietary intake patterns on BMI in European and Mapuches men  and women participants**

| Model Factors | | Tertiles of dietary factors | | | p-values | | |
|---|---|---|---|---|---|---|---|
| **Men / outcome: BMI (kg.m$^{-2}$)** | | **T1 (<2,480)** | **T2 (2,480 – 3,112)** | **T3 (>3,112)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity | Europeans | 25.8 ± 0.69 | 28.5 ± 1.03 | 30.1 ± 1.26 | 0.413 * | 0.0001 * | 0.507 * |
| Energy Intake tertiles (Kcal.day$^{-1}$) Covariates † | Mapuches | 23.3 ± 1.11 | 28.0 ± 0.60 | 30.0 ± 0.48 | 0.446 † | 0.0001 † | 0.585 † |
| | | T1 (<82.3) | T2 (82.3 – 107.1) | T3 (>107.1) | | | |
| Ethnicity | Europeans | 26.4 ± 0.78 | 26.9 ± 1.25 | 29.7 ± 0.92 | 0.117 * | 0.183 * | 0.218 * |
| Protein tertiles (g.day$^{-1}$) Covariates † | Mapuches | 27.8 ± 0.95 | 28.5 ± 0.59 | 28.8 ± 0.53 | 0.402 † | 0.326 † | 0.090 † |
| | | T1 (<60.3) | T2 (60.3 – 89.6) | T3 (>89.6) | | | |
| Ethnicity | Europeans | 27.4 ± 0.72 | 27.0 ± 1.03 | 29.8 ± 1.62 | 0.233 * | 0.006 * | 0.415 * |
| Fat tertiles (g.day$^{-1}$) Covariates † | Mapuches | 29.2 ± 1.03 | 26.7 ± 0.60 | 29.8 ± 0.61 | 0.626 † | 0.088 † | 0.299 † |
| | | T1 (<331.9) | T2 (331.9 – 472.0) | T3 (>472.0) | | | |
| Ethnicity | Europeans | 26.8 ± 0.72 | 28.5 ± 1.15 | 28.9 ± 1.31 | 0.518 * | 0.0001 * | 0.132 * |
| CHO tertiles (g.day$^{-1}$) Covariates † | Mapuches | 22.7 ± 0.84 | 29.4 ± 0.87 | 29.2 ± 0.38 | 0.469 † | 0.0001 † | 0.120 † |
| **Women / outcome: BMI (kg.m$^{-2}$)** | | **T1 (<2,089)** | **T2 (2,089 – 2,752)** | **T3 (>2,752)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity | Europeans | 28.4 ± 0.50 | 28.3 ± 0.91 | 32.1 ± 2.40 | 0.289 * | 0.008 * | 0.791 * |
| Energy Intake tertiles (Kcal.day$^{-1}$) Covariates † | Mapuches | 29.5 ± 1.23 | 29.9 ± 0.93 | 32.0 ± 0.68 | 0.544 † | 0.0002 † | 0.562 † |
| | | T1 (<69.8) | T2 (69.8 – 97.7) | T3 (>97.7) | | | |
| Ethnicity | Europeans | 28.3± 0.60 | 27.64 ± 0.71 | 30.9 ± 1.04 | 0.004 * | 0.010 * | 0.554 * |
| Protein tertiles (g.day$^{-1}$) Covariates † | Mapuches | 30.5 ± 1.08 | 30.2 ± 0.77 | 31.7 ± 0.82 | 0.019 † | 0.002 † | 0.540 † |
| | | T1 (<55.7) | T2 (55.7 – 86.9) | T3 (>86.9) | | | |
| Ethnicity | Europeans | 28.7 ± 0.61 | 28.7 ± 0.68 | 28.8 ± 1.52 | 0.006 * | 0.381 * | 0.612 * |
| Fat tertiles (g.day$^{-1}$) Covariates † | Mapuches | 31.0 ± 1.13 | 29.5 ± 0.79 | 31.6 ± 0.77 | 0.022 † | 0.193 † | 0.921 † |
| | | T1 (<290.9) | T2 (290.9 – 385.6) | T3 (>385.6) | | | |
| Ethnicity | Europeans | 28.6 ± 0.58 | 28.4 ± 0.83 | 29.6 ± 1.38 | 0.079 * | 0.014 * | 0.133 * |
| CHO tertiles (g.day$^{-1}$) Covariates † | Mapuches | 27.5 ± 1.50 | 30.4 ± 0.72 | 32.6 ± 0.72 | 0.171 † | 0.003 † | 0.213 † |

Data are presented as mean ± SEM for untransformed and a ge-adjusted data. P values shown are age -adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (total energy intake, proteins, fat and CHO) and ethnicity x diet interaction effect in an age -adjusted **(\*)** and fully adjusted model **(†)**. Tertiles unit are presented in kca.day$^{-1}$ for energy intake and in g.day$^{-1}$ for proteins, fat and CHO intake. **(\*)** Model adjusted for age.  **(†)** Models adjusted for age, environment, socio -economic level, education level, and smoking status.

**Table 5.5. Summary of the effects of dietary intake patterns on waist circumference in European and Mapuches men and women participants**

| Model Factors | | Tertiles of dietary factors | | | p-values | | |
|---|---|---|---|---|---|---|---|
| **Men / outcome: waist (cm)** | | **T1 (<2,480)** | **T2 (2,480 – 3,112)** | **T3 (>3,112)** | *p*-Ethnicity | *p*-Main effect | *p*-Interaction |
| Ethnicity | Europeans | 97.3 ± 2.51 | 104.0 ± 3.38 | 105.5 ± 3.58 | 0.674 * | 0.0001 * | 0.328 * |
| Energy Intake tertiles (Kcal.day$^{-1}$) | Mapuches | 90.8 ± 3.09 | 101.5 ± 2.00 | 107.8 ± 1.62 | 0.403 † | 0.0001 † | 0.798 † |
| Covariates † | | | | | | | |
| | | T1 (<82.3) | T2 (82.3 – 107.1) | T3 (>107.1) | | | |
| Ethnicity | Europeans | 98.2 ± 2.89 | 98.5 ± 3.83 | 107.0 ± 2.66 | 0.148 * | 0.261 * | 0.250 * |
| Protein tertiles (g.day$^{-1}$) | Mapuches | 101.9 ± 2.53 | 103.8 ± 2.02 | 104.4 ± 2.14 | 0.808 † | 0.349 † | 0.061 † |
| Covariates † | | | | | | | |
| | | T1 (<60.3) | T2 (60.3 – 89.6) | T3 (>89.6) | | | |
| Ethnicity | Europeans | 102.5 ± 2.47 | 97.5 ± 3.60 | 104.2 ± 4.08 | 0.294 * | 0.136 * | 0.769 * |
| Fat tertiles (g.day$^{-1}$) | Mapuches | 101.7 ± 3.36 | 101.1 ± 2.09 | 106.1 ± 1.81 | 0.982 † | 0.639 † | 0.563 † |
| Covariates † | | | | | | | |
| | | T1 (<331.9) | T2 (331.9 – 472.0) | T3 (>472.0) | | | |
| Ethnicity | Europeans | 97.8 ± 2.35 | 106.5 ± 3.34 | 102.3 ± 4.04 | 0.767 * | 0.0001 * | 0.165 * |
| CHO tertiles (g.day$^{-1}$) | Mapuches | 89.5 ± 3.04 | 105.4 ± 2.31 | 105.7 ± 1.53 | 0.418 † | 0.001 † | 0.288 † |
| Covariates † | | | | | | | |
| **Women / outcome: waist (cm)** | | **T1 (<2,089)** | **T2 (2,089 – 2,752)** | **T3 (>2,752)** | *p*-Ethnicity | *p*-Main effect | *p*-Interaction |
| Ethnicity | Europeans | 100.4 ± 1.53 | 100.7 ± 2.93 | 104.9 ± 4.62 | 0.189 * | 0.188 * | 0.757 * |
| Energy Intake tertiles (Kcal.day$^{-1}$) | Mapuches | 101.4 ± 2.98 | 105.1 ± 2.41 | 107.8 ± 1.57 | 0.442 † | 0.064 † | 0.715 † |
| Covariates † | | | | | | | |
| | | T1 (<69.8) | T2 (69.8 – 97.7) | T3 (>97.7) | | | |
| Ethnicity | Europeans | 99.6 ± 2.11 | 102.3 ± 2.32 | 100.9 ± 2.39 | 0.005 * | 0.870 * | 0.820 * |
| Protein tertiles (g.day$^{-1}$) | Mapuches | 106.4 ± 2.63 | 105.4 ± 1.91 | 106.0 ± 2.03 | 0.051 † | 0.827 † | 0.683 † |
| Covariates † | | | | | | | |
| | | T1 (<55.7) | T2 (55.7 – 86.9) | T3 (>86.9) | | | |
| Ethnicity | Europeans | 101.4 ± 1.91 | 102.3 ± 2.16 | 96.2 ± 3.09 | 0.006 * | 0.913 * | 0.084 * |
| Fat tertiles (g.day$^{-1}$) | Mapuches | 105.4 ± 3.05 | 102.9 ± 2.08 | 107.8 ± 1.74 | 0.028 † | 0.908 † | 0.203 † |
| Covariates † | | | | | | | |
| | | T1 (<290.9) | T2 (290.9 – 385.6) | T3 (>385.6) | | | |
| Ethnicity | Europeans | 99.8 ± 1.66 | 102.8 ± 2.82 | 101.4 ± 3.04 | 0.150 * | 0.047 * | 0.412 * |
| CHO tertiles (g.day$^{-1}$) | Mapuches | 98.3 ± 3.61 | 107.1 ± 1.97 | 107.1 ± 1.56 | 0.360 † | 0.023 † | 0.522 † |
| Covariates † | | | | | | | |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (total energy intake, proteins, fat and CHO) and ethnicity x diet interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. Tertiles unit are presented in kca.day$^{-1}$ for energy intake and in g.day$^{-1}$ for proteins, fat and CHO intake. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

**Table 5.6. Summary of the effects of dietary intake patterns on percentage of body fat in European and Mapuche s men and women participants**

| Model Factors | | Tertiles of dietary factors | | | *p*-values | | |
|---|---|---|---|---|---|---|---|
| **Men / outcome: body fat (%)** | | **T1 (<2,480)** | **T2 (2,480 – 3,112)** | **T3 (>3,112)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity Energy Intake tertiles (Kcal.day⁻¹) Covariates † | Europeans | 28.3± 1.22 | 26.4 ± 1.26 | 29.5 ± 1.99 | 0.773 * | 0.458 * | 0.201 * |
| | Mapuches | 26.6 ± 1.62 | 29.0 ± 0.80 | 28.6 ± 0.65 | 0.770 † | 0.130 † | 0.677 † |
| | | T1 (<82.3) | T2 (82.3 – 107.1) | T3 (>107.1) | | | |
| Ethnicity Protein tertiles (g.day⁻¹) Covariates † | Europeans | 29.6 ± 1.09 | 25.4 ± 1.79 | 28.5 ± 1.38 | 0.384 * | 0.723 * | 0.103 * |
| | Mapuches | 27.2 ± 0.84 | 30.0 ± 0.82 | 27.8 ± 0.82 | 0.260 † | 0.448 † | 0.125 † |
| | | T1 (<60.3) | T2 (60.3 – 89.6) | T3 (>89.6) | | | |
| Ethnicity Fat tertiles (g.day⁻¹) Covariates † | Europeans | 28.5 ± 1.12 | 26.5 ± 1.70 | 28.5 ± 1.91 | 0.522 * | 0.973 * | 0.271 * |
| | Mapuches | 27.6 ± 1.82 | 29.2 ± 0.67 | 27.9 ± 0.74 | 0.370 † | 0.816 † | 0.574 † |
| | | T1 (<331.9) | T2 (331.9 – 472.0) | T3 (>472.0) | | | |
| Ethnicity CHO tertiles (g.day⁻¹) Covariates † | Europeans | 27.3 ± 1.19 | 28.3 ± 1.33 | 28.8 ± 2.07 | 0.897 * | 0.327 * | 0.989 * |
| | Mapuches | 26.6 ± 1.62 | 28.5 ± 0.79 | 28.9 ± 0.65 | 0.746 † | 0.096 † | 0.583 † |
| | | | | | | | |
| **Women / outcome: body fat (%)** | | **T1 (<2,089)** | **T2 (2,089 – 2,752)** | **T3 (>2,752)** | *p*-**Ethnicity** | *p*-**Main effect** | *p*-**Interaction** |
| Ethnicity Energy Intake tertiles (Kcal.day⁻¹) Covariates † | Europeans | 31.5 ± 0.61 | 32.1 ± 0.81 | 32.0 ± 1.69 | 0.888 * | 0.816 * | 0.969 * |
| | Mapuches | 31.9 ± 1.46 | 32.2 ± 0.67 | 31.4 ± 0.87 | 0.462 † | 0.174 † | 0.804 † |
| | | T1 (<69.8) | T2 (69.8 – 97.7) | T3 (>97.7) | | | |
| Ethnicity Protein tertiles (g.day⁻¹) Covariates † | Europeans | 31.1 ± 0.82 | 31.4 ± 0.74 | 32.9 ± 0.92 | 0.961 * | 0.172 * | 0.894 * |
| | Mapuches | 31.7 ± 0.95 | 30.8 ± 0.95 | 32.5 ± 0.89 | 0.150 † | 0.124 † | 0.967 † |
| | | T1 (<55.7) | T2 (55.7 – 86.9) | T3 (>86.9) | | | |
| Ethnicity Fat tertiles (g.day⁻¹) Covariates † | Europeans | 31.4 ± 0.67 | 32.0 ± 0.89 | 31.8 ± 1.05 | 0.536 * | 0.320 * | 0.167 * |
| | Mapuches | 34.2 ± 1.29 | 30.7 ± 0.90 | 31.5 ± 0.77 | 0.141 † | 0.338 † | 0.652 † |
| | | T1 (<290.9) | T2 (290.9 – 385.6) | T3 (>385.6) | | | |
| Ethnicity CHO tertiles (g.day⁻¹) Covariates † | Europeans | 31.9 ± 0.62 | 31.6 ± 0.99 | 30.9 ± 1.19 | 0.961 * | 0.396 * | 0.469 * |
| | Mapuches | 30.1 ± 0.91 | 32.5 ± 0.74 | 31.4 ± 1.01 | 0.324 † | 0.087 † | 0.237 † |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for dietary intake factors (total energy intake, proteins, fat and CHO) and ethnicity x diet interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. Tertiles unit are presented in kca.day⁻¹ for energy intake and in g.day⁻¹ for proteins, fat and CHO intake. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

Table 5.7 shows the effect of increasing amount of time spent in sedentary behaviours on obesity-related phenotypes (BMI, waist circumference and percentage of body fat) in European and Mapuche men and women, in age adjusted analyses. A significant trend for body fat (%) to increase with increasing tertiles of time spent in sedentary behaviours was found. A similar effect of sedentary time was observed on BMI and waist circumference for women. When the influence of tertiles of MVPA on obesity traits was examined (Table 5.8), a significant trend to decrease BMI for women and percentage of body fat for both sexes, was observed when participants increased their time spent in MVPA. The effect of fitness on obesity traits was just observed for percentage of body fat but not BMI and waist circumference in both sexes (Table 5.9). Further adjustment for age, environment, smoking status, education and socio-economic level did not alter these finding except for the association between fitness and percentage of body fat in men that become not significant. No evidence of an interaction effect between ethnicity and PA/fitness factors were observed.

**Table 5.7. Summary of the effects of time spent in sedentary behaviours on obesity-related phenotypes in European and Mapuches men and women participants**

| Model Factors | | Tertiles of sedentary time (min.day$^{-1}$) | | | p-values | | |
|---|---|---|---|---|---|---|---|
| **Men** | | **T1 (<466.3)** | **T2 (466.3 – 559.6)** | **T3 (>559.6)** | **p-Ethnicity** | **p-Main effect** | **p-Interaction** |
| **Outcome:** BMI (kg.m$^{-2}$) | | | | | | | |
| Ethnicity | Europeans | 29.0 ± 1.07 | 27.8 ± 0.97 | 29.6 ± 1.54 | 0.911 * | 0.113 * | 0.087 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 26.3 ± 0.73 | 28.0 ± 0.63 | 30.4 ± 1.03 | 0.915 † | 0.171 † | 0.094 † |
| Covariates † | | | | | | | |
| **Outcome:** waist (cm) | | | | | | | |
| Ethnicity | Europeans | 105.2 ± 3.06 | 100.5 ± 3.22 | 108.3 ± 4.55 | 0.343 * | 0.170 * | 0.363 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 98.1 ± 2.72 | 100.4 ± 1.85 | 105.6 ± 2.91 | 0.253 † | 0.058 † | 0.222 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat (%) | | | | | | | |
| Ethnicity | Europeans | 27.3 ± 1.63 | 28.4± 1.22 | 32.6 ± 1.43 | 0.943 * | 0.0002 * | 0.799 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 26.1 ± 1.12 | 28.1 ± 0.74 | 32.5 ± 1.07 | 0.889 † | 0.020 † | 0.445 † |
| Covariates † | | | | | | | |
| **Women** | | **T1 (<485.2)** | **T2 (485.2 – 559.6)** | **T3 (>559.6)** | **p-Ethnicity** | **p-Main effect** | **p-Interaction** |
| **Outcome:** BMI (kg.m$^{-2}$) | | | | | | | |
| Ethnicity | Europeans | 26.5 ± 0.64 | 28.2 ± 0.77 | 32.7 ± 1.13 | 0.008 * | 0.0001 * | 0.084 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 28.9 ± 0.78 | 31.7 ± 0.90 | 33.0 ± 1.36 | 0.114 † | 0.0003 † | 0.068 † |
| Covariates † | | | | | | | |
| **Outcome:** waist (cm) | | | | | | | |
| Ethnicity | Europeans | 94.9 ± 2.24 | 98.5 ± 2.14 | 108.8 ± 2.61 | 0.015 * | 0.004 * | 0.179 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 102.9 ± 2.00 | 106.5 ± 2.16 | 107.8 ± 3.16 | 0.192 † | 0.007 † | 0.377 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat (%) | | | | | | | |
| Ethnicity | Europeans | 30.5 ± 1.02 | 30.3 ± 0.78 | 34.5 ± 0.70 | 0.007 * | 0.510 * | 0.240 * |
| Sedentary tertiles (min.day$^{-1}$) | Mapuches | 28.7 ± 0.93 | 33.7 ± 0.71 | 34.9 ± 1.72 | 0.085 † | 0.733 † | 0.157 † |
| Covariates † | | | | | | | |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for sedentary behaviour and ethnicity x sedentary behaviour interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

**Table 5.8. Summary of the effects of time spent in moderate to vigorous physical activity on obesity-related phenotypes in European and Mapuches men and women participants**

| Model Factors | | Tertiles of MVPA (min.day$^{-1}$) | | | p-values | | |
|---|---|---|---|---|---|---|---|
| **Men** | | **T1 (<21.6)** | **T2 (21.6 – 51.3)** | **T3 (>51.3)** | ***p*-Ethnicity** | ***p*-Main effect** | ***p*-Interaction** |
| **Outcome:** BMI (kg.m$^{-2}$) | | | | | | | |
| Ethnicity | Europeans | 28.2 ± 1.01 | 30.5 ± 1.52 | 27.8 ± 0.91 | 0.587 * | 0.340 * | 0.244 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 28.3 ± 1.03 | 27.9 ± 0.84 | 27.8 ± 0.54 | 0.465 † | 0.302 † | 0.107 † |
| Covariates † | | | | | | | |
| **Outcome:** waist (cm) | | | | | | | |
| Ethnicity | Europeans | 102.1 ± 3.12 | 111.5 ± 3.96 | 100.7 ± 3.18 | 0.183 * | 0.057 * | 0.214 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 101.5 ± 2.85 | 101.8 ± 2.49 | 98.7 ± 1.81 | 0.095 † | 0.083 † | 0.081 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat (%) | | | | | | | |
| Ethnicity | Europeans | 30.6 ± 1.08 | 31.5 ± 1.69 | 24.4 ± 1.57 | 0.744 * | 0.0001 * | 0.108 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 32.1 ± 0.86 | 27.3 ± 0.96 | 26.9 ± 0.91 | 0.974 † | 0.015 † | 0.097 † |
| Covariates † | | | | | | | |
| | | | | | | | |
| **Women** | | **T1 (<20.1)** | **T2 (20.1 – 36.5)** | **T3 (>36.5)** | ***p*-Ethnicity** | ***p*-Main effect** | ***p*-Interaction** |
| **Outcome:** BMI (kg.m$^{-2}$) | | | | | | | |
| Ethnicity | Europeans | 29.4 ± 0.68 | 29.7 ± 1.17 | 27.1 ± 0.79 | 0.0007 * | 0.004 * | 0.079 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 32.4 ± 0.96 | 30.3 ± 0.92 | 29.8 ± 0.95 | 0.025 † | 0.014 † | 0.068 † |
| Covariates † | | | | | | | |
| **Outcome:** waist (cm) | | | | | | | |
| Ethnicity | Europeans | 100.9 ± 2.14 | 102.0 ± 2.72 | 96.8 ± 2.53 | 0.007 * | 0.510 * | 0.240 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 107.8 ± 2.36 | 102.7 ± 2.85 | 105.7 ± 1.90 | 0.085 † | 0.733 † | 0.157 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat (%) | | | | | | | |
| Ethnicity | Europeans | 32.6 ± 0.75 | 31.4 ± 0.94 | 29.8 ± 1.01 | 0.133 * | 0.0005 * | 0.426 * |
| MVPA tertiles (min.day$^{-1}$) | Mapuches | 35.1 ± 1.02 | 32.4 ± 1.02 | 29.6 ± 0.93 | 0.103 † | 0.002 † | 0.363 † |
| Covariates † | | | | | | | |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for MVPA and ethnicity x MVPA interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

