PROTOCOL

A Prospective Cohort Study to Track Changing Lifestyles, Risk Factors and Chronic Disease in Urban and Rural Areas of:

Argentina, Bangladesh, Brazil, Canada, Chile, China, Colombia, Ecuador, India, Iran, Kazakhstan, Kyrgyzstan, Malaysia, Pakistan, Palestine, Peru, Philippines, Poland, Russia, Saudi Arabia, South Africa, Sweden, Tanzania, Turkey, United Arab Emirates, Uruguay, Zimbabwe

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1.0 Introduction

For most populations, the last century has witnessed dramatic improvements in health. Life expectancy has increased from a global average of 46 years in 1950 to 66 years in 1998[1.] During the 20th century, developed countries (early industrializers) experienced rapid declines in deaths from infections and childhood diseases, and marked increases in chronic diseases such as obesity, diabetes and cardiovascular disease (CVD) the latter peaking around 1960-1980[2,3] The health transition in developed countries was associated with industrialization, mechanization, urbanization and economic development[3,4] and these changes were accompanied by improved living standards and infrastructure, water and sanitation, economic growth, nutrition, and immunization. More recently, the epidemic of chronic diseases has shifted from the high income countries (HIC) to developing countries (late industrializers). It is expected that by 2020, >80% of global CVD will be in low and middle income countries (LIC and MIC)[5-7] with the bulk being in countries that are undergoing rapid industrialization and urbanization. Simultaneously, CVD is expected to continue to be a leading disease burden in HICs. The INTERHEART study involving 15,152 myocardial infarction (MI) cases and 14,820 controls from 52 countries documents that 9 individual level risk factors (smoking, ApoB/ApoA-1 ratio, hypertension, diabetes, obesity, psychosocial factors, lack of daily fruits and vegetables, physical inactivity and lack of alcohol consumption) were associated with over 90% of the population attributable risk (PAR) of MI globally, and in every region of the world[8,9.] 99% of subjects had at least one risk factor, suggesting that these risk factors are ubiquitous in urban societies worldwide. Therefore, CVD, obesity and diabetes are likely primary diseases of societies (**sick populations**), within which some individuals (**sick people**) develop disease. This indicates that there is an urgent need to understand why risk factors develop in <u>both</u> individuals and populations, and requires at least 2 lines of investigation. First, given that the 9 risk factors identified in INTERHEART (IH-9) account for >90% of the risk of MI[8], modifying them utilizing <u>individualized</u> interventions (drugs or lifestyle) has been proposed[10]. This concept (the polypill)[10] is being investigated by us in the international multicentre HOPE-3 clinical trial (http://www.phri.ca). However, such an approach is unlikely to be cost-efficient or practical at a societal level as individualized “therapies” are expensive and raises the spectre of “medicalization of entire populations”. A complementary approach may be to shift the entire distribution of risk factors in a population, by societal interventions and policies. However, prior to such interventions which may meet some social resistance, a detailed knowledge of the factors that cause populations as a whole to develop elevated risk factors, needs to be elucidated. Such information is currently sparse and this proposal represents an initial step to understanding the societal determinants of biological risk factors and CVD. Given that CVD was rare 100 years ago, but is epidemic now, suggests that CVD may be due to marked societal changes. Therefore, there is an urgent need to understand how changes within and across different societies in LIC, MIC and HIC lead to a variety of health transitions, which affect biologic risk factors, behaviours, and CVD. At present, it is not clear whether the societal factors are “deterministic” (i.e. always leads to a specific impact) or “contingent” (i.e. contextual). By documenting societal factors and consequent individual adaptive or maladaptive behaviours, the potential exists to develop novel strategies to reduce population levels of risk factors for CVD. We anticipate that some of the lessons will be common across various societies and communities, whereas others may be contextual. Knowledge of both individual and societal factors will lead to a body of information that will allow development of societal interventions adaptable to a range of communities. We

therefore propose a global research program that includes investigation of community level factors (urban-rural differences, built environment, policy environment related to tobacco and food, and social factors), household level factors (family structure, income, housing, etc) and individual level factors (lifestyle behaviours and attitudes, and genetic markers) which lead to the development of the nine risk factors for CVD, and ultimately to CVD (Figs 1 & 2).