**Table 5.9. Summary of the effects of time spent in fitness on obesity-related phenotypes in European and Mapuches men and women participants**

| Model Factors | | Tertiles of fitness (ml.kg.min$^{-1}$) | | | *p*-values | | |
|---|---|---|---|---|---|---|---|
| **Men** | | **T1 (<44.6)** | **T2 (44.6 – 54.9)** | **T3 (>54.9)** | ***p*-Ethnicity** | ***p*-Main effect** | ***p*-Interaction** |
| **Outcome:** BMI | | | | | | | |
| Ethnicity | Europeans | 28.7 ± 0.82 | 27.2 ± 0.82 | 29.4 ± 1.41 | 0.918 * | 0.102 * | 0.869 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 28.3 ± 0.70 | 26.7 ± 0.61 | 29.6 ± 0.64 | 0.721 † | 0.206 † | 0.633 † |
| Covariates † | | | | | | | |
| **Outcome:** waist circumference | | | | | | | |
| Ethnicity | Europeans | 104.0 ± 2.70 | 101.74 ± 3.96 | 106.3 ± 3.69 | 0.999 * | 0.091 * | 0.716 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 101.5 ± 2.01 | 100.95 ± 2.04 | 107.1 ± 1.98 | 0.363 † | 0.120 † | 0.506 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat | | | | | | | |
| Ethnicity | Europeans | 30.1 ± 0.97 | 27.9 ± 1.69 | 25.9 ± 1.86 | 0.238 * | 0.002 * | 0.983 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 30.9 ± 0.76 | 28.6 ± 0.85 | 27.1 ± 0.74 | 0.286 † | 0.111 † | 0.933 † |
| Covariates † | | | | | | | |
| | | | | | | | |
| **Women** | | **T1 (<36.3)** | **T2 (36.3 – 47.1)** | **T3 (>47.2)** | ***p*-Ethnicity** | ***p*-Main effect** | ***p*-Interaction** |
| **Outcome:** BMI | | | | | | | |
| Ethnicity | Europeans | 30.6 ± 0.72 | 27.0 ± 0.59 | 28.0 ± 0.96 | 0.0002 * | 0.184 * | 0.100 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 31.1 ± 1.11 | 30.8 ± 0.87 | 31.2 ± 0.70 | 0.012 † | 0.226 † | 0.322 † |
| Covariates † | | | | | | | |
| **Outcome:** waist circumference | | | | | | | |
| Ethnicity | Europeans | 107.9 ± 1.97 | 99.3 ± 1.93 | 97.5 ± 2.3 | 0.026 * | 0.373 * | 0.105 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 104.0 ± 2.30 | 104.8 ± 2.09 | 107.9 ± 1.78 | 0.176 † | 0.178 † | 0.097 † |
| Covariates † | | | | | | | |
| **Outcome:** body fat | | | | | | | |
| Ethnicity | Europeans | 32.7 ± 0.58 | 31.8 ± 0.83 | 28.9 ± 0.86 | 0.071 * | 0.001 * | 0.609 * |
| Fitness tertiles (ml.kg.min$^{-1}$) | Mapuches | 34.4 ± 1.10 | 32.1 ± 1.06 | 30.6 ± 0.61 | 0.061 † | 0.027 † | 0.435 † |
| Covariates † | | | | | | | |

Data are presented as mean ± SEM for untransformed and age-adjusted data. P values shown are age-adjusted *p*-trend for Europeans and Mapuches, main effect for ethnicity, main effect for fitness and ethnicity x fitness interaction effect in an age-adjusted **(*)** and fully adjusted model **(†)**. **(*)** Model adjusted for age. **(†)** Models adjusted for age, environment, socio-economic level, education level, and smoking status.

## 5.4 Discussion

The main findings of this study were: a) Mapuche are particularly susceptible to the adverse metabolic effects of obesity compared to Europeans, moving from the lowest to the highest percentage adiposity tertile was associated with an approximate doubling in $HOMA_{IR}$ in European Chileans, but a greater than 3.5-fold increase in $HOMA_{IR}$ in Mapuches; b) Sedentary behaviours have a greater effect on insulin resistance in Mapuches than European Chileans – there was 4-5 fold increase in insulin resistance in Chilean men and women of European descent, but a 9-10 fold increase in Mapuche men and women, when they move from the lowest to the highest tertile for sedentary time; c) lower time spent in MVPA and lower cardiorespiratory fitness were associated with a greater detrimental effect on insulin resistance in Mapuches compared to Europeans.

The present data indicate that increasing adiposity is associated with larger increases in insulin resistance in Mapuches than European Chileans. Moving from the lowest to the highest percentage body fat tertile was associated with an approximate doubling in $HOMA_{IR}$ in European Chileans, but a greater than 3.5-fold increase in $HOMA_{IR}$ in Mapuches. Thus, the data suggest that Mapuche are particularly susceptible to the adverse metabolic effects of obesity. This susceptibility may contribute to the disproportionately large difference in insulin resistance (and reported diabetes prevalence) between Mapuches living in rural and urban environments, despite the relatively modest difference in adiposity between these two groups. Similar observations have been made when considering other groups at increased risk of T2D. For example, adults (Razak et al. 2007b) and children (Whincup et al. 2002b) of South Asian origin exhibit larger increases in insulin resistance with increasing adiposity than White Europeans. Our data do not allow conclusions to be drawn about why adiposity had a more potent effect on insulin resistance in the Mapuche group. Speculatively, this could be due to differences in ectopic and regional fat distribution, in adipocyte size, or in adipose tissue signalling, between the groups, but further study is needed to test these hypotheses.

It is well established that high levels of physical activity (Gill and Cooper 2008) and cardiorespiratory fitness (Wei et al. 1999a) are protective against T2D and are

associated with a favourable metabolic risk profile (Gill and Malkova 2006c). Recently, it has also become evident that increasing sedentary time is associated with increased diabetes risk (Dunstan et al. 2004b; Hu et al. 2003d) and an adverse metabolic risk profile (Gill et al. 2011b; Healy et al. 2008d) and that these effects may be independent of time spent in physical activity (Hu et al. 2003c; Gill et al. 2011a). Reports have shown that low levels of self-reported physical activity or low cardiorespiratory fitness are associated with insulin resistance in other aboriginal groups (Kriska et al. 2001b). The findings here are consistent with these observations, and extend them by using objective measures of physical activity and sedentary time and by revealing that the effects on insulin resistance of a low level of moderate-to-vigorous physical activity, or a high level of sedentary time, or (in women) a low level of cardiorespiratory fitness, was greater in the Mapuche than the European Chileans. For example, moving from the lowest to the highest tertile for sedentary time was associated with a 4-5 fold increase in insulin resistance in Chilean men and women of European descent, but a 9-10 fold increase in Mapuche men and women. Thus, the adverse effects of sedentariness and low physical activity appear particularly large in this aboriginal group. Again, we cannot draw conclusions about why Mapuches are particularly susceptible to the adverse effects of low physical activity and high sedentary time on insulin resistance. However, it is clear that this effect is not simply mediated by differences in adiposity, fitness, dietary intake, smoking, or socioeconomic level or education between the Mapuche and European groups: statistically adjusting for these potential confounding factors did not influence the findings. Thus, further studies are needed to address the underlying mechanisms.

There was no significant influence of any dietary component (energy density, protein, fat, CHO, sugar, starch and alcohol) on insulin resistance in either the Mapuche or European populations. This is consistent with other observational (Schulze et al. 2004a; van Dam et al. 2002) and intervention trial (Jebb and Moore 1999) data, in populations of European descent, showing no effect of diet on insulin resistance or diabetes risk once the effects of diet on adiposity are accounted for. Thus, despite the difference in a number of dietary variables between rural and urban populations being greater in Mapuches than Europeans, the present data indicate this

larger dietary difference does not explain the larger effect of environment on insulin sensitivity in the Mapuches.

When the influences of dietary components on obesity related phenotypes (BMI, waist circumference and percentage of body fat) were examined, no ethnicity x dietary intake interactions were observed, indicating that dietary variables influenced adiposity similarly in both ethnic groups. Overall, BMI increased with increasing total energy intake, fat and carbohydrate intake. Waist circumference increased with increasing total energy and carbohydrate intake. However, percentage body fat was not significantly influenced by a dietary variable. Previous cross-sectional observational studies of free-living adult populations have shown a positive and independent association between dietary energy intake and obesity as measured by BMI and waist circumference in populations of different ethnic backgrounds (Mendoza, Drewnowski, and Christakis 2007b; Howarth et al. 2006b; Stookey 2001a; Murakami et al. 2007a). Results from prospective studies of the association between dietary energy intake and weight or BMI change have been contradictory; some report a significant and positive association (Vergnaud et al. 2009a; Savage, Marini, and Birch 2008a; Bes-Rastrollo et al. 2008a) but not all (Iqbal, Helge, and Heitmann 2006b; Du et al. 2009a). Fat and carbohydrate intake have also shown inconsistencies in the association with obesity as measured by BMI or waist circumference, with some studies reporting a positive relationship (Field et al. 2007; Du et al. 2009b; Hare-Bruun, Flint, and Heitmann 2006) but not all (Forouhi et al. 2009). These discrepancies in the association between diet intake and obesity could perhaps be explained in part by the underreporting of dietary intake, bias associated with the questionnaires and data collection methods or to heterogeneous dietary patterns across food cultures.

A change in physical activity patterns as well as increasing sedentary time are other factors that have been related to obesity (Stamatakis, Hirani, and Rennie 2009a; Dunton et al. 2009; Fitzgerald et al. 1997). In this chapter, increasing sedentary behaviours showed an effect to increase percentage of body fat for European and Mapuches men but not for women, whilst in both ethnic groups women's sedentary time was related to an increase in BMI and waist circumference but not body fat. However, in contrast to the data on insulin resistance, no ethnicity x physical

activity/sedentary time interactions were observed for adiposity variables. This agrees, in part, with previous cross-sectional and prospective studies that have reported a positive and significant association of inactivity with obesity in adult men and women populations (Bowman 2006; Cleland et al. 2008; Stamatakis, Hirani, and Rennie 2009b; Hu et al. 2003b; Wijndaele et al. 2010; Jakes et al. 2003). This chapter also revealed a trend to decrease obesity (BMI and body fat, but not waist circumference) by increasing time spent in MVPA. Additionally, higher fitness levels were related to lower percentage of body fat, but not BMI or waist circumference in this study. Previous studies have shown that higher fitness levels were associated with lower risk of obesity in adults (Brien et al. 2007; DiPietro et al. 1998). The inconsistency in the relationship between obesity traits and inactivity, physical activity and fitness, between men and women might be able to be explained by limitations of the accelerometer to capture different types of physical activities, like upper body physical activity, that it is a frequent physical activity for women in their home duties, this maybe modified the previous relationship between sedentary time and obesity in men and women.

A particular strength of the study is the detailed phenotyping of the study populations, particularly with respect to lifestyle measures. In this thesis fitness, physical activity and sedentary time were objectively measured, providing greater validity in these measures than would be obtained from self-report questionnaire (Shephard 2003c).

Some weakness that must be aware are, the measurement of insulin resistance, due to the data collection challenges and sample size, we used $HOMA_{IR}$, rather than a euglycaemic clamp or intra-venous glucose tolerance test, to assess insulin sensitivity. It will be important to seek verification that our conclusions remain valid when insulin sensitivity is assessed using more sophisticated techniques. Because the study is cross-sectional in nature, it is not possible to draw firm conclusions about the causality of associations observed. A randomised controlled trial is needed to address this definitively. However, our comprehensive study design, with relatively precise measures of a number of relevant exposure variables, allowed us to control for a number of potential confounding variables in our analyses.

In conclusion, the present study reveals that adiposity, physical activity and sedentary behaviour influence insulin resistance to a greater extent in Chilean Mapuches than in Chileans of European descent. These associations persist after adjustment for a comprehensive range of potential confounding factors. However, physical activity, sedentary time and dietary factors influenced obesity related variables in a similar manner in both ethnic groups. However the cross-sectional nature of these data does not allow for firm conclusions to be drawn about causality, but the findings highlight the fact that environmental and lifestyle effects on metabolic risk differ between ethnic groups. This has potential implications both for the designng and implementation of lifestyle strategies which aim to reduce metabolic risk in different ethnic groups, and for improving the basic understanding of the mechanisms underpinning human insulin resistance.

# 6 The *FTO* Gene, Ethnicity and Lifestyle Factors: Their Influences on Obesity and Insulin Resistance

## 6.1 Introduction

The previous chapters provided evidence that changes in lifestyle are fuelling the emerging obesity and diabetes problem (Hill et al. 2003a). However, not everyone who is exposed to an obesogenic environment develops obesity, insulin resistance (IR) or T2D (T2D), highlighting the multifactorial nature of these conditions. Although obesity, IR and T2D could be strongly influenced by lifestyle factors, these do not seem to explain all of the variance in their respective prevalence. This suggests that genetic factors could also play an important role, explaining the increased prevalence of diabetes and obesity.

In 2007, two high density genome-wide association studies (GWAS) confirmed *FTO* (fat mass and obesity-associated gene) as the first gene indisputably associated with obesity and related traits (Frayling 2007b; Scuteri et al. 2007b). To date, the *FTO* gene has the largest effect of all the genes found to be associated with BMI in individuals of European descent. Each risk allele increase BMI by 0.10 – 0.13 standard deviation (equivalent to about 0.40-0.66 $kg.m^{-2}$) and the risk increase by 1.18-fold and 1.32-fold for overweight and obesity, respectively. Homozygotes for the risk allele of *FTO* gene weighed about 3 kg more and had a 1.67-fold increased risk for obesity compared to those who did not inherit a risk allele (Scuteri et al. 2007a; Frayling et al. 2007c).

The frequency of the *FTO* risk genotypes is high in populations of European descent; 63% carry at least one risk allele and 16% are homozygous for this allele. Although the population attributable risk for overweight (~13%) and obesity (~20%) is high, the *FTO* locus explains only <1% of the variation in BMI (Frayling et al. 2007g).

Initially, findings of the influence of *FTO* on BMI were not replicated in groups of other ethnicities (Oceanic and Chinese) (Ohashi et al. 2007; Li et al. 2008a). However, there is increasing evidence that effect sizes are similar to those observed for European populations (Hotta et al. 2008; Cha et al. 2008a; Chang et al. 2008b; Al Attar et al. 2008; Villalobos-Comparan et al. 2008b; Wing et al. 2009c).

The *FTO* gene was reported first in a GWAS for T2D in which variants in the first intron of the *FTO* gene showed a highly significant association with T2D. Although the relationship between *FTO* and T2D was initially found to be mediated by its effect on obesity-related phenotypes in white European populations (Frayling 2007c; Frayling et al. 2007i). Recent studies suggest that *FTO* is associated with an increased T2D risk independent of adiposity factors in populations of different ethnic background out of white European population. A study conducted in a Pakistani population reported an association between *FTO* and T2D risk; each extra copy of the risk allele was associated with a 22% increased risk of T2D, independent of adiposity-related phenotypes (Rees et al. 2011e). Similarly, a study in an Asian Sikh population from North India reported a strong T2D risk conferred by the *FTO* gene independent of BMI; those subject homozygous for the risk allele showed a 45% increase risk of T2D (Sanghera et al. 2008). Other studies, in Chinese populations, have also replicated this finding, showing a ~30% increase in T2D risk in a Chinese population, among those who carried two copies of the risk allele independent of obesity-related phenotypes (Liu et al. 2010d). To date, just one study has reported a significant association between *FTO* and insulin resistance, measured by $HOMA_{IR}$. This study of 1,514 Japanese found a 0.74-unit of $HOMA_{IR}$ increase for each extra copy of the risk allele, and a 0.39-unit increase in insulin secretion per each allele; both associations were independent of age, sex, and obesity-related factors (Shimaoka et al. 2010c).

It is well-recognised that western lifestyles are the major driving force behind the obesity and diabetes epidemics in both developed and developing countries. However, genetically susceptible individuals will gain more weight in this obesogenic environment than those that are genetically protected. Recent studies have reported a gene- environment interaction, showing that the association between *FTO* variants and obesity-related phenotypes is more pronounced in individuals that

are more physically inactive, whereas the association is diminished in those who have physically active lifestyles (Vimaleswaran et al. 2009c; Lee et al. 2010b; Scott et al. 2010; Andreasen et al. 2008d). These observations suggest that genetic susceptibility towards obesity induced by variations in *FTO* can be overcome, by adopting a physically active lifestyle. So far, lifestyle intervention studies have not confirmed an interaction with *FTO* in relation to weight loss (Haupt et al. 2008a; Franks et al. 2008a; Lappalainen et al. 2009b).

The gene-environment relationship is a key issue not only in understanding the pathogenesis of multifactorial diseases, but also in designing appropriate treatments for the affected population. The previous chapters provided evidence that some populations such as South Asians, Pima Indians and Mapuches have higher obesity rates and an increased T2D susceptibility when they adopt a westernised lifestyle. Despite the emerging evidence of an increased susceptibility in some ethnic minorities, there are no published reports on gene-environment interaction studies in these populations. Understanding how genes and lifestyle factors interact may help to elucidate how lifestyle factors could modulate genetic contributions to risk of complex diseases such as obesity and T2D. Thus, the aims of the present chapter are:

(a) To investigate the association between the *FTO* gene and obesity-related phenotypes (measured by BMI, waist circumference and body fat) in Chilean populations.

(b) To assess the influences of *FTO* genotypes on insulin resistance, as a marker of T2D risk, in Chilean populations.

(c) To assess the influence of *FTO* genotypes on total energy intake, fat and carbohydrate intake in Chilean populations.

(d) To investigate how physical activity levels and cardio-respiratory fitness modulate the association between genetic variation in *FTO* and obesity-related phenotypes as well as insulin resistance in Chilean populations.

# 6.2 Methods

A total of 411 individuals were included in this study (Europeans n = 208 and Mapuches n = 203). Volunteers were recruited and screened as detailed in section 2.1.5. The assessment of anthropometric characteristics, body composition, physical activity, fitness, metabolic testing, socio-economic status, health and cultural screening was described in detail in chapter 2.

## 6.2.1 DNA Preparation and Genotyping Methods

A whole blood sample was collected by venepuncture from each individual. Blood samples were collected directly in 10 ml $K_3$EDTA tubes (BD, vacutainer System, Franklin Lakes, NJ USA). Whole blood was dispensed in 1.5 ml aliquots into labelled 2 ml sterilised screw cap tubes (Iberica Laboratories Ltd, Iberica, Chile). Six aliquot samples of whole blood were stored at -20ºC for 11 months pending DNA extraction.

## 6.2.2 Genomic DNA: Extraction, Storage, Concentration and Quality Checking Methods.

Genomic DNA (gDNA) for each individual was isolated from 2 ml of whole blood using the QIAamp DNA blood midi kit (QIAGEN, Ltd. UK). DNA was eluted in approx. of 900 µl on average and a range between 86.3 and 237.5 ng of DNA yield was obtained for the whole blood samples. The extracted gDNA was dispensed in four 200 µl aliquots into 2 ml sterilised tubes and stored at -80°C. One aliquot (100 µl) was stored at 4°C for further quality checking and genotyping. DNA concentration was quantified on a NanoDrop ND-8000 Spectrophotometer (ThermoFisher, Waltham, MA) using 1.5 µl of gDNA sample as per the manufacturer's instructions. After determining the samples' DNA concentrations, 20 random samples were selected and run in an agarose gel to verify the readings of the Spectrophotometer and to check DNA quality. A mean of three readings was used to determine the final gDNA concentration for each sample. After calculating the final gDNA concentrations, samples were diluted and standardised to a concentration of 10 ng.µl$^{-1}$, using the elution buffer AE (10 mM Tris-Cl; 0.5 mM EDTA; pH 9.0)

provided by the manufacturer. A re-reading was made to verify the target concentration (10 ng.µl$^{-1}$) for each sample. These samples were dispensed into sterile 2 ml 96 deep well plates (STARLAB, Ltda. UK) and stored temporarily at 4°C for future genotyping.