2.0 Overarching Hypothesis:

We hypothesize that behaviours (activity and nutrition) and psychosocial transitions secondary to urbanization, industrialization, and changes in the built and commercial environment **(primordial determinants)** are associated with the development of obesity, elevated BP, glucose, abnormal lipid levels and stress. These factors interact with tobacco use, leading to increased CVD. These processes are negatively influenced by low socio-economic status and partially mitigated by adaptive changes that "cushion" the harmful effects of the primordial determinants. This overall hypothesis can be elaborated as involving:

2.1 **Maladaptive Changes**: including i) The physical activity transition due to changes in occupation (more individuals in manufacturing and service jobs, fewer in agricultural jobs), which result in less energy expenditure in utilitarian activities due to automation, and an unfavorable "built environment" leading to weight gain and CVD. ii) Changes in nutrition involving increased energy consumption, decreased use of coarse and unprocessed cereals and an increase in saturated and trans-fats, due to changes in food, and agricultural policies. iii) Psychosocial changes involving breakdown of traditional family supports, pressures of a "faster" urban life, inability to cope, low social capital and/or low perceived control leading to increased depression and adoption of poorer lifestyles leading to higher risk factor levels. iv) Increased tobacco use due to peer pressure, increased psychosocial stressors, advertising and affordability.

2.2 **The Adaptive Processes** potentially mitigating the maladaptive influences include: i) Adaptations in physical activity with increased leisure time activities, favourable societal changes in the "built" environment (within buildings, urban layout) that promote utilitarian physical activity. ii) Adaptations in nutrition at the individual level (increased fruits and fresh vegetables, and fish or n-3 polyunsaturated fatty acids; smaller portion size due to health awareness, etc) and at the societal level (appropriate food policies that affect pricing and availability). iii) Psychosocial adaptations with increased social networking and family support, and other forms of coping. iv) Decreased tobacco use due to tobacco control legislation and its enforcement, and greater health awareness, etc. **The balance of the maladaptive and adaptive processes will likely determine the net impact on risk factors (by affecting behaviours and interactions with genes) and CVD**

Table 1: Percentage of Individuals Living in Urban Settings in 1970 and 2025[2]

Region	1970	1994	2025
World	36.6	44.8	61.1
Developed Countries	67.5	74.4	84.0
Economies in transition*	25.1	37.0	57.0
Developing countries	12.6	21.9	43.5

*Current term for Eastern Europe

Table 2: Data for PURE countries ordered by GDP per capita, summarizing human development index and percent of population in urban areas in 1975, 2003, and 2015.

		Human Development Indices						
		Population (millions) 2003	Life expectancy at birth (yrs) 2003	Adult literacy rate (% 15 yrs and above) 2003	GDP per capita (PPP US$) 2003	% Urban population 1975	2003	2015
High Income Countries (HIC)	**Canada**	31.6	80.0	N/A*	30,677	75.6	80.4	84.0
	Sweden	9.0	80.2	N/A*	26,750	82.7	83.4	84.3
	UAE	4.0	78.0	77.3	22,420	83.6	85.1	87.2
Middle Income Countries (MIC)	**Argentina**	38.0	74.5	97.2	12,106	81.0	90.1	92.2
	S Africa	46.9	48.4	82.4	10,346	48.0	56.9	62.7
	Chile	16.0	77.9	95.7	10,274	78.4	87.0	90.2
	Poland	38.6	74.3	99.7	11,379	55.4	61.9	64.0
	Brazil	181.4	70.5	88.4	7,790	61.2	83.0	88.4
	Thailand	63.1	70.0	92.6	7,595	23.8	32.0	36.7
	Colombia	44.2	72.4	94.2	6,702	60.0	76.4	81.3
	China	1,300.0	71.6	90.9	5,003	17.4	38.6	49.5
	Iran	68.2	70.4	77.0	6,995	45.8	66.6	73.9
	Turkey	71.3	68.7	88.3	6,772	41.6	66.3	71.9
Low Income Countries (LIC)	**Zimbabwe**	12.9	36.9	99.0	2,443	19.6	35.0	41.4
	India	1,070.8	63.3	61.0	2,892	21.3	28.3	32.2

* Not Applicable

3.0 Rationale:

In 1990, CVD were the leading causes of mortality worldwide[5,11]. By 2020, they will also make up the largest contribution to global disease burden. Approximately 80% of the CVD burden will occur in LIC and MIC, but will still remain the leading cause in HIC. The increase in CVD is partially explained by the increased proportion of older adults, but it is

also influenced by broader societal factors (urbanization, industrialization, policies affecting activity, nutrition and tobacco) which change job structures, related efforts, and other daily (utilitarian) activities, diet and psychosocial stressors. Some of these factors may be "adverse" and others "protective", and it is their balance that influences the development of risk factors and CVD. Below we describe a number of transitions that likely influence these processes:

2.1 ***The Urban Transition*** is one of the most dramatic shifts in environment that most populations have experienced in the last century. In 1970, 37% of the world's population lived in urban areas; by 2025 it is projected to be 61%. In HIC, the corresponding projections are 68% and 84%, whereas in LIC and MIC, they are 13% and 44% respectively (Table 1).[2] While urbanization has increased worldwide, its societal and health impact may be heterogeneous. In HIC, urbanization is accompanied by economic growth, planned development of urban infrastructure, and increased spending on social services, education and health care. In poor countries, rapid urbanization is occurring without adequate infrastructure, creating urban slums, and leading to greater socio-economic disparity. Consequently, **urbanization could involve varied societal and health changes in different regions**. For example, urbanization and adoption of a free market economy have resulted in rapid economic growth in China, but an economic downturn and a decrease in per capita income in Russia[12]. Concomitantly, nutritional status and life expectancy improved in China, but both worsened in Russia[12,13]. Although several countries are already highly urbanized (e.g. Canada, Chile, Sweden), their urban societies are also likely to evolve further so that additional adaptive or maladaptive societal and lifestyle changes may occur in these countries over the next few decades. By contrast, other countries will show more rapid or intensive urbanization (e.g**.** China, India) (Table 2). The rates of urbanization, the responses to them and their health effects are likely to vary both between and within countries.

2.1 ***The Nutrition Transition***. The poor are most likely to be obese in developed countries and least likely in poor countries[14]. In MIC, the relationship is more variable. When there is rapid economic group in poor countries, the poor start consuming more calories, and obesity in that group starts to rise[15]. Changes in diet have been attributed to economic growth[15], changes in food production, food policies affecting price and availability.[16] Media influences[17] and industry[18] affect changes in diets. Food policies can markedly affect the relative costs of different foods and consequently its availability and consumption[16]. For example, following economic liberalization in Poland in 1989, removal of subsidies on dairy foods resulted in marked increases in the prices of dairy products and a decline in the relative cost of vegetables and fruits, leading to less meat and increased fruits and vegetable consumption. This was associated with a 20% decline in CVD rates within 5 years[16],[19]. Similar data are not available from other countries, but in general in most societies the cost of energy dense foods is less than that of less dense foods, such as fruits and vegetables[20]. Our preliminary work in India

suggests that the major nutrition shift is an increase in energy consumption in urban areas, with an increase in all types of fats, and a decrease in whole grains and fibre, compared to rural areas[21] However, there is increased consumption of fruits and vegetables in urban areas, which may partially offset some of the adverse dietary changes. Whether these differences observed in one region in India will occur in other regions or countries is unknown.

2.2 ***The Activity Transition: Mechanization and Impact on Physical Activity***. Reduced physical activity and sedentary behaviours have been associated with obesity, diabetes, and CVD in HIC. Reduced activity occurs due to changing job types, increased mechanization at work for the same job and at home, increased sedentary behaviours in leisure time (e.g. time with computers and televisions), less energy expenditure in transportation (increased number of vehicles), all of which lead to markedly reduced energy expenditure during utilitarian activities. All of these changes increase sedentary behaviours and are related to changes in the built environment (i.e. urban sprawl, commuting by mechanized transportation instead of walking, lack of connectivity and land-use mix in residential areas), and influence activity throughout the whole day. Societal changes that increase sedentary behaviours include a shift in jobs from those that expend more energy (e.g. agriculture) to those that expend less energy (e.g. service or manufacturing)[22], increased vehicle ownership, and changes in structure of communities and architecture of buildings, that discourage walking but encourage using energy saving devices (cars, elevators, escalators, etc).[22,23] This results in dramatic declines in energy expenditure, without a complementary increase in leisure time physical activity. While adaptive behaviours, such as even moderate physical activity reduce the risk of CVD in HIC[24] the built environment constrains the overall level of activity that is possible for most individuals, unless there are marked changes in current urban structures. In HIC, factors such as sidewalks, mixed residential and commercial tenants, street connectivity and safety from crime correlate with more physical activity[25]. However, understanding the complex interplay of built environment, changing energy expenditure at work and at home, and changes in leisure time activity, and their relationship to risk factors requires multilevel and multidimensional exploration in a range of diverse and contrasting communities. While some preliminary information relating the built environment to obesity is available from Western countries, very little is known about the built environment and its impact on risk factors and CVD in LIC and MIC.