## 6.2.3 Genotyping

Genotyping of *FTO* SNPs rs3751812 and rs17817449, located in chromosome 16, was performed using TaqMan SNP Genotyping Assays C__27476887_10 and C__34511515_10, respectively (Applied Biosystems, Warrington, UK) and fluorescence was measured using endpoint reads and allele determination using ABI 7900 sequence Detection System, following the manufacturer's instruction (Applied Biosystems, Warrington, UK). The genotyping success rate was 98.5% (n = 405) for both SNPs, and no discordant genotypes were observed in 40 random duplicate samples.

## 6.2.4 Data and Statistical Analysis

A goodness-of fit chi-square test ($x^2$) (with 1 degree of freedom) was performed to confirm whether the observed genotype counts were in Hardy-Weinberg equilibrium. As previously mentioned, one of the selection criteria for Mapuches was based on the ABO blood group classification; Mapuches were included if the were of type O blood group (~95% of the Mapuches have this blood group), to verify weather a potential confounding effect existed between the *ABO* gene and *FTO* gene. The position of both genes were investigated; ABO gene was located in the chromosome 9 while the variants of the FTO gene were on chromosome 16. No other T2D or variants were found on this chromosome. This reduced the potential confounding effect of the recruitment selection criteria for the FTO analysis.

A General Linear Model (GLM) approach was used to test quantitative variables for differences between genotype groups. The main outcomes used in our analyses were obesity-related phenotypes (weight, BMI, waist circumference, body fat), dietary intake patterns (total energy intake, carbohydrates, fat and protein intake) and HOMA$_{IR}$. First, an ANOVA with no genetic model was performed for each SNP.

After an additive genetic model was verified, the genotypes were recoded (0, 1 and 2) and regression analysis was performed under an additive genetic model.

Multiple regression analyses were performed to assess the independent association between *FTO* genotypes and each specific outcome. A systematic approach was taken (Table 6.1). When obesity-related phenotypes were the outcome, firstly, an univariate model including *FTO* genotype only was run (base model). Each of the covariates and categorical factors of interest (age, sex, environment, ethnicity, SES, sedentary time, MVPA and fitness) was then added individually to the base model. Then, all six covariates and factors were simultaneously added to the base model. Finally, a series of interaction terms were included one at time (these tested the interaction between categorical covariates and *FTO* genotype: ethnicity * *FTO*, sex * *FTO*, environment * *FTO*). These tested whether the association between *FTO* and the obesity-related phenotypes differed by ethnicity, sex, *etc*. The same statistical approach was applied when $HOMA_{IR}$ was the outcome. However, due to previous studies reported that *FTO* effect on T2D risk could be mediated by BMI; additional covariates were included into the model (BMI, waist, body fat). Similarly, alcohol consumption and carbohydrate intake were also included into the model as confounding factors, due to previous literature suggesting that these lifestyle factors are associated with an increased risk of T2D risk.

**Table 6.1. Model building approach.**

| Models | Variable in model (outcome =Obesity-related phenotypes and $HOMA_{IR}$) |
|---|---|
| base model | Outcome = *FTO* genotype |
| Model 1 | Outcome = *FTO* genotype + covariates (added individually) |
| Model 2 | Outcome = *FTO* genotype + covariates (added simultaneously) |
| Model 3 | Outcome = *FTO* genotype * factors + covariates (added simultaneously) |

Outcome= Obesity-related phenotypes and $HOMA_{IR}$.
  Covariates: (age, sex, environment, ethnicity, SES, sedentary time, MVPA and fitness)
   Covariates additionally added for $HOMA_{IR}$ (BMI, waist, body fat, alcohol and CHO intake)
[‡] Factors (sex, environment, ethnicity).

The *FTO* genotype * lifestyle interactions, (such as physical activity, MVPA, sedentary behaviour and fitness), were examined by creating categorical activity

variables representing tertiles of each measure, which were included in the models using a GLM approach. To assess the independent influence of genotype * ethnicity * environment interactions on obesity-related phenotypes or insulin resistance, the same systematic approach was taken. All analyses were performed using Statistica (version 8.0; StataSoft, Tulsa, USA) and STATA (version 11.0; College Station. Texas. USA). For all analyses statistical significance was defined as $p < 0.05$.

## 6.3 Results

No recombinations were observed between the two *FTO* SNPs rs3751812 and rs17817449, assuming perfect linkage disequilibrium (LD) between the two *FTO* genotypes (Table 6.2 and Table 6.3). Further analyses were performed for the SNP rs17817449. Genotypes were in Hardy-Weinberg Equilibrium for both the combined cohort (Mapuches + Europeans) and when the cohort was stratified by ethnicity. The minor allele frequency (MAF) for the variant rs17817449 was 32.8% and 26.8% for Europeans and Mapuches, respectively. Due to perfect LD between both SNPs, further analyses are reported for the rs17817449.

**Table 6.2.** *FTO* **haplotypes frequency between the SNPs rs3751812 and rs17817449 in the European Cohort**

| | | | European (n=203) | | | |
|---|---|---|---|---|---|---|
| | | | FTO rs17817449 (MAF=0.328) | | | |
| | | **GG \*** n=19 | fq (n , %) | **GT** n=95 | fq (n , %) | **TT** n=89 | fq (n , %) |
| FTO rs3751812 (MAF=0.328) | **GG** n=89 | GG | 0 | GG | 0 | TG | 89 / 0.438 |
| | | GG | 0 | TG | 0 | TG | 89 / 0.438 |
| | **GT** n=95 | GG | 0 | GT | 95 / 0.468 | TG | 0 |
| | | TG | 0 | TG | 95 / 0.468 | TT | 0 |
| | **TT \*** n=19 | GT | 19 / 0.094 | GT | 0 | TT | 0 |
| | | GT | 19 / 0.094 | TT | 0 | TT | 0 |

\* Ancestral allele

**Table 6.3.** *FTO* **haplotypes frequency between the SNPs rs3751812 and rs17817449 in Mapuches Cohort**

| | | **Mapuches** (n=203) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **FTO rs17817449** (MAF=0.268) | | | | | |
| | | **GG \*** n=19 | fq (n , %) | **GT** n=71 | fq (n , %) | **TT** n=113 | fq (n , %) |
| **GG** n=113 | | **GG** | 0 | **GG** | 0 | **TG** | 113 / 0.557 |
| | | GG | 0 | TG | 0 | TG | 113 / 0.557 |
| **GT** n=71 | | **GG** | 0 | **GT** | 71 / 0.349 | **TG** | 0 |
| | | TG | 0 | TG | 71 / 0.349 | TT | 0 |
| **TT \*** n=19 | | **GT** | 18 / 0.094 | **GT** | 0 | **TT** | 0 |
| | | GT | 18 / 0.094 | TT | 0 | TT | 0 |

(Left axis label: **FTO rs3751812** (MAF=0.268))

\* Ancestral allele

**Table 6.4.** *FTO* **rs17817449 genotype frequencies for Mapuches and Europeans.**

| | Genotype | N observation | Genotype frequency | Allele frequency (%) | $^2$ *p*-value |
|---|---|---|---|---|---|
| ***Europeans*** | *GG* $^*$ | 19 | 0.094 | *(G\*)* 0.328 | 0.374 |
| | *GT* | 95 | 0.468 | | |
| | *TT* | 89 | 0.438 | *(T)* 0.672 | |
| | | | | | |
| ***Mapuches*** | *GG* $^*$ | 19 | 0.094 | *(G\*)* 0.268 | 0.118 |
| | *GT* | 71 | 0.349 | | |
| | *TT* | 113 | 0.557 | *(T)* 0.732 | |

$^2$ test, Hardy-Weinberg equilibrium was accepted at >0.05. Ancestral allele was denoted as $^*$

## 6.3.1 Association Between FTO Genotype and Obesity-related Traits

All subjects were initially tested together to establish the influences of the *FTO* genotyping on obesity traits. The unadjusted statistical analyses revealed that the rs17817449 G allele was significantly associated, using an additive genetic model, with increased body weight [3.69-kg increase per allele (SE: 0.773); $p=2 \times 10^{-6}$], BMI [1.40-unit (SE: 0.273); $p=4 \times 10^{-7}$], waist circumference [2.49-cm (SE: 0.813); $p<0.003$] and body fat [1.88-kg (SE: 0.375); $p=1 \times 10^{-6}$] in this sample (Figure 6.1).

To determine whether the *FTO* effect on the obesity-related phenotypes was independent of other confounding factors, several additional covariates were included into the model (age, sex, environment, ethnicity, smoking status, SES, sedentary time, MVPA and fitness). After adjustment for all of these, the association between *FTO* and obesity-related traits remained significant but with an attenuated effect size per risk allele for body weight [0.261-kg increase per allele (SE: 0.792); $p$=0.001], BMI [0.80-unit (SE: 0.279); $p$=0.004], waist circumference [2.01-cm (SE: 0.913); $p$<0.028] and body fat [1.61-kg (SE: 0.403); $p$=8x10$^{-5}$]. To assess whether the effect of *FTO* on obesity-related phenotypes differed by sex, environment or ethnicity, a series of interactions were tested (*FTO*\*sex, *FTO*\*environment and *FTO*\*ethnicity). No evidence of an independent interaction effect between *FTO*\*sex (body weight, $p$=0.135; BMI, $p$=0.259; waist, $p$=0.201; body fat, $p$=0.118), *FTO*\*Environment (body weight, $p$=0.311; BMI, $p$=0.121; waist, $p$=0.419; body fat, $p$=0.298) and *FTO*\*ethnicity (body weight, $p$=0.229; BMI, $p$=0.638; waist, $p$=0.546; body fat, $p$=0.592) on obesity-related phenotypes was found.
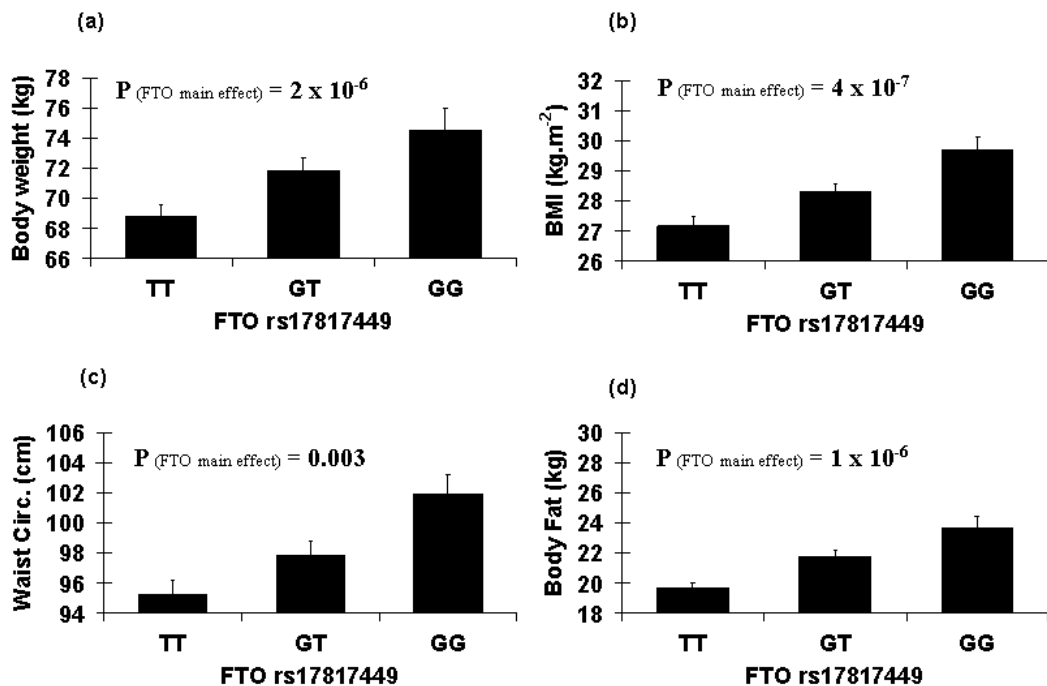


**Figure 6.1. Association between rs17817449 and obesity-related phenotypes.**
Unadjusted mean and SEM are presented for each genotype group. GLM was used to examine the main *FTO* genotype affect on each obesity-related trait, under an additive genetic model.

## 6.3.2 Association Between FTO Genotype and Dietary Intake

To examine whether the rs17817449 influences dietary intake patterns, total energy intake (Kcal), carbohydrates (CHO), fat and protein intake were included as outcomes in models. No significant associations between *FTO* and dietary intake were found [total energy intake 36.2 kcal increase per allele (*SE*: 71.0); *p=0.610*; CHO intake 5.48-g increase per allele (*SE*: 10.8); *p=0.614*; Fat intake -0.004-g increase per allele (*SE*: 3.33); *p=0.998*; Protein intake -1.17-g increase per allele (*SE*: 3.42); *p=0.732*] (Figure 2.1). Further examination for *FTO*\*ethnicity (Energy intake, *p=0.657*; CHO, *p=0.303*; fat, *p=0.733*; protein, *p=0.352*), *FTO*\*sex (Energy intake, *p=0.332*; CHO, *p=0.288*; fat, *p=0.134*; protein, *p=0.938*) and *FTO*\*environment (Energy intake, *p=0.614*; CHO, *p=0.801*; fat, *p=0.241*; protein, *p=0.840*) did not reveal any significant effect on any of the dietary factors.
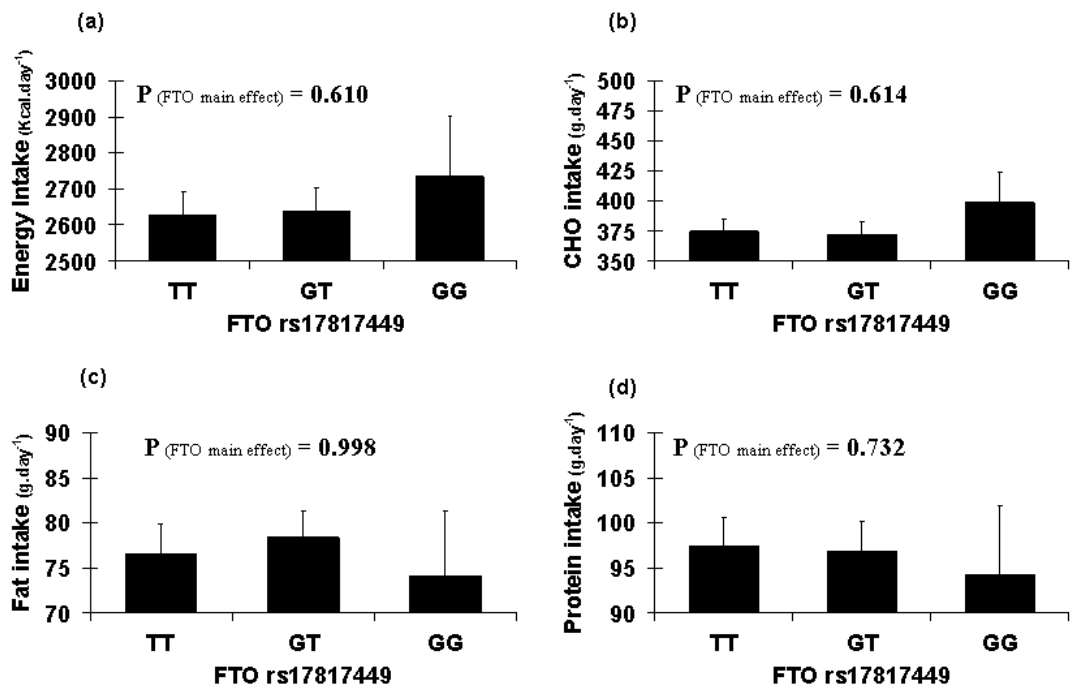


**Figure 6.2. Association between rs17817449 and components of dietary intake.**
Unadjusted mean and SEM are presented for each genotype group. GLM was used to examine the main *FTO* genotype affect on each diet intake factor, under an additive genetic model.

### 6.3.3 Association Between FTO Genotype and Insulin Resistance

All individuals were firstly examined together to establish the influence of the *FTO* gene on insulin resistance (IR), measured by $HOMA_{IR}$. We found that the rs17817449 G allele showed a highly significant association with $HOMA_{IR}$ [1.08-unit increase per allele (*SE*: 0.222); $p=1x10^{-6}$]; using an additive genetic model the *FTO* genotype explained 6.7% of the variance in IR. To determine whether the *FTO* effect on the $HOMA_{IR}$ was independent of other factors, several additional covariates were included in the model (age, sex, environment, ethnicity, smoking status, SES, sedentary time, MVPA, fitness, CHO intake and obesity-related phenotypes). After adjustment for all these, the association between *FTO* and $HOMA_{IR}$ remained highly significant but with an attenuation on the effect size per risk allele [0.73-unit increase per allele (*SE*: 0.126); $p=2x10^{-6}$]. After adjusting for confounding factors, *FTO* genotype explained 3.1% of the variance in IR.

To assess whether the effect of *FTO* on insulin resistance differed by sex, environment or ethnicity, the following interactions were tested (*FTO*\*sex, *FTO*\*environment and *FTO*\*ethnicity). No evidence of an interaction between *FTO*\*sex ($p=0.227$) or *FTO*\*environment ($p=0.140$) was found. The analysis, illustrated in Figure 6.3, revealed a significant gene\*ethnicity interaction for insulin resistance ($p<0.0002$); in subsequent analyses stratified by ethnicity there was a significant association between the genotype and IR for Mapuches but not Europeans. The per-allele genotype effect size on IR was 2.04-unit (*SE*: 0.369; $p=9x10^{-10}$) for Mapuches and 0.265-unit (*SE*: 0.125; $p=0.189$) for Europeans. To determine whether the *FTO*\*ethnicity interaction effect for $HOMA_{IR}$ was independent of other confounding factors, the following covariates were included in the model (age, sex, environment, smoking status, SES, sedentary time, MVPA, fitness, CHO intake, alcohol consumption, BMI, waist circumference and body fat). Subsequent to adjustment, the association between *FTO* and $HOMA_{IR}$ remained highly significant for Mapuches [1.20-unit increase per allele (*SE*: 0.209); $p=4x10^{-6}$] and remained not significant for Europeans [0.14-unit (*SE*: 0.099); $p=0.123$]. After adjustment for several confounding factors *FTO* genotype explained 6.5% and <0.1% of the IR variance in Mapuches and European respectively.
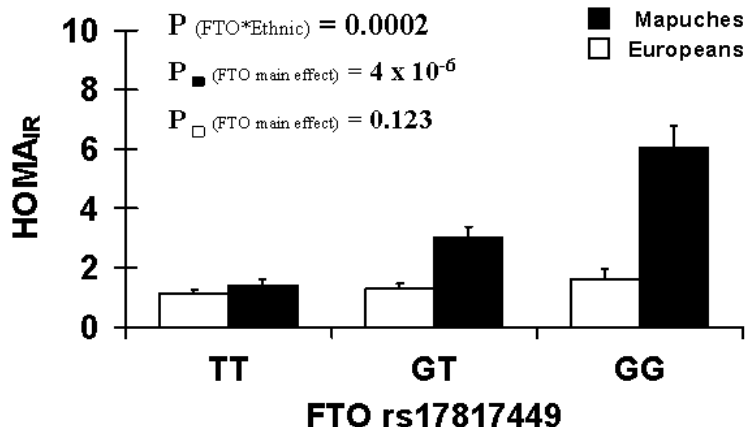
**Figure 6.3. Association between rs17817449 and insulin resistance for Mapuches and Europeans population.**
Adjusted Mean and SEM are presented for each genotype group. Data presented was adjusted for several potential confounding factors (age, sex, environment, SES, smoking status, sedentary time, MVPA, fitness, CHO intake, alcohol consumption and obesity-related traits). Transformed $HOMA_{IR}$ (ln) was used for statistical analysis.

## 6.3.4 FTO, Physical Activity and Fitness

The following section examines the influence of different subcomponents of PA, (sedentary behaviours, time spent in MVPA and fitness) on the relationship between *FTO* and obesity-related traits (BMI, waist circumference and body fat).

### 6.3.4.1 *FTO* Genotype and Sedentary Behaviour Interaction: Its Influences on Obesity-related Traits

No association between rs17817449 and time spent in sedentary behaviours was found ($p = 0.334$). The interaction effect between *FTO*\*sedentary behaviour on BMI, waist and body fat was examined (with sedentary behaviour classified into tertiles; lower, middle, upper). Although the interaction was not statistically significant for any of the outcomes (BMI, $p=0.965$; waist, $p=0.962$; body fat, $p=0.697$), analysis revealed that the strength of the association between *FTO* and the obesity traits increased with increasing time spent in sedentary behaviours (Figure 6.4) [BMI: Lower tertile, 0.72-unit per allele (*SE*: 0.449); $p=0.110$; Middle tertile, 1.02-unit per allele (*SE*: 0.510);  $p=0.049$; Upper tertile, 1.21-unit per allele (*SE*: 0.481); $p=0.014$]. A similar magnitude of association was found for waist circumference

[Lower, 1.55-cm per allele (*SE*: 1.42); *p=0.277*; Middle, 2.24-cm per allele (*SE*: 1.48); *p=0.132*; Upper, 3.23-cm per allele (*SE*: 1.60); *p=0.047*] and Body Fat [Lower, 1.21-kg per allele (*SE*: 0.683); *p=0.079*; Middle, 1.36-kg per allele (*SE*: 0.690); *p=0.050*; Upper, 2.17-kg per allele (*SE*: 0.636); *p=0.0006*].

To determine whether these associations were independent of confounding factors, covariates were included in the model (age, sex, ethnicity, environment, smoking status, SES, accelerometer wearing, MVPA and fitness). Subsequent to adjustment, the influence of sedentary behaviour on the *FTO* and obesity relationship was slightly reduced but remained significant for BMI [Lower, 0.59-unit per allele (*SE*: 0.462); *p=0.195*; Middle, 0.60-unit per allele (*SE*: 0.512); *p=0.251*; Upper , 1.10-unit per allele (*SE*: 0.494); *p=0.029*] and body fat [Lower, 1.13-kg per allele (*SE*: 0.739); *p=0.128*; Middle, 1.34-kg per allele (*SE*: 0.739); *p=0.073*; Upper , 1.79-kg per allele (*SE*: 0.621); *p=0.004*] but was abolished for waist circumference [Lower, 1.53-cm per allele (*SE*: 1.51); *p=0.313*; Middle, 1.30-cm per allele (*SE*: 1.52); *p=0.396*; Upper , 2.71-cm per allele (*SE*: 1.70); *p=0.115*]. The effect of ethnicity on the association between *FTO*, MVPA and obesity traits was explored adding the ethnicity factor under a 3-way interaction model. This model shows no evidence of an *FTO*\*ethnicity\*sedentary time interaction on any of the obesity related traits was found (BMI, *p=0.998*; waist, *p=0.998*; body fat, *p=0.956*).
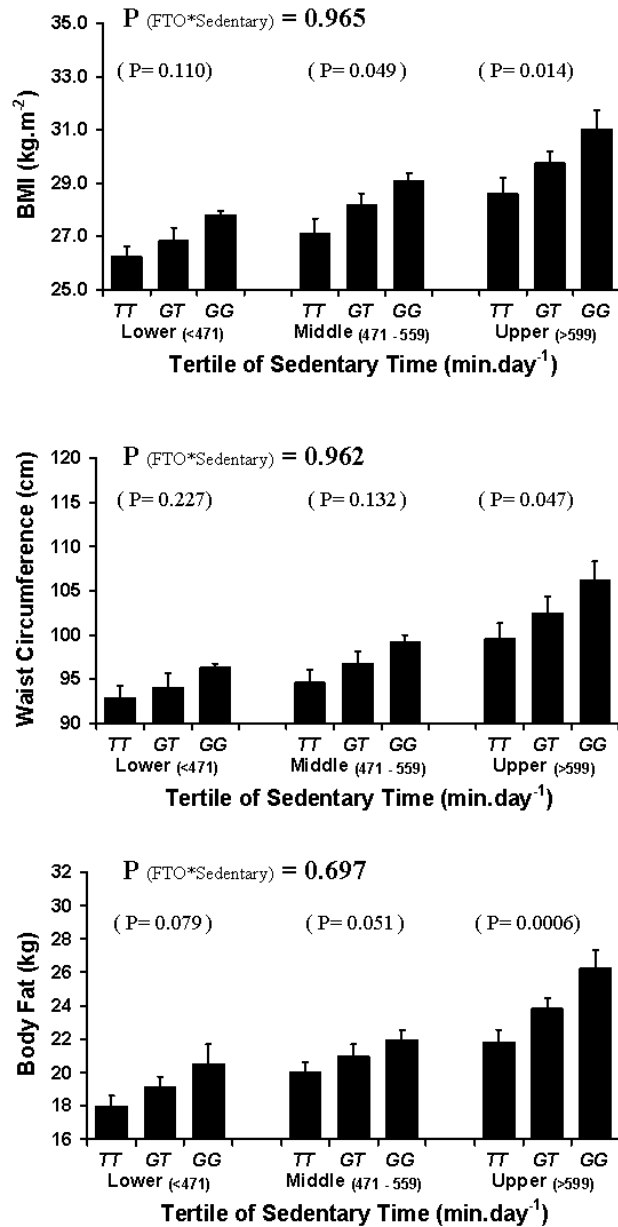
**Figure 6.4. Effect of the interaction between rs17817449 and sedentary behaviour on obesity-related traits.**
Unadjusted mean and SEM are presented for each genotype group across the tertiles of sedentary time (lower, middle, upper). GLM was used to examine a *FTO*\*PA interaction and a PA-stratified GLM analysis was performed to determine the *FTO* genotype main effect under an additive genetic model (*p*-value denoted in brackets).

### 6.3.4.2 *FTO* Genotype and Moderate to Vigorous Physical Activity Interaction: Its Influences on Obesity-related Traits

No association between rs17817449 and Moderate to Vigorous physical activity (MVPA) was found ($p=0.252$). Subsequently the effect of the *FTO*\*MVPA interaction on obesity traits was examined across the tertiles of MVPA (lower, middle, upper). The unadjusted analysis revealed that the interaction (*FTO*\*MVPA) was not statistically significant for any of the obesity outcomes (BMI, $p=0.186$; waist, $p=0.721$; body fat, $p=0.668$) (Figure 6.5).

Even though no significant interactions were found, the analysis revealed that the strength of the association between *FTO* and body fat was attenuated with increasing time spent in MVPA [Lower, 2.12-kg per allele (*SE*: 0.645); $p=0.002$; Middle, 2.02-kg per allele (*SE*: 0.688); $p=0.004$; Upper, 1.51-kg per allele (*SE*: 0.729); $p=0.0006$]. However, this trend was not observed for BMI [Lower, 0.10-unit per allele (*SE*: 0.504); $p=0.058$; Middle, 1.52-unit per allele (*SE*: 0.523); $p=0.004$; Upper, 0.838-unit per allele (*SE*: 0.); $p=0.060$] and waist circumference [Lower, 2.49-cm per allele (*SE*: 1.62); $p=0.128$; Middle, 4.22-cm per allele (*SE*: 1.64); $p=0.011$; Upper, 1.39-cm per allele (*SE*: 1.29); $p=0.283$]. Further adjustment for age, sex, ethnicity, environment, smoking status, SES, accelerometer wearing time, sedentary time and fitness, abolished the association and trend found between *FTO* and MVPA on body fat [Lower, 1.17-kg per allele (*SE*: 0.642); $p=0.069$; Upper, 1.78-kg per allele (*SE*: 0.723); $p=0.115$]. The effect of ethnicity on the association between *FTO* and MVPA and obesity traits was explored adding the ethnicity factor under a 3-way interaction model. No evidence of an interaction between *FTO*\*ethnicity\*MVPA on obesity related traits was found (BMI, $p=0.614$; waist, $p=0.956$; body fat, $p=0.340$).

**Figure 6.5. Effect of the interaction between rs17817449 and MVPA on obesity-related traits.**

Unadjusted mean and SEM are presented for each genotype group across the tertiles of MVPA (lower, middle, upper). GLM was used to examine a *FTO*\*PA interaction and a PA-stratified GLM analysis was performed to determine the *FTO* genotype main effect under an additive genetic model (*p*-value denoted in brackets).

### 6.3.4.3 *FTO* Genotype and Fitness Interaction: Its Influences on Obesity-related Traits

The analyses showed that there is no association between *FTO* rs17817449 and fitness ($VO_{2max}$ ml.kg.min$^{-1}$) (*p*=0.495). The interaction of *FTO*\*fitness on obesity traits was examined for each level of fitness tertiles. The interaction (*FTO*\*fitness) was not statistically significant for any of the obesity outcomes (BMI, *p*=0.495; waist, *p*=0.539; body fat, *p*=0.662). However the unadjusted analyses (Figure 6.4) revealed that the strength of the association between *FTO* and obesity-related traits decreased with increasing fitness level in a similar way that the previous section [BMI: Lower 1.40-unit per allele (*SE*: 0.503); *p*=0.005; Middle, 1.19-unit per allele (*SE*: 0.487); *p*=0.020; Upper, 1.17-unit per allele (*SE*: 0.474); *p*=0.015]. A similar strength of association was found for waist circumference [Lower, 3.20-cm per allele (*SE*: 1.56); *p*=0.043; Middle, 2.91-cm per allele (*SE*: 1.41); *p*=0.040; Upper, 2.67-cm per allele (*SE*: 1.39); *p*=0.056] and Body Fat [Lower, 2.29-kg per allele (*SE*: 0.665); *p*=0.0008; Middle, 2.15-kg per allele (*SE*: 0.623); *p*=0.0007; Upper, 1.58-kg per allele (*SE*: 0.643); *p*=0.014].

To determine whether these associations were independent of confounding factors, covariates were included in the model (age, sex, ethnicity, environment, smoking status, SES, MVPA and sedentary time). After adjustment for all these, the influence of fitness on the *FTO* and obesity relationship was slightly reduced but remained borderline significant for BMI [Lower, 0.89-unit per allele (*SE*: 0.455); *p*=0.051; Middle, 0.87-unit per allele (*SE*: 0.486); *p*=0.074; Upper, 0.66-unit per allele (*SE*: 0.512); *p*=0.195] and body fat [Lower, 1.84-kg per allele (*SE*: 2.27); *p*=0.025; Middle, 1.76-kg per allele (*SE*: 0.719); *p*=0.015; Upper, 1.15-kg per allele (*SE*: 0.596); *p*=0.056] but was abolished for waist circumference [Lower, 1.88-cm per allele (*SE*: 1.51); *p*=0.242; Middle, 1.73-cm per allele (*SE*: 1.48); *p*=0.247; Upper, 1.59-cm per allele (*SE*: 1.64); *p*=0.102]. No evidence of an interaction between *FTO*\*ethnicity\*sedentary time and obesity related traits was found (BMI, *p*=0.770; waist, *p*=0.458; body fat, *p*=0.936).
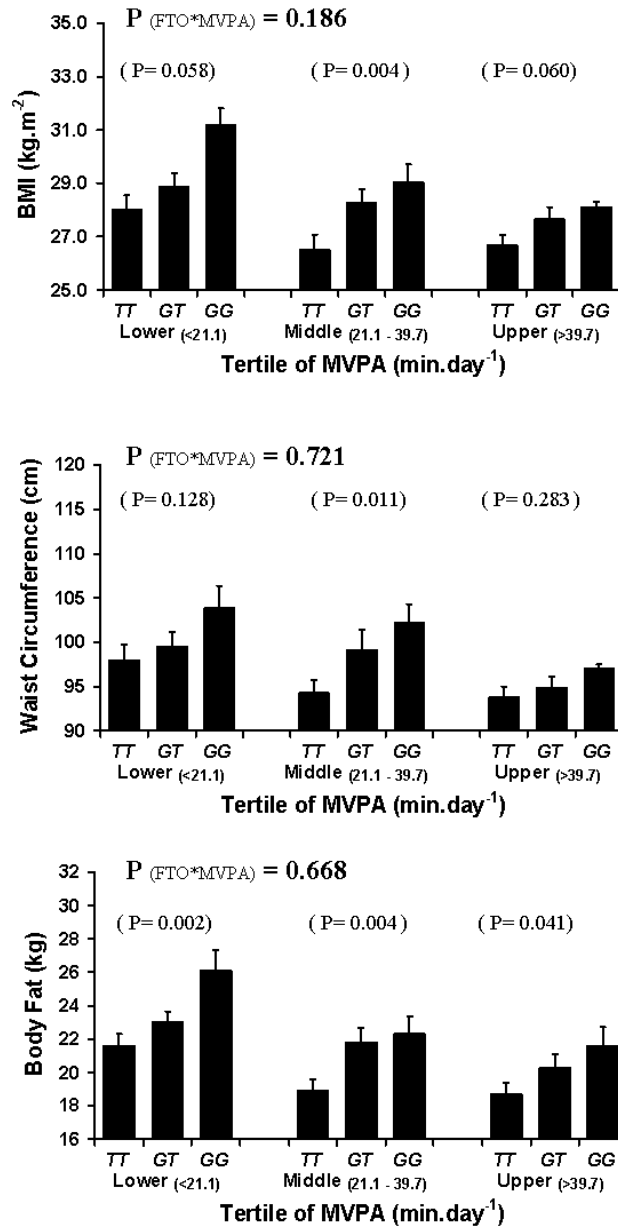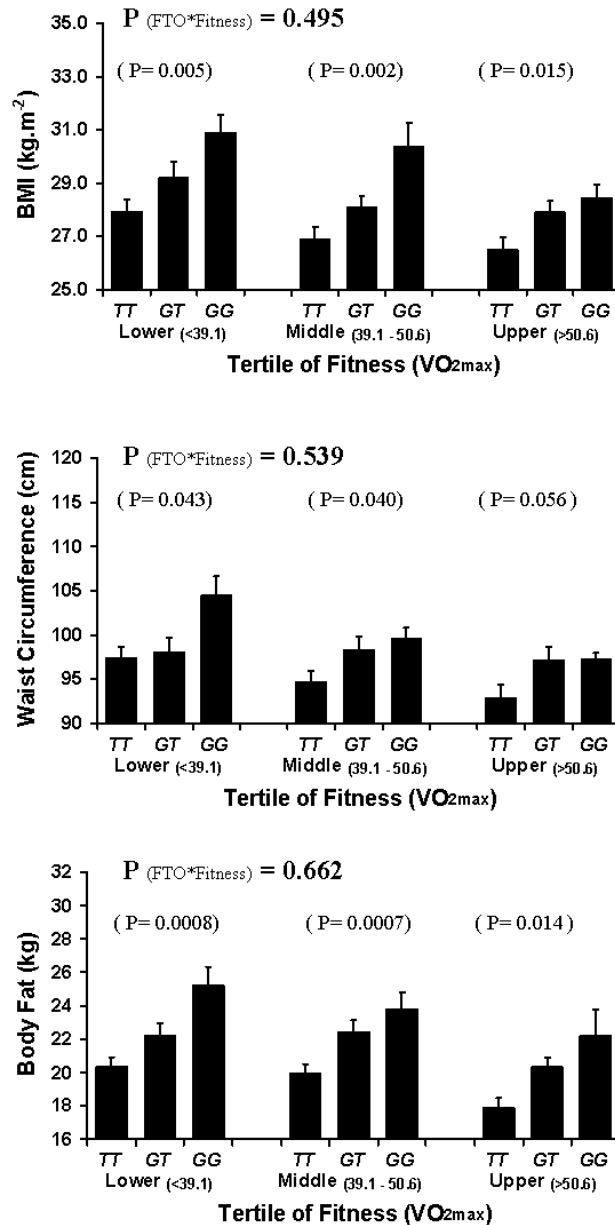
**Figure 6.6. Effect of the interaction between *FTO* and fitness on obesity-related traits.**
Unadjusted mean and SEM are presented for each genotype group across the tertiles of fitness (lower, middle, upper). GLM was used to examine a *FTO*\*fitness interaction and a fitness-stratified GLM analysis was performed to determine the *FTO* genotype main effect under an additive genetic model (*p*-value denoted in brackets).

### 6.3.5 Interaction Between FTO, Ethnicity and Physical Activity/Fitness on Insulin Resistance

Previously in this chapter was reported a highly significant *FTO* and ethnicity interaction effect on insulin resistance (IR), measured by $HOMA_{IR}$. This result showed that genetic variant *FTO* influenced IR in Mapuches but not in Europeans. To examine whether sedentary behaviour, MVPA and fitness could modulate the *FTO* genotype effect on IR a 3-way interaction term between *FTO*, ethnicity and PA/fitness levels was built.

Although sedentary time did not have a statistically significant interaction with *FTO* and ethnicity on IR ($p$=0.964) (Figure 6.7), the unadjusted analysis showed that the strength of the association between *FTO* and IR increased with increasing time spent in sedentary behaviour in Mapuches [Lower, 0.25-unit per allele (*SE*: 0.041); $p$=7x10$^{-9}$; Middle, 1.30-unit per allele (*SE*: 0.361); $p$=0.0008; Upper, 2.57-unit per allele (*SE*: 0.462); $p$=4x10$^{-6}$]. The association between *FTO* and $HOMA_{IR}$ was significant in Europeans only in the higher sedentary tertile [Lower, 0.02-unit per allele (*SE*: 0.045); $p$=0.619; Middle, 0.08-unit per allele (*SE*: 0.138); $p$=0.558; Upper, 0.61-unit per allele (*SE*: 0.236); $p$=0.012]. After accounting for several potential confounding factors (age, sex, environment, smoking status, SES, accelerometer wearing time, fitness, MVPA, alcohol consumption, CHO intake, BMI, waist circumference and body fat), the influence of sedentary time on the *FTO* and IR relationship remained significant for Mapuches [Lower, 0.23-unit per allele (*SE*: 0.075); $p$=3x10$^{-6}$; Middle, 1.09-unit per allele (*SE*: 0.328); $p$=0.005; Upper, 1.29-unit per allele (*SE*: 0.438); $p$=0.034] and remained significant for the higher tertile of sedentary time in Europeans [Lower, 0.01-unit per allele (*SE*: 0.069); $p$=0.444; Middle, 0.05-unit per allele (*SE*: 0.214); $p$=0.656; Upper, 0.44-unit per allele (*SE*: 0.301); $p$=0.021].
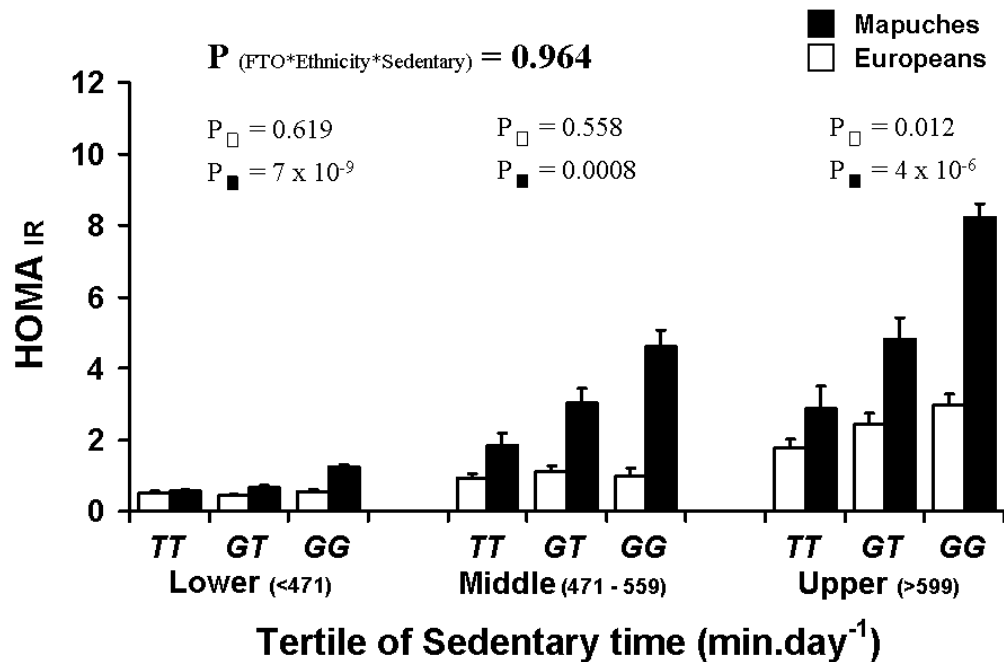
**Figure 6.7. Effect of the interaction between rs17817449, ethnicity and sedentary behaviour on insulin resistance.**
Unadjusted mean and SEM are presented for each genotype group across the tertiles of sedentary behaviour. GLM was used to examine a *FTO*\*fitness\*ethnicity interaction. Ethnicity and sedentary time stratified GLM analysis was performed to determine the *FTO* genotype main effect for Mapuches and Europeans under an additive genetic model. Transformed $HOMA_{IR}$ (ln) was used for statistical analysis.