2.3 ***The Psychosocial and Socioeconomic Transition*** includes changes in psychosocial factors such as anxiety and depression in response to changing life events such as migration to a new environment, new job, and marital discord. The response to changing life events may be modified by inherent characteristics of an individual (coping, perceived lack of control), and by external factors (family structures, networks and cohesiveness; social disparity, services; and family and community support). Depression, perceived lack of control in the home and at work, perceived stress, social isolation, and lack of social support (at individual levels) are related to MI in HIC[26], MIC, and LIC[9], with a surprisingly large PAR of about 30% globally and in most regions (Table 3a & b)[9]. However, international or intercommunity comparisons of psychosocial risk factors have been rare and produced inconsistent results suggesting that the associations between population levels of psychosocial stresses and biologic risk factors for

CVD may differ across countries, perhaps depending on their stage of economic and urban transition. In INTERHEART perceived stress levels were higher in controls from HIC, compared to MIC or LIC; and higher among those at higher than lower income. These curious findings would not be predicted by theories linking psychosocial factors to CVD based on studies in HIC[27,28], so it is important to investigate these issues through international comparisons in prospective studies. Preliminary data from the present Prospective Urban Rural Epidemiology (PURE) pilot study suggests that although life events (business and crop failures) were more common in rural (and poorer) areas, depression and anxiety were paradoxically more common in urban (and richer) areas. (Table 4a). It is also possible that family networks, (common in rural areas), may be more important than social networks, (more common in urban areas). Thus, the patterns of perceived stress and their association with other psychosocial and biologic risk factors for CHD are likely very complex and dependent on country and cultural context. This type of information is important in understanding the factors that may "cushion" against psychosocial stressors and will assist developing novel and culturally appropriate approaches to prevention that can mitigate the adverse impact of increasing stressors during urban transitions.

2.4 ***Variations in Tobacco use and CVD*:** Smoking is one of the two most important risk factors for CVD, but these data are largely from HIC[29]. The INTERHEART study extended these findings to LIC and MIC; and showed that various forms of tobacco use (cigarettes, pipes, beedies and chewing) were harmful, and accounted for a PAR of 44.0% in men and 16.0% in women (36% overall)[8]. Global consumption of cigarettes has steadily risen in the 20th century. While consumption is leveling off and even decreasing in some HIC, it is increasing in LIC and MIC[30]. Even if current patterns of tobacco use persist, the number of tobacco related deaths is likely to be about one billion in the 21st century, compared to about 100 million in the 20th century[31] Of these deaths, about half will be from vascular causes, with the majority occurring in LIC and MIC, but with substantial numbers of deaths in HIC. Changes in tobacco control policy can be expected in coming years as many countries ratify the Framework Convention on Tobacco Control (FCTC). There are several contextual issues regarding tobacco. First, the types of tobacco used vary by region. For example, in South Asia, the commonest form of tobacco (which are one-tenth the price of cigarettes; do not carry health and safety warnings, and are not regulated) and chewing tobacco[32]. Second, societal "norms" that influence tobacco use may vary. For example, chewing tobacco may be socially acceptable in rural societies in men and women, whereas smoking may be considered taboo among women in some urban societies. Conversely, in some rapidly emerging urban societies, smoking, especially in young women, may be a sign of financial independence and newly gained "liberty". Third, both beedies and chewing tobacco are more common in rural areas, where few tobacco control policies are implemented. Fourth, it is likely that declining tobacco use in urban areas will offset the adverse health impact of increases in other risk factors, whereas high tobacco use in rural areas will mitigate some of the benefits of greater activity and healthier diets in LIC and MIC. Fifth, in HIC (e.g. Canada or Sweden), there is a clustering of high rates of tobacco use, with multiple other adverse lifestyles characterized by sedentary behaviours and high fat diets, especially in rural areas[33] or among those of low SES, such that the worst impact of tobacco use may be among rural poor people in HIC.