No evidence of a significant *FTO*\*ethnicity\*MVPA interaction effect on IR was found ($p=0.924$). However, there was an attenuation of the association between *FTO* and IR by increasing time spent in MVPA was observed for Mapuches under an adjusted model [Lower, 2.14-unit per allele (*SE*: 0.526); $p=0.0006$; Middle, 1.57-unit per allele (*SE*: 0.332); $p=0.0002$; Upper, 0.68-unit per allele (*SE*: 0.229); $p=0.013$] but not for Europeans [Lower, 0.51-unit per allele (*SE*: 0.262); $p=0.056$; Middle, 0.41-unit per allele (*SE*: 0.173); $p=0.011$; Upper, 0.07-unit per allele (*SE*: 0.115); $p=0.538$]. After accounting for several potential confounding factors (age, sex, environment, smoking status, SES, accelerometer wearing time, fitness, sedentary time, alcohol consumption, CHO intake, BMI, waist circumference and body fat), the influence of MVPA on the *FTO* and IR relationship remained significant for Mapuches [Lower, 1.54-unit per allele (*SE*: 0.331); $p=0.0001$; Middle, 1.02-unit per allele (*SE*: 0.218); $p=0.051$; Upper, 0.43-unit per allele (*SE*: 0.218); $p=0.056$].

$P_{(FTO*Ethnicity*MVPA)} = 0.924$

$P_{\square} = 0.056$  $P_{\square} = 0.011$  $P_{\square} = 0.538$
$P_{\blacksquare} = 0.0006$  $P_{\blacksquare} = 0.0002$  $P_{\blacksquare} = 0.538$

■ Mapuches
□ Europeans
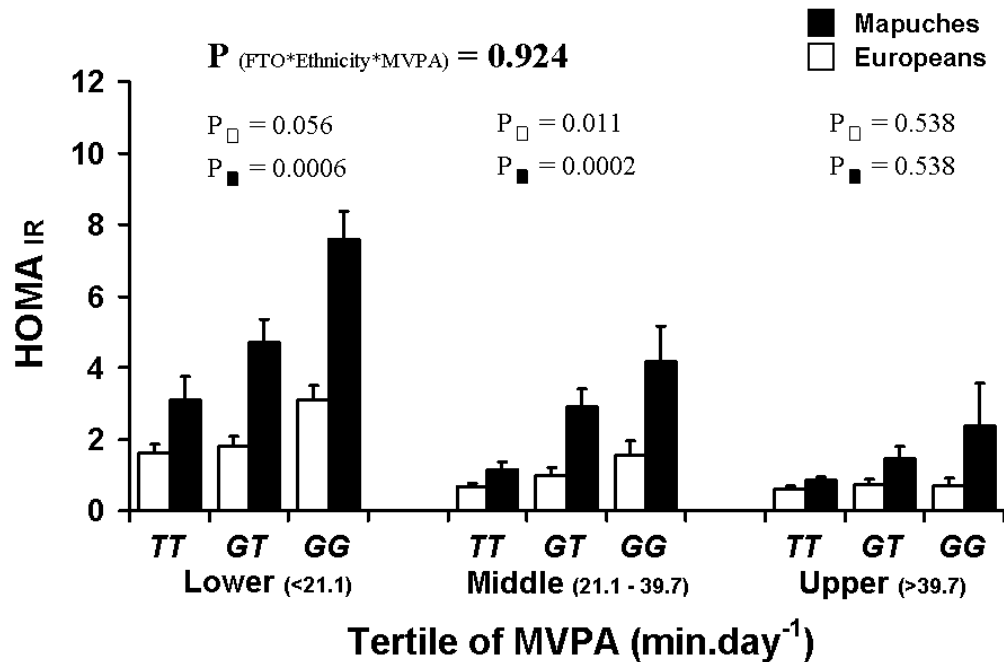
**Figure 6.8. Effect of the interaction between rs17817449, ethnicity and MVPA on insulin resistance.**
Unadjusted mean and SEM are presented for each genotype group across the tertiles of MVPA. GLM was used to examine a *FTO*\*MVPA\*ethnicity interaction. Ethnicity and MVPA stratified GLM analysis was performed to determine the *FTO* genotype main effect for Mapuches and Europeans under an additive genetic model. Transformed HOMA$_{IR}$ (ln) was used for statistical analysis.

Similarly to sedentary time and MVPA, no evidence of a significant *FTO*\*ethnicity\*fitness interaction effect on IR was found (*p*=0.513). However there was a significant trend; the association between *FTO* and IR decreased with increasing fitness level for both Mapuches [Lower, 3.27-unit per allele (*SE*: 0.499); *p*=5x10$^{-6}$; Middle, 1.53-unit per allele (*SE*: 0.440); *p*=0.0002; Upper, 0.38-unit per allele (*SE*: 0.229); *p*=0.043] and Europeans [Lower, 0.46-unit per allele (*SE*: 0.220); *p*=0.025; Middle, 0.14-unit per allele (*SE*: 0.093); *p*=0.061; Upper, 0.01-unit per allele (*SE*: 0.083); *p*=0.858]. The effect of fitness on the association between *FTO* and IR was independent of covariates and factors (sex, environment, smoking status, SES, accelerometer wearing time, sedentary time, MVPA, alcohol consumption, CHO intake, BMI, waist circumference and body fat) for Mapuches [Lower, 2.39-unit per allele (*SE*: 0.669); *p*=0.019; Middle, 1.07-unit per allele (*SE*: 0.326); *p*=0.002; Upper, 0.083-unit per allele (*SE*: 0.177); *p*=0.417] but was abolished for

Europeans [Lower, 0.18-unit per allele (*SE*: 0.178); *p*=0.305; Middle, 0.06-unit per allele (*SE*: 0.092); *p*=0.514; Upper, 0.02-unit per allele (*SE*: 0.094); *p*=0.756].
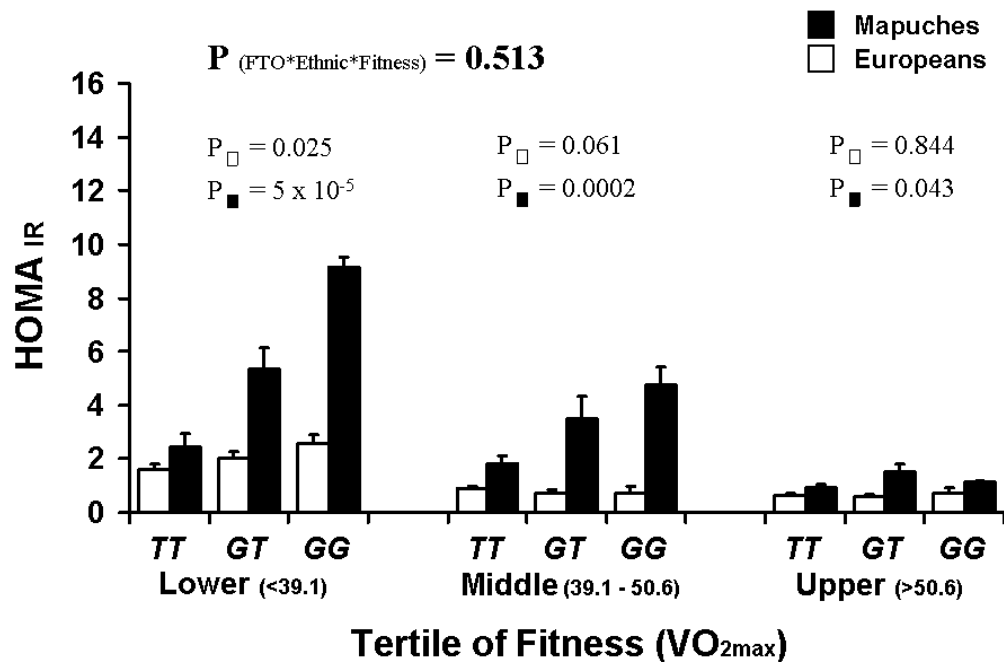


**Figure 6.9. Effect of the interaction between rs17817449, ethnicity and fitness on insulin resistance.**
Unadjusted mean and SEM are presented for each genotype group across the tertiles of fitness. GLM was used to examine a *FTO*\*fitness\*ethnicity interaction. Ethnicity and fitness stratified GLM analysis was performed to determine the *FTO* genotype main effect for Mapuches and Europeans under an additive genetic model. Transformed $HOMA_{IR}$ (ln) was used for statistical analysis.

## 6.4 Discussion

The main findings of this chapter were: a) the genetic variation of the rs17817449 of the *FTO* gene was associated with increased obesity level (measured by BMI, waist circumference or body fat; b) Interestingly, the rs17817449 was associated with a substantial increase in insulin resistance for Mapuche but not European populations, independent of several potential confounding factors including lifestyle and adiposity-related traits and; c) this chapter provided evidence that lifestyle factors such as physical activity, sedentary behaviours and fitness can modulate the genetic effect of the rs17817449 on obesity and insulin resistance.

This study found perfect linkage disequilibrium between the two *FTO* SNPs rs3751812 and rs17817449 in both Mapuches and Chileans of European descent. No recombination was found between the tow FTO SNPs and only two haplotypes were observed for both ethnic groups at both SNPs (GT and TG). This perfect linkage disequilibrium could potentially be explained in several ways; one of them could be related to the out of Africa model of human evolution, this postulated that modern humans were originated in Africa and that a smaller subset of this population later migrated to other parts of the world. During and after migration, some variants would have been lost and, as the separation time was still short, non-african populations were not yet acquired higher nucleotide diversity (Stoneking et al. 1997). A second reason could be related to the low admixture in the Mapuche and Chilean of European descent population recruited for this study, due to strict selection criteria. Those populations with high level of admixture show higher nucleotide diversty than those isolated or more pure. These reasons could explain why the degree of LD for FTO in our study is higher than in African-ancestry populations, which report low LD in comparison to populations of European and Asian origins (Reich et al. 2001).

Previous reports indicated that both SNPs located in the chromosome 16q12.2 and genotyped in this study are part of a large cluster of >40 single nucleotide polymorphism (SNPs) that are highly correlated (linkage disequilibrium (LD): $r^2$ >0.80 in populations outside of African ancestries in the HapMap). It is therefore expected that the result would have been similar if we have genotyped rs8050136, rs7193144, rs1421085, rs1121980 or rs9939609, because all these SNPs fall in a region of strong linkage disequilibrium (LD) in intron 1 of the *FTO* gene in populations outside of Africa, so they are perfect proxies for each other (Loos and Bouchard 2008e). In addition, the derived allele (G) was lower in the Chilean European population (32.8%) than that reported in the HapMap for White European populations (39 - 45%). For Mapuches the derived allele frequency was 26.8%, similar to the frequency reported in the HapMap for a Gujarati Indian population (25.9%). In both groups, Mapuches and Europeans, the derived allele frequency was higher than those reported in the HapMap for Asians (16-18%) and Mexicans (~18%), and lower than Africans (~39%).

The results of this study were consistent with previous findings, which showed an unequivocal association between *FTO* variants and body weight (Andreasen et al. 2008c; Dina et al. 2007e; Frayling et al. 2007j; Sonestedt et al. 2009d). In this chapter, a modest *FTO* genotype effect was found for the combined population (3.69-kg per allele). However, the *FTO* genotype effect size was obscured to some extent after accounting for several confounding factors, but remained significant (0.26-kg per allele). This adjusted effect size was lower than what has been reported for other ethnic populations. White Europeans populations had an increase in body weight per allele ranging between 1.7 and 3.3 kg (Andreasen et al. 2008b; Dina et al. 2007d; Frayling et al. 2007f; Sonestedt et al. 2009c), while for other populations such as old order Amish, the affect was 3.5-kg (Rampersaud et al. 2008e). In South Asians *FTO* shows a modest effect of 0.6 kg in normal populations and 1.3 in T2D subjects (Yajnik et al. 2009d). The reduction in the effect size of *FTO* observed in this study after adjustment highlights the need to account effectively for other confounding factors when estimating the strength of genotype effects on body weight.

The association between the *FTO* SNPs and BMI and the risk of being overweight or obese has been unequivocally confirmed in multiple ethnic populations (Fawcett and Barroso 2010b). This study confirmed and extended these previous findings for a South American population. Our results revealed an *FTO* effect size on BMI of 1.4-units per allele in the cohort. However, the strength of the association was reduced after accounting for several confounding factors (0.80-unit per allele) but remained significant. Previous studies have reported similar and modest *FTO* effects on BMI, showing a varied effect across different ethnic populations; those of White European descent and Hispanic Americans showed an increased effect of ~0.4 to ~1.1-units (Fawcett and Barroso 2010a; Frayling et al. 2007h; Dina et al. 2007b; Sonestedt et al. 2010c; Wei et al. 1999f). Although results were initially inconsistent in East Asian populations (Li et al. 2008b), there is increasing evidence that the genotype effect sizes are similar (~0.3 to ~1.2-units) to those observed for the European populations (Hotta et al. 2008; Cha et al. 2008c; Chang et al. 2008c; Lee et al. 2010c; Tabara et al. 2009a; Tan et al. 2008b). Similar effect sizes have been described for South Asians (Rees et al. 2011d; Yajnik et al. 2009c). However, in African populations the genetic variation in *FTO* does not seem to affect BMI in a similar way to White

European populations, this could be in part explained because the *FTO* gene shows significant differences in allele frequency and LD patterns in populations of African ancestry compared with other continental populations. To date several studies have found associations between *FTO* and obesity in African Americans and Africans (Wing et al. 2010; Hassanein et al. 2010; Bollepalli et al. 2010; Adeyemo et al. 2010; Bressler et al. 2010; Wing et al. 2009b), but not all (Hennig et al. 2009).

As seen with BMI, the *FTO* variant was significantly and independently associated with body fat (1.88-kg per allele) and waist circumference (2.01-cm per allele). A limited amount of evidence has been reported on the effect of *FTO* on waist circumference and body fat, compared to the substantial evidence for BMI. The effect of the *FTO* risk allele on waist circumference has varied between populations; for white Europeans the effect size was between 0.6 and 2.3-cm (Andreasen et al. 2008a; Sonestedt et al. 2009b; Loos and Bouchard 2008a; Loos 2009d; Vimaleswaran and Loos 2010), while Asians and South Asians had a smaller genotype effect, lying between ~0.3-cm and ~1.4-cm (Lee et al. 2010d; Rees et al. 2011c). Fewer studies have reported a significant association between body fat and *FTO,* with a genotype effect size of ~0.4-kg (Peeters et al. 2008; Vimaleswaran and Loos 2010; Frayling et al. 2007l). However, differences in the genotype size effect between our study and previous studies could be explained due to differences in methods used to estimate adiposity.

Previous studies have reported that *FTO* is highly expressed in the hypothalamus, a region involved in appetite regulation. *FTO* has been shown to be associated with increased energy intake, especially fat intake (Cecil et al. 2008; Timpson et al. 2008; Wardle et al. 2009) and impaired satiety responsiveness (Wardle et al. 2008) in children. Studies of adults have also shown that risk allele carriers consume more energy (Speakman, Rance, and Johnstone 2008b), whereas the genotypes does not seem to influence energy expenditure (Berentzen et al. 2008; Franks et al. 2008c). These results indicate that *FTO* could modulate obesity mainly by influencing appetite regulation. However, no evidence of a relationship was found between *FTO* genotype and dietary intake patterns (total energy, CHO, fat, proteins) in our study. This lack of association could be explained by bias in the food intake measures. Studies have indicated that obese individuals generally reported to consume less or

the same amount of energy as normal weight individuals (Hill and Davies 2001), but studies using the doubly labeled water technique have provided evidence of systematic misreporting of dietary intake among overweight or obese individuals (Lissner 2002; Lissner, Heitmann, and Lindroos 1998).

Previously in this thesis, we provided evidence that *FTO* was first associated to T2D, but this relationship was modulated by adiposity (Frayling et al. 2007b). Although the relationship between *FTO* and T2D seems to be mediated by BMI in white European populations, the results reported in this chapter provide evidence of a significant *FTO* and ethnicity interaction effect on insulin resistance, a well known proxy of T2D risk. This genotype effect was independent of obesity-related traits and lifestyle factors ($p$=0.0002). The extent of the effect of the *FTO* risk allele on insulin resistance was 2.04-unit for Mapuches, but no significant association was detected for Europeans. This finding is in agreement with several recently published studies that reported a significant association between *FTO* variants and risk of T2D in populations of no white European backgrounds. Several studies in South Asian populations have reported a significantly increased risk of T2D independent of obesity-related traits in subjects that carried two copies of the *FTO* risk allele, with an odds ratio ranging between 1.18 and 1.46 (Rees et al. 2011b; Sanghera et al. 2008). Similarly T2D risk has been reported for Chinese and other Asian populations (OR: 1.18 - 1.30) independent of BMI (Liu et al. 2010a; Liu et al. 2010c; Ng et al. 2008; Xi et al. 2011b; Yajnik et al. 2009b). To date, just one study has reported a significant association between *FTO* and insulin resistance, measured by $HOMA_{IR}$; this study of 1514 Japanese found a 0.74-unit of $HOMA_{IR}$ increase for each extra copy of the risk allele, and a genotype effect size of 0.39-unit increase in insulin secretion per allele. Both associations were independent of age, sex, and obesity-related factors (Shimaoka et al. 2010b). This could suggest that the mechanism by which *FTO* influences insulin resistance and increased T2D risk are not explained in a straight forward manner by increasing adiposity. However, the design of this study does not allow conclusions to be drawn about why *FTO* influences diabetes risk in populations of non-white European background.

International guidelines recommend a physically active lifestyle, indicating that increasing time spent in moderate to vigorous PA could reduce the risk of obesity

and other cardiovascular disease, such as T2D (Gill and Malkova 2006b; O'Donovan et al. 2010; Barwell et al. 2008a). However, no recommendations have been focused on reducing time spent in sedentary risk behaviours. Previously in this section was described an effect of the *FTO* gene on obesity traits and insulin resistance. However, genetically susceptible individuals could respond in different ways depending on their exposure to different lifestyle factors. These suggest that PA could modulate the genetic effect of *FTO* on obesity-related traits. Although we did not find evidence of an *FTO*, ethnicity and PA interaction effect on obesity, we observed that sedentary behaviours strengthen the extent of the *FTO* effect on BMI and body fat, but not for waist circumference, after accounting for several confounding factors; *i.e.* the effect size per copy of the risk allele for BMI and body fat for individuals with higher time spent in sedentary activities (tertile 3) was 1.86-fold and 1.58-fold higher, respectively compared to the less inactive ones (tertile 1).

Increasing time spent in MVPA is the major recommendation in public health to decrease the risk of obesity and cardiovascular disease (Pate et al. 1995a). We examined whether time spent in MVPA could modulate the genetic susceptibility to obesity. Although no significant *FTO*\*Ethnicity\*MVPA interactions were found, an attenuation in the genotype effect on body fat but not BMI or waist circumference was observed. Those individuals that spent less time in MVPA activities showed a 1.40-fold increase in the genotype effect size for body fat compared to more active individuals (tertile 3). This association became borderline significant after accounting for confounding factors, highlighting the need to account effectively for other confounding factors when estimating the real strength of genotype effects on obesity-related traits. Cardiorespiratory fitness showed similar attenuation of the effect of the *FTO* genotype on body fat and BMI but not for waist circumference, independent of confounding factors. The genotype effect size in those individual with lower fitness showed a 1.20-fold increase in BMI and 1.60-fold increase in body fat, respectively compared to the fitter group.

The results provided in this section results are in agreement with the majority of studies that have investigated *FTO* and lifestyle interactions. These studies showed a similar effect of PA on the association between *FTO* gene variant and BMI as our study (Andreasen et al. 2008f; Sonestedt et al. 2010b; Vimaleswaran et al. 2009b;

Rampersaud et al. 2008d). A large ethnically homogenous population-based cohort (~20,300) from the EPIC-Norfolk study found a significant interaction between the *FTO* rs1121980 variant and self-reported PA (*p*=0.004), where the BMI increase per risk allele was more pronounced (69%) in physically inactive individuals (0.44-unit) compared to active individuals (0.25-unit). This study has been the only one that also reported a similar increase in the *FTO* effect on waist circumference (62%) in inactive (1.04-cm per allele) versus active individuals (0.64-cm per allele). Another study, reported by Andreasen et al, in middle aged Danes also found an interaction between the *FTO* rs9939609 variant and self-reported PA (*p*=0.007), where the BMI increase per risk allele was strengthened 4.1-fold in those more inactive individuals (1.95-unit) than active individuals (0.47-unit). Likewise, a study of 704 old Order Amish (Rampersaud et al. 2008c) found an interaction (*p*=0.01) between the *FTO* variant (rs1861868) and PA, where the BMI increase per risk allele was more pronounced (3.7-fold) in individuals within the lower half of the PA distribution (1.12-unit) than individuals in the upper half of the PA distribution (0.30-unit). A recent gene and PA interaction study in 21,675 apparently healthy Caucasian women found a significant 2.3-fold increase in the effect of the *FTO* (rs8050136) gene on BMI for inactive versus active women. In this study, each risk allele was associated with a rise in BMI of 0.73-unit among inactive women ( , 8.8 MET-hours/week), compared with 0.31-unit among active women (>8.8 MET-hours/week) (Ahmad et al. 2011b). In contrast, other studies in adult populations have failed to show evidence of an interaction between *FTO* variants and the environment (Cornes et al. 2009; Holzapfel et al. 2010).