2.5 ***Interaction Between Societal Transitions and Genetics***: Data from family studies initially suggested that genetic factors may explain up to 50% of the susceptibility to CVD. However, population based studies (including INTERHEART), increasingly suggest that the majority (over 90%) of CVD susceptibility can be explained by nine modifiable risk factors (Table 3a). Therefore, it seems likely that the most important susceptibility genes with regard to population health may be those influencing these risk factors. Data from the PURE pilot studies suggest that transition from rural to urban living is a critical environmental mediator of levels of these risk factors (Table 4). Thus, we hypothesize that the genes with the largest effects on population risk will be those which determine differences in the physiological response of individuals to this environmental transition – which will require the study of **gene-environment interactions**. Although many studies have attempted to study gene-environment interactions in CVD, few robust conclusions have resulted[34-36]. This is for several reasons: 1) their small size, 2) design issues i.e. cross-sectional or retrospective case-control studies, 3) Poor characterization of the environment, and 4) limited variation in the environmental factors studied. For example, studies of gene-environment interactions in Western populations in which the dietary intake is homogenous limits the exploration of how a particular genotype may vary in response to a range of dietary intakes. The same can be said for variability in other environmental factors. Therefore increasing contrasts in environmental exposure increases the potential to discover gene-environment interactions. Although allele frequencies may vary significantly between ethnic groups, the strength of the relationship between genetic variants and disease do not appear to be heterogenous[37] which is consistent with the INTERHEART findings of similar directional impact of risk factors in all ethnic groups. The PURE study will establish a unique cohort suitable for examining whether the interactions between a variety of candidate genes for hypertension, dyslipidemia, diabetes/glucose intolerance, obesity, and CVD with contrasting lifestyles, is of similar strength, and due to the same or different alleles, in different populations. In order to explore gene-environment interactions, at present we only request funding for large-scale DNA collection and storage. This is for a number of reasons. First, it is highly likely that linkage disequilibrium patterns both in candidate genes and throughout the human genome in all the major ethnic groups will be much better characterized in 3 to 5 years than they presently are (e.g. the currently planned genetic analysis in INTERHEART on the stored DNA on 11,000 MI cases and 11,000 controls) which will render genetic analysis more cost-effective at that time[38,39] Second, the approaches for genetic analyses (e.g. haplotype mapping) and whole genome scanning are rapidly evolving and will likely be substantially more cost efficient in 5 years. Third, some years will elapse before sufficient numbers of CVD cases have occurred in the PURE study to permit robust case-control genetic analyses. Thus establishment of a bank of DNA from individuals living in contrasting environments, coupled with extensive information characterizing their environment will provide an efficient approach to providing clear answers on gene-environment interactions.

Table 3a : Risk of AMI associated with risk factors in the overall population

Risk Factor	Prevalence		OR (99% CI) adjusted for age, sex and smoking (OR 1)	PAR (99% CI)	OR (99% CI) adjusted additionally for all other risk factors (OR 2)	PAR 2 (99% CI)
	%Controls	%Cases				
Overall						
Current Smoking	26.76	45.17	2.95(2.72,3.20)	--	2.87(2.58,3.19)	--
Current+Former Smoking	48.12	65.19	2.27 (2.11,2.44)	36.4(33.9, 39.0)	2.04(1.86,2.25)	35.7 (32.5,39.1)
Diabetes	7.52	18.45	3.08(2.77,3.42)	12.3(11.2,13.5)	2.37(2.07,2.71)	9.9 (8.5,11.5)
Hypertension	21.91	39.02	2.48(2.30,2.68)	23.4(21.7,25.1)	1.91(1.74,2.10)	17.9 (15.7,20.4)
Abd Obesity (2 v 1)[&]	33.40	30.21	1.36(1.24,1.48)	--	1.12(1.01,1.25)	--
Abd Obesity (3 v 1)[&]	33.32	46.31	2.22(2.03,2.42)	33.7(30.2, 37.4)	1.62(1.45,1.80)	20.1 (15.3,26.0)
All psychosocial[§]	-	-	2.51(2.15,2.93)	28.8(22.6,35.8)	2.67(2.21,3.22)	32.5 (25.1,40.8)
Veg and fruits daily[†]	42.36	35.79	0.70(0.64,0.77)	12.9(10.0,16.6)	0.70(0.62,0.79)	13.7 (9.9,18.6)
Exercise[†]	19.28	14.27	0.72(0.65,0.79)	25.5(20.1,31.8)	0.86(0.76,0.97)	12.2 (5.5,25.1)
Alcohol Intake[†]	24.45	24.01	0.79(0.73,0.86)	13.9(9.3,20.2)	0.91(0.82,1.02)	6.7 (2.0,20.2)
ApoB/ApoA ratio(2 v 1)[&&]	19.99	14.26	1.47(1.28,1.68)	--	1.42(1.22,1.65)	--
ApoB/ApoA ratio(3 v 1)[&&]	20.02	18.05	2.00 (1.74,2.29)	--	1.84(1.58,2.13)	--
ApoB/ApoA ratio(4 v 1)[&&]	19.99	24.22	2.72 (2.38,3.10)	--	2.41(2.09,2.79)	--
ApoB/ApoA ratio(5 v 1)[&&]	20.00	33.49	3.87 (3.39,4.42)	54.1(49.6,58.6)	3.25(2.81,3.76)	49.2 (43.8,54.5)
All above Risk Factors combined[$]	-	-	129.20* (90.24,184.99)	90.4(88.1,92.4)*	129.20* (90.24,184.99)	90.4(88.1,92.4)*