The findings provided for this thesis revealed a novel effect of physical activity-related lifestyle factors on the relationship between *FTO* and insulin resistance for the Mapuche population. Previously, an independent and highly significant *FTO* and ethnicity interaction effect on IR for the Mapuche population was described. Although no evidence for an *FTO*, ethnicity and PA interaction were found, the analyses revealed that higher levels of PA substantially attenuated the effect of *FTO* on IR, and that increasing time spent in sedentary behaviours increased this association for Mapuches but not for Chilean Europeans. In Mapuches, highly sedentary individuals (upper tertile) showed an approximately 5.6-fold increase in the genotype effect size on IR compared to the less sedentary ones (lower tertile).

Conversely, increasing time spent in MVPA (upper tertile) diminished the *FTO* genotype effect on IR by 72% compared to less active Mapuches (lower tertile). While, increasing fitness levels, substantially reduced the effect of the *FTO* genotype on IR by ~96% compared to more unfit Mapuches (lower tertile). Considering that cross-sectional nature of this study, it is not possible to draw a dose-effect conclusion. However, it could be speculated that adopting an active lifestyle could reduce the *FTO* effect on diabetes risk in populations of non-white European backgrounds.

Despite recent progress, the mechanisms by which SNPs in *FTO* influence human body mass remain elusive. Multiple processes could plausibly contribute to the risk of obesity, including neurological circuits governing appetite and whole-body energy expenditure, as well as peripheral pathways involved in energy expenditure. Loss-of-*FTO* function appears to reduce fat mass in mice, at least in part, through increased energy expenditure but not decreased energy intake (Church et al. 2009c; Church et al. 2010). However, the studies of intermediate phenotypes in humans showed that *FTO* SNPs are associated with appetite and food intake but not energy expenditure (Haupt et al. 2009; Speakman, Rance, and Johnstone 2008a; Speakman 2010). Interestingly, data from rodent studies suggest that *FTO* might affect neuropeptide Y expression in the hypothalamus, which in turn is known to impact on feeding behaviour (Larder et al. 2011). An investigation of the link between *FTO* SNP expression and neuropeptide levels in the human hypothalamus might therefore provide a mechanism for the modulatory effect of *FTO* SNPs on appetite. At present, the strongest associations between *FTO* SNPs and obesity belong to intronic SNPs; it is possible that fine-mapping the causal variant(s) could shed light on the biological mechanism impacting obesity. However, fine-mapping the association signal might be difficult because the obesity-associated SNPs lie within a 47 kb LD block in which the effects of causal variant(s) could be indistinguishable from others. Under these circumstances it might prove more important to understand the biological effect of the risk haplotype (rather than the causal variants themselves) on genes and pathways. It is clear that the research undertaken by *FTO* has generated many questions as well as answers. Perhaps the most important lesson from the extensive work on *FTO* over the last three years is the promise that other obesity loci identified

by GWAS could lead to a new understanding of the underlying biology of human adiposity.

In conclusion, bearing in mind the cross-sectional nature of this study which does not allow conclusions to be drawn about the causality of these interactions, our findings were: 1) *FTO* rs17817449 influences insulin resistance in Mapuches but not Chileans of European descent. These associations were independent of adiposity-related phenotypes and persist after adjustment for a comprehensive range of social and lifestyle factors; 2) this study also confirmed and expanded the evidence on the influence of *FTO* on obesity-related traits in populations of different ethnic backgrounds in South America. However, these relationships were obscured to some extent when lifestyle factors were taken into account; this should be considered for further research studies that try to address the real genetic effect of *FTO* and its contribution to obesity; 3) the results in this chapter also highlighted the relevance and the key role that physical activity could have in preventing and reducing the genetic predisposition to obesity and insulin resistance; this observation has important public health implications, because this study emphasizes that being physically active can overcome, at least in part, the genetic predisposition to obesity induced by variations in the *FTO* gene; those subjects with higher levels of fitness could substantially reduce the genetic effect of *FTO* on insulin resistance and T2D risk; 4) finally we suggest that further studies into the mechanisms underpinning this effect are needed. This has potential implications both for the design and implementation of lifestyle strategies to reduce metabolic risk in different ethnic groups, and for advancing the basic understanding of the mechanisms underpinning human obesity and insulin resistance.

# 7 General Discussion

Through the chapters of this thesis, a number of research questions have been considered relating to the influence of environment and lifestyle factors on obesity and insulin resistance in populations of two different ethnic backgrounds. First, a validation of a subjective questionnaire-based method to estimate physical activity patterns was undertaken using an objective measurement of physical activity; second, the effect of the environment on metabolic risk markers were determined in Mapuches and Europeans living in rural and urban settings; third, the influence of lifestyle such as diet and physical activity related factors on obesity and insulin resistance were investigated in the two populations; and finally, the effect of the *FTO* gene on obesity, dietary intake factors and insulin resistance was examined. In addition, the influence of lifestyle factors on the association between *FTO* with obesity and insulin resistance was investigated. This thesis focused particularly on an indigenous population that showed an increased risk of obesity, diabetes and related cardio-metabolic phenotypes when exposed to a 'Westernised' environment.

The main findings described in this thesis were: (a) that Mapuches men and women are more insulin resistant (as assessed by $HOMA_{IR}$) than Chilean men and women of European descent; (b) urbanisation appears to have a greater effect on insulin resistance in indigenous Mapuches than Europeans Chileans; (c) increasing adiposity, inactivity levels and/or decreasing fitness and MVPA, had a greater influence on insulin resistance in Mapuches than Europeans; (d) the association between the *FTO* gene and insulin resistance in Mapuches but not European population, this association was independent of obesity-related phenotypes and several confounding factors; (e) the results of this thesis did not find a significant interaction between *FTO* and environment, however the analysis revealed that lifestyle factors (increased fitness or MVPA could attenuate the genetic effect of the *FTO* gene on insulin resistance in Mapuches. Similarly, being physically active decreased the magnitude of the *FTO* genotype effect on adiposity.

In common with developed countries, South America is experiencing a rapid and severe increase in the prevalence of obesity, T2D and cardiovascular disease (Tejero 2010b; Barcelo and Rajpathak 2001b; Barcelo 2006b; Albala, Vio, and Yanez 1997e; Escobedo et al. 2009a; Schargrodsky, Escobar, and Escobar 1998b). During the past three decades, Chile has moved from the bottom to the top three positions in levels of obesity and T2D within the South American population. This could be explained by the rapid epidemiological transition, which could have a significant impact on lifestyle and health profile leading to demographic, socioeconomic, epidemiologic and nutritional changes (Kain, Vio, and Albala 2003; Albala, Vio, and Yanez 1997d). Chile is now a leading country in terms of obesity and T2D rates in South America, with a ~61% prevalence of overweight and obesity. This is accompanied by a substantial increase in the prevalence of T2D, which shifted from 4.2% in 2003 to 9.4% in 2010 (MINSAL 2010). Despite this higher level of obesity and significant increase in T2D, no studies have been published in order to determine the contribution of lifestyle and environmental factors, as well as genetic influences, in the increased prevalence of these two conditions. Regardless of the high proportion of indigenous population in the country (~6% of the ~18 million total population) and the higher number of people with mixed backgrounds between aboriginal and populations of white Europeans descent (Aprox. 45% of the total population) (INE 2002b), there is a lack of information concerning ethnicity and health in Chile. This highlights the relevance of this study that aimed to elucidate the contribution of lifestyle and other environmental factors, and the interplay between genes and lifestyle on obesity and T2D in Chilean population of different ethnic backgrounds.

It is clear that urbanisation is a key feature in the rise of diabetes and cardiovascular disease (Hossain, Kawar, and El Nahas 2007; Leal and Chaix 2010). Chile in the last decades has significantly increased the urbanisation of the country; by 2002 around ~86% of the population was living in urban environment, of those ~87% were European and 64% were Mapuches (INE 2002a; INE 2002b). However it has been estimated that by 2012, more than 95% of the population will live in an urban setting (INE 2002a). This urbanisation process could have important health implications and changes in the epidemiology of the non-communicable diseases such as obesity and T2D. These health implications of urbanisation are supported by the results shown in this thesis, where urbanisation appears to have a greater effect on insulin resistance

in Mapuches than European Chileans, with a significant ethnicity x environment interaction for this effect in both men and women. It is not unreasonable to hypothesise that this increase could be due to changes in lifestyle factors such as physical activity or changes in dietary intake patterns. However, the relationship between environment and ethnicity on insulin resistance was independent of MVPA, sedentary time and nutritional factors, suggesting that maybe environment captures other potential risk factors associated with insulin resistance in an urban setting which our study was not able to measure or quantify. This finding is in agreement with a growing body of evidence that suggests that rural areas of developing countries have low levels of insulin resistance and prevalence of T2D, but that these levels rapidly increase with increasing urbanization or modernisation. This is the case in rural Bangladesh where diabetes increased from 2.3% to 6.8% in 5 years (Rahim et al. 2007). An additional example of this is the drastic increase in the prevalence of diabetes in India, a developing country, where the prevalence of diabetes has increased from 5.2% in 1984 to 14.3% in 2003 due to urbanization (Mohan et al. 2006; Ramachandran et al. 2001). A similar study that compared cardiovascular risk factors between people living in a city, town and periurban villages (PUVs) in India, showed that the prevalence of diabetes was lower in people who lived in PUVs (9.2%) compared to those who lived in the city or town (18.6% and 16.4%, respectively). This study reported a similar finding to our results, showing that the shift from the PUVs (or traditional environment) to the urban city was associated with an increase of 84% in the risk of diabetes; similarly moving from the PUVs to the towns was associated with a 38% increased risk of diabetes (Ramachandran et al. 2008c). However, compare prevalence of T2D between different periods of time has some limitations. The main one is the use of different T2D diagnosis criteria; this could explain why some countries shows such a drastic increase in prevalence of T2D. Additionally, this drastic response of the Mapuches to a westernised environment is consistent with the pattern observed in Pima Indians and other ethnic groups that also show a T2D susceptibility associated with the shift of environments. It has recently been reported that Pima Indians in the USA with normal glucose tolerance have four-fold higher $HOMA_{IR}$ values than Mexican Pimas (Esparza-Romero et al. 2010c). In addition, US Pima Indians have been shown to be more insulin resistant than age- and adiposity-matched Americans of European descent (Stefan et al. 2004b). In the present study the greater insulin resistance in the

urban, compared to rural, Mapuche occurred in the absence of a higher BMI, although percentage body fat was greater in the urban Mapuche group. However, although percentage body fat was greater in the urban compared to rural groups in the present study, the extent of the difference did not differ between the Mapuche and Chileans of European descent, indicating that increasing adiposity, in itself, could not explain the disproportionately large increase in insulin resistance associated with urbanisation in the Mapuche population.

Urbanisation has been related to the adoption of a westernized lifestyle, which at the same time is linked to the increased prevalence of obesity worldwide (WHO 2006d). WHO's latest projections indicate that globally in 2008, the prevalence of overweight adults was ~1.5 billion, of these, more than 200 million men and nearly 300 million women were obese (WHO 2006d). This accounts for one quarter of the world's total population being at increased risk for developing cardiovascular disease and diabetes. The projections for the year 2015 expect the prevalence of overweight adults to be as high as 2.3 billion and that of obese adults to be ~700 million (WHO 2006d). Public health statistics show that westernised countries have high levels of obesity; in Europe, Scotland has one of the highest prevalences, with the proportion of adults classified as either overweight or obese increasing markedly between 1995 and 2003 (from 55.6 to 64.0 % for men; 47.2 to 57.3 % for women) (Hirani 2003). Interestingly, the obesity epidemiological change is not that different in developing countries like Chile. The 2010 Chilean Health Survey shows that the prevalence of overweight and obesity in the adult Chilean population was 64.3% and 64.6% for women and men, respectively. The prevalence of central obesity was also drastically higher for both sexes (~33%) (MINSAL 2010). Considering this epidemiological evidence, and the strong link between obesity and insulin resistance, additional to the recent and fast demographic and nutritional transitions of the Chilean population, it is relevant to determine which environmental factors could contribute to this high prevalence of obesity in Chilean population. Through this thesis subcomponents of dietary intake (total energy, fat and carbohydrates intake) have been positively associated with BMI, but not waist circumference or percentage of body fat. These findings are in agreement with previous cross-sectional observational studies of free-living adult populations that have shown a positive and independent association between dietary energy intake and obesity as measured by BMI in populations of

different ethnic backgrounds (Mendoza, Drewnowski, and Christakis 2007c; Howarth et al. 2006a; Stookey 2001b; Murakami et al. 2007b). Findings from prospective studies of the association between dietary energy density and weight or BMI change have been contradictory; some report a significant and positive association (Vergnaud et al. 2009b; Savage, Marini, and Birch 2008b; Bes-Rastrollo et al. 2008b) but not all (Iqbal, Helge, and Heitmann 2006a; Du et al. 2009a). These discrepancies in the association between diet intake and obesity could perhaps be explained partly by the underreporting of dietary intake, bias associated with the questionnaires and data collection methods or to heterogeneous dietary patterns across food cultures.

Another factor that could be modulated by the urbanisation is physical activity and fitness. Several studies have been conducted in relation to physical activity and health, these provide evidence that high levels of physical activity (Gill and Cooper 2008) and cardiorespiratory fitness (Wei et al. 1999e) are protective against T2D (Gill and Malkova 2006a). Krista and collaborators have shown that low levels of self-reported physical activity, or low cardiorespiratory fitness are associated with insulin resistance in other aboriginal groups (Kriska et al. 2001a). The findings in this thesis are consistent with these observations, showing that the effects of a low level of moderate-to-vigorous physical activity or low level of cardiorespiratory fitness was associated to an increase in insulin resistance, this increase was greater in the Mapuche population, than the European Chileans population. Similarly previous studies, have shown that MVPA is inversely associated with insulin resistance in European Chileans, Native American, African-American and Caucasian women (Irwin et al. 2000; Arteaga et al. 2010). This protective effect of physical activity (MVPA or light intensities PA) against insulin resistance levels is clinically relevant, since fasting insulin levels as well as insulin resistance are strong predictors of T2D and cardiovascular disease in populations with different ethnic backgrounds (Bunt et al. 2007; Weyer et al. 2001). This becomes even more relevant, if we remember that the prevalence of T2D is higher among ethnic minorities, particularly among native Americans such as Pimas Indians and Mapuches (Gilliland et al. 1997; Harjo et al. 2011; Carrasco et al. 2004c). The potential health impact of increased physical activity on reducing diabetes risk in ethnic minorities is substantial. In studies with Pima Indians (a population that as Mapuches shows an increased T2D risk) a

diabetes prevalence of 70% has been reported (Charles, Eschwege, and Bennett 1997), Kriska *et al.* have shown among the 1,054 Pima Indians who reported 2.5 h per week of leisure PA in the previous year, the prevalence of diabetes was reduced by 32% compared with those reporting less activity (Kriska et al. 1993). Manson *et al.* also showed that vigorous PA was associated with a lower risk of diabetes among Caucasian women. In their sample of ~87,000 women aged 34–59 years enrolled in the Nurses Health Study, those who engaged in vigorous physical activity at least once per week had a 33% reduction in diabetes risk compared with women who did not exercise weekly (Manson et al. 1991).

On the other hand, urbanization could also be related to the adoption of sedentary behaviours. Physical inactivity has been strongly related with detrimental effects on health. The Chilean Health Survey (2010) reported a higher level of sedentary behaviour in the Chilean adult population, the prevalence of inactivity was 84% and 93% for men and women respectively; this indicates that overall ~89% of the population did not exercise in their leisure time more than 150-min per week. Despite, the high prevalence of inactivity in Chile, no studies have been conducted to determine the association between sedentary behaviours and markers of metabolic health in this population. This thesis provides new evidence related to the harmful association between physical inactivity and insulin resistance, revealing that the effects on insulin resistance of a high level of sedentary behaviour, was greater in the Mapuche than the European Chileans. However, both ethnic groups spent similar proportions of their day in sedentary activities, Mapuches spent ~8.6 hours and Europeans ~8.7 hours in sedentary behaviours, this remains with a similar magnitude if environment is taken into account. However, moving from the lowest to the highest tertile for sedentary time was associated with a 4-5 fold increase in insulin resistance in Chilean men and women of European descent, but a 9-10 fold increase in Mapuche men and women. These findings provide extra evidence to the importance of sedentary behaviours and their implication on health, particularly in the Mapuche population, by using objective measures of sedentary time. To date, emerging epidemiological evidence strongly supports the harmful implications that sedentary lifestyle has on the risk of developing cardiovascular disease, diabetes and other vascular and metabolic disorders (Thorp et al. 2010; Hu et al. 2003a; Hu et al. 2001a). Taking into consideration the apparent T2D susceptibility of ethnic

minorities, the potential impact of increasingly inactive lifestyles could have serious clinical implications, attributable to sedentary behaviours. However, most of the attention to prevent the increase of physical activity related disorders is focused on increasing time spent in moderate to vigorous physical activity, but not in reduce time spent in sedentary activity, even considering that prolonged periods of inactivity and absence of whole body movements, is distinctly related to risk of chronic disease independent of physical activity levels (Healy et al. 2008c; Healy et al. 2008k; Dunstan et al. 2004a; Dunstan et al. 2005; Hamilton, Hamilton, and Zderic 2007a).

In this thesis several evidence have been provided in relation to how lifestyle factors contribute to the rapid increase in obesity and T2D in two Chilean populations of different ethnic backgrounds. It is clear that this drastic increase in obesity and T2D during the past five decades must be ascribed to changes in environment and lifestyle factors rather than genes, as the genetic background has not changed during this period. However, the genetic background determines how we respond in a specific environment. Evidence of gene-lifestyle interactions in the development of obesity and T2D was first provided by descriptive epidemiological studies such as migration studies that compared the risk of disease between genetically related populations who have adopted different lifestyles. The best and clearest illustration of gene-environment interaction is the comparison of risk in obesity and T2D in Pima Indian native America populations Pimas living in the obesogenic environments of U.S. Arizona, showed a prevalence of 69% for obesity and 55% for T2D, while those living in traditional environments of the remote Mexican Sierra Madre Mountains reported a prevalence of 13% of obesity with only 6% having T2D. Our study result showed a similar feature of the contribution of genetic components in the increased risk of T2D in Mapuches, who are more susceptible to develop T2D in an obesogenic environment than their counterparts living in the traditional rural environment (~8% vs. ~1%) (Larenas et al. 1985a; Carrasco et al. 2004b). However, the obesogenic environment influences are not reflected in adiposity-related phenotypes. These finding show that despite a similar genetic predisposition different lifestyles result in a different prevalences of obesity and T2D. White Americans in U.S living in a similar obesogenic environment but who have a different genetic background are much less susceptible to developing obesity (~32%) and T2D (~8%) compared with the Pimas Indians living in Arizona (Ravussin et al. 1994b; Ravussin 1993b; Esparza

et al. 2000b), similar, is the evidence between the risk of T2D in Chilean Europeans (~5%) compared to Mapuches indigenous living in urban setting (Carrasco et al. 2004a; Perez-Bravo et al. 2001a).

Through this study we examined how genes, specifically *FTO* contribute to obesity and insulin resistance, additionally it was explored how lifestyle factors could modulate the relationship between *FTO* and the two conditions mentioned above. The association between *FTO* SNPs and BMI and the risk of being overweight or obese has been clearly confirmed in multiple ethnic populations (Fawcett and Barroso 2010e; Loos and Bouchard 2008b; Vimaleswaran and Loos 2010; Loos 2009a). This thesis confirmed and extended these previous associations between *FTO* and obesity-related traits for a South American population. Previous studies have reported similar and modest *FTO* effects on BMI, showing a diverse effect across different ethnic populations showing an increased effect by ~0.3 to ~1.1-units (Wing et al. 2009a; Hotta et al. 2008; Cha et al. 2008d; Chang et al. 2008d; Lee et al. 2010e; Dina et al. 2007a; Fawcett and Barroso 2010f; Sonestedt et al. 2009a; Frayling et al. 2007a; Tabara et al. 2009b; Tan et al. 2008a). The association between *FTO* and obesity in the population studied in this thesis shows a higher genotype effect size (BMI: 1.40-unit increase per allele) when counfounder factors were not taken it into account, than those reported previouslly in the literature (BMI: ~0.3 to ~1.1-units). However after adjustment for several confounding factors the assocaiton between *FTO* and obesity was similar to those reported for previous studies (BMI: 0.80 unit increase per allele). This highlights the need to account effectively for other predictor factors when estimating the strenght of genotyping effects on obesity-related traits.

GWAS for T2D identified for first time the *FTO* gene in a cluster of common SNPs in the first intron of *FTO* showing a strong association with T2D. After adjusting for BMI, the association with T2D was completely abolished, suggesting that the *FTO* and T2D association – was mediated through BMI (Frayling 2007e; Frayling et al. 2007e). However, recent studies in other ethnic groups have revealed a genetic effect of the *FTO* gene on T2D risk, independent of obesity-related phenotypes. One of the main findings of our study was related to a robust significant *FTO* and ethnicity interaction effect on insulin resistance ($p$=0.0002), a well known proxy of T2D risk:

interestingly this association was independent of obesity-related traits and lifestyle factors. The unadjusted extent of the effect of the *FTO* genotype on insulin resistance was ~1.08 units for the Mapuche population, but no significant association was detected for Europeans. However, after accounting for several lifestyle factors and other covariates the genotype size effect was reduced by ~32% (to 0.73 units per allele). This finding is in agreement with a recent study conducted in a Japanese population were *FTO* was associated with insulin resistance, reporting a similar genotype effect (0.74 unit per allele) (Shimaoka et al. 2010a). Additionally, there is emerging evidence that reported a significant association between *FTO* variants and risk of T2D in population of non-white European background. Several studies in South Asian populations have reported a significant increased risk of T2D independent of obesity-related traits in those subjects that carry two copies of the *FTO* risk allele, with an odds ratio ranging between and 1.18 and 1.46 (Rees et al. 2011a; Sanghera et al. 2008; Yajnik et al. 2009a). Similar T2D risk has been reported for Chinese and other Asian populations (OR: 1.18 – 1.30) independent of BMI (Liu et al. 2010b; Liu, Liu, and He 2011; Xi et al. 2011a).

It is well recognized that that a western lifestyle is one of the major driving forces behind the obesity and T2D epidemic. However, genetically susceptible individuals as we describe in the previous paragraph, could respond in a different way to an obesogenic environment compared to those genetically protected. As we described before, sedentary time appears to be one of the major factors behind obesity and T2D. Taking this into account this thesis examined the interplay between *FTO* and sedentary behaviours and its influences in obesity-traits and insulin resistance. Our study did not show evidence of an *FTO*-ethnicity-physical activity interaction effect on obesity traits or insulin resistance. However, we observed that the genetic effects of *FTO* on obesity traits (BMI and body fat) was strengthened by increasing time spent in sedentary behaviours. The genotype size effect on obesity (BMI, body fat) for those individuals more inactive (upper tertile) was 1.58 and 1.86-fold higher, respectively compared to the less inactive ones (lower tertile). An opposite effect was found for MVPA, the genotype effect size on obesity was attenuated by increasing time spent in MVPA. The magnitude of the genotype effect on body fat was reduced by 28.7% in the more active individuals compared to less active ones (upper vs lower tertile). The findings described previously agree with those few studies that have

investigated the effect of *FTO* and lifestyle interaction on obesity. These studies showed a similar effect of PA on the association between *FTO* gene variant and BMI (Sonestedt et al. 2010a; Vimaleswaran et al. 2009a; Andreasen et al. 2008g; Rampersaud et al. 2008b). A large ethnically homogenous population-based cohort (~20,300) from the EPIC-Norfolk study found a significant interaction between *FTO* rs1121980 variant and self-reported PA ($p = 0.004$), where the BMI increase per risk allele was more pronounced (69%) in physically inactive individuals (0.44 unit) compared to active individuals (0.25 unit). This study has been the only one that also reported a similar increase of the *FTO* effect on waist circumference (62%) in inactive (1.04 cm per allele) versus active individuals (0.64 cm per allele). Another study, reported by Andreasen et al, in middle aged Danes also found an interaction between the *FTO* rs9939609 variant and self-reported PA ($p = 0.007$), where the BMI increase per risk allele was strengthened by 4.1-fold on those more inactive individuals (1.95 units) than active individuals (0.47 units). Similarly, a study of 704 old Order Amish (Rampersaud et al. 2008a) found and interaction ($p = 0.01$) between the *FTO* variant (rs1861868) and physical activity, where the BMI increased per risk allele where more pronounced (3.7-fold) in individuals within the lower half of the physical activity distribution (1.12 units) than individuals in the upper half of the PA distribution (0.30 units). A recent gene and physical activity interaction study in apparently healthy 21,675 Caucasian women increased the evidence that support a significant increase by 2.3-fold of the effect of the *FTO* (rs8050136) gene on BMI for inactive versus active women. In this study, each risk allele was associated with a rise in BMI by 0.73 units among inactive women ( 8.8 MET-hours/week), compared with 0.31 units among active women (> 8.8 MET-hours/week) (Ahmad et al. 2011a).

Additionally, this thesis revealed a novel finding on the effect of physical activity related factors, such as sedentary behaviours, MVPA and fitness on the relationship between *FTO* and insulin resistance for the Mapuche population. Highlighting that physical activity substantially attenuated the genetic effect of *FTO* genotype on insulin resistance, at the same time that increasing time spent in sedentary behaviours strengthens this association. Those individuals who spent the most time sedentary (tertile 3) showed a 5.6-fold increase in the genotype effect size on insulin resistance compared to those who spent the least time in sedentary activities (tertile 1) for

Mapuches. Similarly, increasing time spent in MVPA was associated with a reduction of ~96% in the genotype size effect on insulin resistance when less active individuals were compared to more active ones.

A major challenge is the translation of this new knowledge into public health and clinical practice. The recent discoveries of genetic variants that predispose to different complex diseases has raised hopes of the development of genetic risk profile that, based on the obesity-T2D-susceptibility variants, would predict early in life who will be at risk of developing obesity or diabetes. However, effect sizes, risk and predication - despite strongly significant association and consistency and repeated replication - of each of the recently identified loci on BMI and T2D risk has been small. Furthermore, as illustrated in the analyses in Chapter 6, the real effect sizes are likely to be smaller than reported due to the fact that most of the genetics analyses did not fully account for confounding factors, and thus could give a misleading impression of the real genetic effect of *FTO* in the development of obesity and T2D. One of the advantages of the present study is that rigorous adjustment for several confounding factors revealed the real effect of the genetic variants. As discovery is the first aim of the genome-wide association studies, significance of the association and replication has been the main focus. The replication of previous associations is relevant for a better understanding of the role of the susceptibility loci towards variation in obesity and T2D.

Despite the evidence of high heritability of T2D (50 – 70%) and obesity (40 – 80%) that come from the higher concordance of this two conditions in monozygotic twins studies (Vimaleswaran and Loos 2010). Recent genetic association studies are beginning to explain some of this heritability, as they have identified a number of SNPs associated with an increased risk of T2D and obesity. However, these SNPs only account for ~10% and ~1% of the heritability of T2D and obesity, respectively (McCarthy 2008). Although it is well established that the risk of an individual developing obesity or T2D depends on the interaction between their genotype and lifestyle factors (such as an energy-rich diet and sedentary behaviours), the increase in obesity and T2D over the past two decades has occurred too rapidly to be explained solely by such factors. There is now substantial evidence that the fetal and early postnatal environments strongly influence the risk of developing obesity and

T2D, and that altered epigenetic regulation of specific genes. (Gluckman, Hanson, and Low 2011). The term epigenetic refers to the process that induces heritability changes in gene expression without altering the gene sequence. Alteration in the epigenetic regulation of genes, may lead to profound changes in phenotype. Godfrey *et al*. investigated how epigenetic mechanism operate in humans (Godfrey, Gluckman, and Hanson 2010a). By extracting DNA from the umbilical cord tissue of two cohorts of unselected children who were well phenotyped in childhood, they have were able to correlate a number of methylation changes in the promoter of biologically plausible genes, to a range of phenotypes in childhood. For example, they have demonstrated that methylation in one specific cluster of CpGs within the promoter of *RXRA,* which encodes a transcription factor implicated in fat metabolism and insulin sensitivity correlates with body adiposity, as measured by imaging at age 6 or 9 years in two independent cohorts (Godfrey, Gluckman, and Hanson 2010b). The predictive effects of methylation of *RXRA* explained over 20% of the variance in body composition, an effect greater than any known genetic polymorphism. The methylation at this site was in turn strongly associated with maternal carbohydrate intake during early pregnancy. This domain of research investigating how differences in neonatal human epigenotype may be linked to a functional outcome later in life is still in its infancy. However, this suggests that the developmental component of phenotype determination, is very important in humans, and provides a mechanistic proposal to explain the variable risk of individuals living in an obesogenic world. This epigenetic theory could be a potential explanation of the drastic increase in T2D and CVD in population of non-white European origin, whom have migrated recently from their traditional environment to an obesogenic setting. This may explain why Mapuches sharing a similar western environment and lifestyle with Europeans, showed an increased risk of obesity and T2D.

The prevalence of the risk allele in the population is another important determinant of their contribution toward public health. For example, the frequency of the *FTO* risk allele is high in Europeans; ~63% carry at least 1 copy of the risk allele and ~16% are homozygous for the risk allele. As such the population attributable risk (PAR) of the *FTO* locus for obesity was estimated at ~20% (Frayling et al. 2007d). This could imply from a public health perspective that at least 20% of the obese cases could be prevented if the negative effect of the *FTO* risk allele were attenuated.

More valuable risk estimates for clinical practice could be derived from prospective cohort studies. For example a 9-year follow up study that examined the influence of the *FTO* loci found that each additional risk allele increase the incidence of obesity by 24% (Cauchi et al. 2009).

The results reported through this thesis have several strengths and weakness that must be considered. Data collection in this study presented a number of logistical challenges. The rural Mapuches generally live in remote areas far from road access or mains electricity. Data collection from some of these villages required transport of all equipment (including generators to provide electricity and centrifuges to spin blood) by horseback riding from site to site (this collection trips were on average 3 - 6 hours by horseback). Given these issues, the study is relatively large, with the final sample including 247 Mapuches and 225 European Chileans. A particular strength of the study is the detailed phenotype data of the populations, particularly with respect to lifestyle measures. Fitness, physical activity and sedentary time were objectively measured, providing greater validity in these measures than would be obtained from self-report questionnaire (Shephard 2003a). Samuels *et al*. suggested that diagnostic error rates of just a few percent have such a detrimental effect on power that massive increases in sample size would be required to offset their adverse effects (Samuels, Burn, and Chinnery 2009a). The authors therefore concluded that the accuracy of phenotyping is a major factor limiting the ability of genetic association studies to identify novel genes in complex traits (Samuels, Burn, and Chinnery 2009b). This could explain why our study with a small sample size was able to detect interaction effect between *FTO* and ethnicity in the development of obesity and insulin resistance. Additionally, another potential limitation may be related to the power of the study, and in particular to detect interactions and the potential increased effect of type 1 and type 2 error when multiple test have been conducted and a p-value <0.05 was used to define statistical significance. The chance of false positive and false negative could appear when the p-value defining statistical significance is not corrected for multiple testing correction factor. However, the main finding of this study shows strong evidence of significance and the most relevant results were far from a nominal or borderline significant p-value.

Some of the weaknesses of this study were: dietary intake, energy intake was objectively measured by 7-day weighed food records – the best available method. Although underreporting is a common problem in the measurement of dietary intake, this does not appear to have been a major factor here as reported energy intakes (means of ~2900 kcal per day in men and ~2500 kcal per day in women) were relatively high. Due to the data collection challenges and sample size, we used $HOMA_{IR}$, rather than a euglycaemic clamp or intra-venous glucose tolerance test, to assess insulin sensitivity. It will be important to seek verification that our conclusions remain valid when insulin sensitivity is assessed using more sophisticated techniques. Additionally, a higher insulin and $HOMA_{IR}$ variability were observed in Mapuches living in urban environment compared to the remainder groups, however, sensitivity analysis were conducted to exclude any potential outliers in the data that could affect the results and conclusion of this study. Moreover, those variables with a skewed distribution were logarithmically transformed to bring the data into a normal distribution, thus giving enough confidentce to believe that the results are not related to outliers confounding effects. Due to cultural, political and ethnic differences between the recruited groups, a potential bias could be present in our recruitment process. However, the collection of detailed socioeconomic and cultural characteristic allows us to check and control for bias related to recruitment process.

Another limitation of the present study is related to multiple testing corrections. Several statistical tests were performed in this thesis, and the significant threshold accepted was not corrected for multiple testing ($p < 0.05$). However, the robustness evidence of the main finding presented in this thesis, suggests that the main finding will remain significant after corrections. One of the main finding described in this thesis was related to physical activity patterns, however an important number of cases with physical activity measurement where excluded. The author of the thesis believe that the bias coming from this exclusion criteria are minor compared to bias that could come from a more flexible inclusion criteria. The inclusion of those cases with less than 10 hours of data recorder per day or less than 4 days of physical activity data per week, could lead to a less accurate estimation and characterisation of physical activity patterns in the population. Several confounding factors were not measured in this study, most of them related to time of migration from rural to urban settings, birth weight or more detailed information related to parental and family

history of diseases. This information should be included in further studies that aim to elucidate the effect of environment on cardio metabolic-related diseases.

The main findings described in this thesis are relevant, but the findings may not be translated into new public health policies or the design of obesity and type 2 diabetes prevention strategies in Chilean population yet. Since this study is cross-sectional in nature; it is not possible to draw firm conclusions about the causality of associations observed. A large scale randomised controlled trial is needed to address this definitively. However, our comprehensive study design, with relatively precise measures of a number of relevant exposure variables, allowed us to control for a number of potential confounding variables in our analyses. Although potential confounding effects cannot be excluded, the robustness of our findings to these adjustments supports our conclusions.

In conclusion the main findings presented in this thesis are as follow:

(a) Urbanisation, adiposity, physical activity and sedentary behaviours influence insulin resistance to a greater extent in the Chilean Mapuches than in Chileans of European descent. These associations persist after adjustment for a comprehensive range of potential confounding factors.

(b) *FTO* genotype influences insulin resistance in the Mapuche but not European Chilean population. These associations persist after adjustment for a comprehensive range of potential confounding factors.

(c) *FTO* influences obesity-related traits in Chilean population. However, these relationships were not all independent of lifestyle factors and these factors should therefore be taken into account in future research studies, which try to address the real genetic effect of *FTO* and its contribution to obesity.

(d) That physical activity plays a key role in modulating the genetic predisposition to obesity and insulin resistance. This observation has important public health implications, because the present data indicate that being physically active can

overcome, at least in part, the genetic predisposition to obesity induced by variation in the *FTO* gene.

(e) That further studies into the mechanisms underpinning the previous effects are needed. This has potential implications both on the design as well as on the implementation of lifestyle strategies to reduce metabolic risk in different ethnic groups, and for advancing the basic understanding of the mechanisms behind human obesity and insulin resistance.

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# 9 Appendices

Appendix A: Subject Information Sheet

Appendix B: Consent Form

Appendix C: Health Screening Questionnaire

Appendix D: Socio-economic Questionnaire

Appendix E: Physical Activity Questionnaire (IPAQ)

Appendix F: Dietary Intake Diary

Apendix G: Sensitivity Analysis for outliers

# 9.1 Appendix A: Subject Information Sheet

## Influence of genetic background and lifestyle on risk factors for cardio-metabolic diseases in Chilean populations of different ethnic origin

### Investigators

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You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

**What is the purpose of the study?**

Diabetes is a disorder in which levels of sugar in the blood are higher than they should be. This can have serious effects on other aspects of health and is an important public health problem in Chile. Research into the causes of late-onset diabetes is therefore important as it may be able to help us to prevent the disease. It is well known that people from some ethnic groups have a greater risk of developing late-onset diabetes than those from other ethnic groups. This is likely to be due to a combination of genetic factors and lifestyle factors (*i.e.* what we eat, how much activity we do and how much we weigh). The purpose of this study is to investigate the differences between people of different ethnic backgrounds in different environments in relation to these risks of developing diabetes, obesity and other related conditions. We shall do this by studying four groups of subjects - Chileans of European ethnic origin and of Mapuche/Peuenche ethnic origin living in both rural and urban environments.

**Who are organising this study?**

This study is managed by four Institutions: The lead institution is the Faculty of Biomedical and Life Sciences at the University of Glasgow, UK; other institutions involved are the Institute of Nutrition and Food Technologies at University of Chile and the Pharmacy Faculty at the University of Concepcion, Chile. All these centres have extensive research

programmes into the causes and prevention of late-onset diabetes, obesity and heart disease.

**Why have I been chosen?**

We are recruiting volunteers in each of four different groups and we have tried to recruit a random sample of adults aged between 20 and 55, with both parents of either European, Mapuche origin. You are suitable for inclusion in one of these four groups based on what you have told us about your ethnic background and on where you live.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

**What will happen if I take part in the study?**

If you agree to help us with this research, you will visit our research Unit at the University of Concepcion. There will be two visits, each lasting 2-3 hours, separated by ~1 week. On each occasion we will ask you to give us information and ask you to take part in a set of tests.

On the first visit, we shall:

- *Ask about your diet and other lifestyle patterns:* you will complete questionnaires relating to your medical history, physical activity level and background.
- *Measure your body composition:* Your height and weight will be measured, followed by measurements around your waist and hips. Then we shall measure the thickness of the skin and fat on your arms, your back and your hips/side (a measure of the amount of fat that you carry). These measurements will take about 25 minutes and cause no discomfort. For this set of measurements you must dress in light clothes to facilitate taking the measurements with as little disturbance to you as possible.
- *Measure your blood pressure*
- *Measure your fitness level:* Your fitness will be measured using the 'Chester Step Test'. This involves you stepping on to and off a low step at a rate set by a metronome or music beat tape for a maximum of 5, 2-minute stages. This test is not maximal and will stop if your heart rate reaches >85% of your age-predicted maximum heart rate.

Between Visits, you will be asked to:

- *Wear a movement and heartbeat measuring device*: This is called an 'ActiTrainer' and when worn for continuous periods provide a good measure of your physical activity level. The ActiTrainer comprises a box about the size of a mobile phone

which you wear around your waist and an elastic strap which you wear around your chest. We ask you to wear this at all times when you are awake (except for when showering/bathing etc) for 7 days.

- ***Keep a food diary***: This will involve you weighing all of the food that you eat for 3 days (two weekdays and one weekend day) and recording this in diary. It is important to our study that you do this accurately, so please remember to record everything and not to change your diet from what you normally eat.

On the second visit you will:

- **Return your ActiTrainer and food diary.**

- **Have a glucose tolerance test**: We will ask you to come to the laboratory after an overnight fast and drink a sugary drink. We will take a blood sample from a vein in your arm before the drink and another blood sample two hours after the drink to assess how your body deals will this sugar. We will take approximately 30 ml (about two tablespoons) of blood over the course of this test, which will help us to determine how well the insulin in your body is working. We will also measure levels of other substances in these blood samples such as the amount of fat and cholesterol in your blood, and the amounts of certain substances which are produced by the liver and fat tissue and released into the bloodstream which are relevant to risk of diabetes, heart disease and related conditions. In addition, if you agree to this on the consent form, part of the blood sample will be stored for potential future use in a bigger study we are planning that will look into genetic factors that influence risk of diabetes, obesity, heart disease and other related conditions. Any future use of your samples will require us to obtain further approval from a research ethics committee and genetic analysis will be performed in such a way that the results will not be directly traceable to you. If you would prefer that your samples are not used in this future research, please indicate this on the consent form and we will destroy your samples after analysis for the present study is completed.

**What are the possible disadvantages and risks of taking part?**

- The exercise test will be at a sub-maximal level and there is a small possibility that certain changes may occur to you during or shortly after the test. They include high or low blood pressure, fainting or a change in the normal rhythm of the heartbeat. Any such effects are likely to be mild and short-lived.
- Blood sampling via the cannula may cause minor bruising, an inflammation of the vein or haematoma (a small accumulation of blood under the skin). Good practice, however, minimises this risk. Some people may feel faint when they give blood but this usually passes quickly.

**What are the possible benefits of taking part?**

The information gained during the study will allow us to give you detailed feedback about your fitness level, body fat, dietary intake, blood pressure, cholesterol, blood sugar and level of "insulin resistance". In addition, the knowledge gained from your participation may benefit people with diabetes in the future by increasing our understanding of how genetic background and environment influence diabetes and other metabolic risk factors in Chilean populations. It will also help guide future research investigating how to reduce this increased risk through lifestyle changes and may also help in the development of treatments for diseases like heart disease and diabetes.

**What if something goes wrong?**

The chance of something going wrong is extremely small. All of the procedures involved in this study are low risk. In the unlikely event that you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it.

**Will my taking part in this study be kept confidential?**

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves Santiago, Concepcion, Temuco and Glasgow Universities will have your name and address removed so that you cannot be recognised from it.

**What will happen to the results of the research study?**

When we have completed our studies, we shall publish the findings in a relevant research journal. In any such publications, we shall not reveal the identity of any of our volunteer participants and you will not be able to be recognised from any data in the publication. Copies of papers published can be obtained from Carlos Celis if you are interested, but they may not be written in language that is easy for the non-scientist to understand. It may be some time before the papers are published and made available to the researchers.

**Who is organising and funding the research?**

This Research Proposal will be carried out by the University of Concepcion, Chile, the University of Chile, Chile and the Institute of Biomedical and Life Sciences at the University of Glasgow, UK. All funding comes from charitable or government sponsored sources and none of the researchers has a vested interest in the outcome of the research.

**What will happen to my samples after the study has finished?**

The blood samples that you provide for this study may be useful for future research into the prevention and treatment of diabetes and cardio-metabolic disease and will be kept stored in our freezers if you indicate your consent form that you give your permission for this. Any use

of your samples for future research will require further approval from a Research Ethics Committee and samples will be analysed in such a way that the results will not be directly traceable to you.  If you do not wish your samples to be used for future research, please indicate this on the consent form. If you do not give permission for storage of samples for future use, the samples you have given will be destroyed once the analysis for this study is complete, and in any case no later than 5 years after they have been given.

**Who has reviewed the study?**

This study has been reviewed and approved by the Medical School Ethics Committee at University of Concepcion, Ethical Committee at University of Chile and by the Institute of Biomedical and Life Sciences Ethics Committee at Glasgow University, UK.

**I might like to volunteer, what I do now?**

If you are interested in volunteering for this study, please complete the attached "Consent Form". The information you provide is confidential.

**Contact for Further Information**

You may ask any questions you like now or at any time about your rights as a participant in a research study or about the research study itself.  Mr. Carlos Celis Morales will be available to discuss these issues with you if you phone him on 07985 117900 or E-mail  c.celis-morales.1@research.gla.ac.uk.

**You will be given a copy of this information sheet and a copy of the signed consent form to keep for your records.**

**It is important that you think carefully about whether you are able to commit your time to the study schedule before you volunteer, because the all of the information collected at each visit and between visits is essential for this study.**

## 9.2 Appendix B: Consent Form

**SUBJECT IDENTIFICATION CODE** ☐☐☐☐☐☐☐☐☐☐

### CONSENT FORM

Project: "Influence of genetic background and lifestyle on risk factors for cardio-metabolic diseases in Chilean populations of different ethnic origin"

Name of Researcher taking consent: _____

**Please initial box**

1. I confirm that I have read and understand the Information sheet dated September 2007 for the above study and have had the opportunity to ask questions.

   Yes | No

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

   Yes | No

3. I agree to take part in the above study.

   Yes | No

4. I agree for my samples to be stored for future research into the prevention and treatment of diabetes and heart disease.

   Yes | No

_____     _____     _____
Name of Patient                Date             Signature

_____     _____     _____
Researcher                     Date             Signature

Copies: 1 for patient; 1 for researcher.

## 9.3 Appendix C: Health Screening Questionnaire

**SUBJECT IDENTIFICATION CODE** [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

Please complete this brief questionnaire about your current and previous health:

**At present, do you have any health problem for which you are:**

    (a) on medication, prescribed or otherwise yes [   ]      no [   ]

    (b) attending your general practitioner      yes [   ]      no [   ]

    (c) on a hospital waiting list      yes [   ]      no [   ]

**In the past two years, have you had any illness which required you to:**

    *(a) Consult your family doctor*      *yes [   ]*      *no [   ]*

    *(b) Attend a hospital outpatient department*      *yes [   ]*      *no [   ]*

    *(c) Be admitted to hospital*      *yes [   ]*      *no [   ]*

**Have you ever had any of the following?**

    *(a) Convulsions/epilepsy*      *yes [   ]*      *no [   ]*

    *(b) Asthma*      *yes [   ]*      *no [   ]*

    *(c) Eczema*      *yes [   ]*      *no [   ]*

    *(d) Diabetes*      *yes [   ]*      *no [   ]*

    *(e) A blood disorder*      *yes [   ]*      *no [   ]*

    *(f) Head injury*      *yes [   ]*      *no [   ]*

    *(g) Digestive problems*      *yes [   ]*      *no [   ]*

    *(h) Hearing problems*      *yes [   ]*      *no [   ]*

*(i)  Problems with bones or joints*                yes [    ]        no [    ]

*(j)  Disturbance of balance/co-ordination*        yes [    ]        no [    ]

*(k)  Numbness in hands or feet*                    yes [    ]        no [    ]

*(l)  Disturbance of vision*                            yes [    ]        no [    ]

*(m) Thyroid problems*                              yes [    ]        no [    ]

*(n)  Kidney or liver problems*                      yes [    ]        no [    ]

*(o)  Chest pain or heart problems*                     yes [    ]        no [    ]

*(p)  Any other health problems*                    yes [    ]        no [    ]

4.  **Have any of your family (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins) ever had any of the following?** (if yes please give details including age of first diagnosis)

*(a)  Any heart problems*                                yes [    ]        no [    ]

*(b)  Diabetes*                                      yes [    ]        no [    ]

*(c)  Stroke*                                            yes [    ]        no [    ]

*(d)  Any other family illnesses*                    yes [    ]        no [    ]

5.  **Do you currently smoke?**                      yes [    ]        no [    ]

*If not, have you ever smoked?*                      yes [    ]        no [    ]

If you still smoke, how much do you currently smoke? ………………………..

If you used to smoke, for how long did you smoke and when did you stop?.................

If YES to any question, please describe briefly if you wish (*e.g.* to confirm whether problem was short-lived, insignificant or well controlled.) (Use a separate sheet if necessary)

.....................................................................................................

...............................................................................................................................................

...............................................................................................................................................

....................................................…......

Name and address of family doctor (or primary health care provider)

……………………………………………………………………………………………………

……………………………………………………………………………………………………

……………………………………………………………………………………………………

…………….……………………………………………………………………………………

……………………

Blood pressure measured at screening

Systolic Pressure…………………..mm Hg

Diastolic Pressure…………………..mm Hg

# 9.4 Appendix D: Socio-economic Questionnaire

## SOCIO-ECONOMIC QUESTIONNAIRE

SUBJECT IDENTIFICATION CODE ☐☐☐☐☐☐☐☐☐

|  |  |  | **O** | **O** |
|---|---|---|---|---|
| Date of Birth | Age | Current Address | Urban | Rural |
|  |  |  | Zone | |

**1** What is your Ethnic origin?

- ☐ Mapuche
- ☐ Pehuenche
- ☐ European
- ☐ Other ethnicity
- ☐ Unknown

**2** What is the ethnic origin of your parents?

**Mother**
- ☐ Mapuche
- ☐ Pehuenche
- ☐ European
- ☐ Other ethnicity
- ☐ Unknown

**Father**
- ☐ Mapuche
- ☐ Pehuenche
- ☐ European
- ☐ Other ethnicity
- ☐ Unknown

**3** How long have you been living at your present address?

☐ years    (If it is more than 2 years, skip to question 5)

**4** What was your previous address and how long did you live there?

|  | **O** | **O** |  |
|---|---|---|---|
| Past Address | Urban | Rural | years |
|  | Zone | | |

**5** How many people normally live with you?

☐ Adults    ☐ Children (under 18 years old)

**6** What is the highest grade of education you have attended?
- ☐ Primary school (complete)
- ☐ Primary school (incomplete)
- ☐ Secondary School (complete)
- ☐ Secondary School (incomplete)
- ☐ Technical education (complete)
- ☐ Technical education (incomplete)
- ☐ University (undergraduate) (complete)
- ☐ University (undergraduate) (incomplete)
- ☐ University (Postgraduate) (complete)
- ☐ University (Postgraduate) (incomplete)
- ☐ None    (if never had attended skip to question 9-10)
- ☐ Other _____

**7** What age do you completed your education? ☐

**8** Number of years of formal education? (primary, secondary, university etc.) ☐

**9** Do you know how to write?
- ☐ yes Spanish
- ☐ yes Mapudungun
- ☐ yes other language _____
- ☐ No

**10** Do you know how to read?
- ☐ yes Spanish
- ☐ yes Mapudungun
- ☐ yes other language _____
- ☐ No

**11** What is the highest grade of education that the main income earner of your home have achieved?

| | | | |
|---|---|---|---|
| ☐ | Primary school (complete) | ☐ | University (undergraduate) (complete) |
| ☐ | Primary school (incomplete) | ☐ | University (undergraduate) (incomplete) |
| ☐ | Secondary School (complete) | ☐ | University (Postgraduate) (complete) |
| ☐ | Secondary School (incomplete) | ☐ | University (Postgraduate) (incomplete) |
| ☐ | Technical education (complete) | ☐ | None |
| ☐ | Technical education (incomplete) | ☐ | Other _____ |

**12** Do you have a job?
   ☐ yes
   ☐ No (If, NO skip to question 14)

**13** How many job do you have? ☐

**14** If, you don't have a job please indicate reasons?
   ☐ Unemployed (if, yes answer the question 15)
   ☐ Disability _____
   ☐ other reason _____

**15** How long have you been unemployed?
   ☐ Months

**16** What kind of job do you have?
   ☐ Employed professional
   ☐ General management, director or top management with responsibility for 5 employees or less
   ☐ General management, director or top management with responsibility for 6 employees or less
   ☐ Middle management, other management with responsibility for 5 employees or less
   ☐ Business proprietor, owner (full/partner) of company OR owner of a shop, craftsman, and other self employed person with responsibility for 6 employees or more
   ☐ Employed position, working mainly at desk
   ☐ Business proprietor, owner of company or owner of a shop, craftsman, and other self employed person with responsibility for 5 employees or less
   ☐ Student
   ☐ Employed non-manual position, not at a desk but travelling or in a service job
   ☐ Farmer&Fisherman
   ☐ Responsible for ordinary shopping and looking after the home, housewife
   ☐ Supervisor&skilled manual worker
   ☐ Other (unskilled) manual worker, servant
   ☐ Retired or unable to work through illness, unemployment or temporarily not working.

**17** Which of the following categories represent your total level of monthly income ?   (Chilean currency)

| | | | | | |
|---|---|---|---|---|---|
| ☐ | Less than $100.000 | ☐ | Between 200.000 - 300.000 | ☐ | Between 1.000.000 - 1.300.000 |
| ☐ | Between 100.000 - 120.000 | ☐ | Between 300.000 - 400.000 | ☐ | Between 1.300.000 a 2.000.000 |
| ☐ | Between 120.000-150.000 | ☐ | Between 400.000 - 700.000 | ☐ | Between 2.000.000 - 3.000.000 |
| ☐ | Between 150.000 - 200.000 | ☐ | Between 700.000 - 1.000.000 | ☐ | Between 3.000.000 - 6.000.000 |
| | | | | ☐ | More than 6.000.000 |

**18** Which of the following categories represent the total level of monthly income of your home ?   (Chilean currency)

| | | | | | |
|---|---|---|---|---|---|
| ☐ | Less than $100.000 | ☐ | Between 200.000 - 300.000 | ☐ | Between 1.000.000 - 1.300.000 |
| ☐ | Between 100.000 - 120.000 | ☐ | Between 300.000 - 400.000 | ☐ | Between 1.300.000 a 2.000.000 |
| ☐ | Between 120.000-150.000 | ☐ | Between 400.000 - 700.000 | ☐ | Between 2.000.000 - 3.000.000 |
| ☐ | Between 150.000 - 200.000 | ☐ | Between 700.000 - 1.000.000 | ☐ | Between 3.000.000 - 6.000.000 |
| | | | | ☐ | More than 6.000.000 |

**19** Normally, How many people depend on this total income? ☐

**20** How many people do that financially contribute to the household's income? ☐

| | Relationship |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |

**21** What is the occupation of the main income earner (M.I.E) in the household?

- [ ] General management, director or top management with responsibility for 6 employees or more
- [ ] Self-employed professional
- [ ] Employed professional
- [ ] General management, director or top management with responsibility for 5 employees or less
- [ ]  General management, director or top management with responsibility for 6 employees or less
- [ ] Middle management, other management with responsibility for 5 employees or less
- [ ] Business proprietor, owner (full/partner) of company OR owner of a shop, craftsman, and other self employed person with responsibility for 6 employees or more
- [ ] Employed position, working mainly at desk
- [ ] Business proprietor, owner of company or owner of a shop, craftsman, and other self employed person with responsibility for 5 employees or less
- [ ] Student
- [ ] Employed non-manual position, not at a desk but travelling or in a service job
- [ ] Farmer&Fisherman
- [ ] Responsible for ordinary shopping and looking after the home, housewife
- [ ] Supervisor&skilled manual worker
- [ ] Other (unskilled) manual worker, servant
- [ ] Retired or unable to work through illness, unemployment or temporarily not working.

**22** Do you or your family get any of the following services at your home or where you are living currently?

| | | | |
|---|---|---|---|
| [ ] Electricity | [ ] TV cable | [ ] PC or laptop | [ ] Micro Oven |
| [ ] Public Water system | [ ] TV colour | [ ] Internet connection | [ ] Refrigerator |
| [ ] Natural Water system | [ ] Phone-Mobile | [ ] film camera | [ ] heating system |
| [ ] Shower | [ ] Automobile | [ ] Washing machine | |

**notes:**

# 9.5 Appendix E: Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

LONG LAST 7 DAYS FORMAT (**SELF-ADMINISTERED**)

**SUBJECT IDENTIFICATION CODE**

**Date** _____

## *Instruction*

*The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items.*

*The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.*

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

### 9.5.1.1.1 PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1.      Do you currently have a job or do any unpaid work outside your home?

             Yes

             No      ➡️ *Skip to PART 2: TRANSPORTATION*

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2.　　During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

　　　　\_\_\_\_\_ **days per week**

　　　　☐　No vigorous job-related physical activity

3.How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

\_\_\_\_\_ **hours per day**

\_\_\_\_\_ **minutes per day**

4.Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

　　　　\_\_\_\_\_ **days per week**

　　　　☐　No moderate job-related physical activity

5.　　How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

　　　　\_\_\_\_\_ **hours per day**

　　　　\_\_\_\_\_ **minutes per day**

6.During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

　　　　\_\_\_\_\_ **days per week**

　　　　☐　No job-related walking

7.How much time did you usually spend on one of those days **walking** as part of your work?

　　　　\_\_\_\_\_ **hours per day**

　　　　\_\_\_\_\_ **minutes per day**

## PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8.      During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

\_\_\_\_\_   **days per week**

☐   No traveling in a motor vehicle

9.      How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

\_\_\_\_\_   **hours per day**

\_\_\_\_\_   **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10.      During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

\_\_\_\_\_   **days per week**

☐   No bicycling from place to place          ***Skip to question 12***

11.      How much time did you usually spend on one of those days to **bicycle** from place to place?

\_\_\_\_\_   **hours per day**

\_\_\_\_\_   **minutes per day**

12.      During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

\_\_\_\_\_   **days per week**

☐   No walking from place to place          ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

*13.      How much time did you usually spend on one of those days walking from place to place?*

\_\_\_\_\_   **hours per day**

\_\_\_\_\_   **minutes per day**

### PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home     *Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY*

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

## PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20.        Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____     **days per week**

☐     No walking in leisure time

21.        How much time did you usually spend on one of those days **walking** in your leisure time?

_____     **hours per day**

_____     **minutes per day**

22.        Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____     **days per week**

☐     No vigorous activity in leisure time              ***Skip to question 24***

23.        How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____     **hours per day**

_____     **minutes per day**

24.        Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____     **days per week**

☐     No moderate activity in leisure time              ***Skip to PART 5: TIME SPENT SITTING***

25.        How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____     **hours per day**

_____     **minutes per day**

### PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**

_____ **minutes per day**

**This is the end of the questionnaire, thank you for participating.**

# 9.6 Appendix F: Dietary Intake Diary

## FOOD INTAKE RECORD FORM

### INSTRUCTIONS

Please record your normal diet for three days. These days need not be consecutive but should include two weekdays and one weekend day.

Please (i) start a separate page for each day.
(ii) start a separate line for each item.

<u>Column 1</u>
Record meal and time and place of eating.

<u>Column 2</u>
Describe each item as accurately as possible, stating where relevant:
(i) type and brand
(ii) whether food is fresh, dried, canned, frozen, salted, smoked, etc.
(iii) whether food is cooked, if so give method of cooking *e.g.* fried, baked, etc.

<u>Column 3</u>
Record the weight of each item after cooking:
(i) place scales on a level surface
(ii) place plate or container on top of scales
(iii) press 'ON/Reset' button to turn on scales
(iv) once zero appears, add first item of food
(v) record weight displayed
(vi) press reset button before weighing next item
Wherever possible, record weights in grams. If this is not possible, record weights in household measures (*e.g.* sugar or jam in teaspoons, stating whether level, rounded, or heaped).

<u>Column 4</u>
Record the weight of any leftovers, such as food remaining on plate, weight of container in which food has been weighed, apple cores, etc.

<u>Columns 5 and 6</u>
Please leave blank.

If food consists of several items, please list each on a separate line *i.e.* instead of writing 'one cheese sandwich', record separately the weights of bread margarine, cheese, etc.

Please remember to record all drinks, as well as food, giving weights where possible, or volumes if these are known. Record separately the weights of added milk and sugar.

An example is shown overleaf.

# Food Inventory - Example

**SUBJECT IDENTIFICATION CODE**

Date _____

| 1. Time/Place | 2. Description of food/drink | 3. Weight of food/drink (g) | 4. Weight of container/ leftovers (g) | Leave Blank | |
|---|---|---|---|---|---|
| Breakfast | Cornflakes (Kelloggs) | 28 | | | |
| 8:30am | Soprole milk | 48 | | | |
| Home | Bread (Mothers Pride, large white sliced, toasted) | 76 | | | |
| | Flora margarine | 7 | | | |
| | Robinsons lemon marmalade | 12 | | | |
| | Coffee (instant) | 2 | | | |
| | Milk (whole pasteurised) | 10 | | | |
| | | | | | |
| Lunch | Cheese (Cheddar) | 55 | | | |
| 1:00pm | Bread (white, crusty) | 76 | | | |
| Pub | Butter | 4 | | | |
| | Chutney (2 teaspoons) | | | | |
| | | | | | |
| Snack | Coffee (instant) | 2 | | | |
| 3:30pm | Coffee-mate | 6 | | | |
| Office | Mars Bar | 35 | | | |
| | Apple | 76 | 8 (core) | | |
| | | | | | |
| Dinner | Turkey Fillet (frozen, grilled) | 102 | | | |
| 6:30pm | Potatoes, old, boiled | 320 | 74 | | |
| Home | | | (leftover) | | |
| | Peas (Birds Eye, frozen, boiled) | 50 | | | |
| | Heinz tomato ketchup | 14 | | | |
| | Yoghurt (Ski strawberry thick and creamy) | 162 | 10 | | |
| | | | (carton) | | |
| | Coffee, filter | 148 | | | |
| | Milk (soprole virtually fat-free) | 8 | | | |
| | | | | | |
| Snack | Banana | 107 | | | |
| 7:45pm | Orange Tango (can) | 330 | | | |

# Food Inventory

**SUBJECT IDENTIFICATION CODE**

**Date _____**

| 1.<br>Time/Place | 2.<br>Description of food/drink | 3. Weight of<br>food/drink<br>(g) | 4. Weight of<br>container/<br>leftovers (g) | Leave<br>Blank | |
|---|---|---|---|---|---|
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# 9.7 Appendix G: Sensitive Analysis for outliers (Dot Plot) of Insulin and HOMA$_{IR}$ by Ethnic and Environment

**Fig. 1-G. Dot plot for insulin and HOMAIR by ethnic and environment**. Graph (a) and (c) showed the raw data for insulin and HOMA$_{IR}$. The logarithmic transformer data are shown in graph (b) and (d).