The model is saturated, so adjusted and unadjusted estimates are identical for "all risk factors". The OR of 129.20 is derived from combining all risk factors together, including current plus former smoking vs never smoking, the top two thirds vs the lower third of abdominal obesity, and the top four fifths vs the bottom fifth of ApoB/ApoA. If however, the model includes only current smoking vs never smoking, the top third versus lower third for abdominal obesity, and the top fifth versus the bottom fifth for ApoB/ApoA, the OR for the combined risk factors increases to 333.7 (99% CI of 230.2 to 483.9).

[†]For protective factors (diet, exercise and alcohol), the PAR are provided for the group without these factors

[§]A model dependent index combining positive exposure to depression, perceived stress at home or work (global stress), low locus of control and major life events, all referenced against non-exposure for all 5 factors. Percent of controls with 4 or 5 factors positive is 22.2% compared to 29.2% in cases.

The median waist to hip ratio was 0.93 in cases and 0.91 in controls ($p<0.0001$), and the median ApoB/ApoA ratio was 0.85 in cases and 0.80 in controls ($p<0.0001$).

OR=Odds Ratio, PAR=Population Attributable Risk, CI=Confidence Intervals

[&]Top and middle third vs lowest third [&&]Second, third, fourth or fifth quintiles v bottom fifth.

Note that the PARs for smoking, abdominal obesity and ApoB/ApoA ratios are based on a comparison of all smokers vs never, upper two thirds vs lowest third, and upper four fifths vs lowest one fifth respectively.

Table 3b: "Population Attributable risk" (etiologic fraction) for myocardial infarction based upon 27,500 individuals from 52 countries (The INTERHEART Study) indicating that 9 simple risk factors predicts about 90% of the PAR globally, and in most regions of the world.

Men and Women

	LIFESTYLE FACTORS					OTHER RISK FACTORS					
Region	**%**	**Fr/vg %**	**Exer %**	**Alcohol %**	**All LS %**	**HTN %**	**Diabetes %**	**Abd Obes %**	**All PS %**	**Lipids %**	**All 9 RF**
W. Europe	29.3 (17.8,44.1)	12.4 (5.2, 26.9)	38.4 (28.3, 49.6)	18.7 (10.3, 31.6)	67.6 (56.1, 77.3)	21.9 (15.6, 30.0)	15.0 (11.0, 20.1)	63.4 (51.3, 74.1)	38.9 (20.2, 61.5)	44.6 (23.5, 67.8)	93.9 (84.6, 97.7)
E/C. Europe	30.2 (23.0,38.6)	10.2 (3.8, 24.8)	11.3 (2.9, 36.3)	12.9 (4.8, 30.4)	49.6 (36.5, 62.7)	24.5 (19.2, 30.6)	9.1 (6.6, 12.4)	28.0 (18.3, 40.3)	4.9 (0.0, 96.0)	35.0 (19.2, 54.9)	72.5 (53.0,86.0)
Middle East	45.5 (39.3,51.9)	7.3 (1.7, 26.8)	4.2 (0.0, 98.0)	-1.0 (-41.7, 39.7)	47.6 (23.3, 73.1)	9.2 (5.7, 14.4)	15.5 (12.2, 19.4)	25.9 (13.7, 43.5)	41.6 (25.2, 60.1)	70.5 (57.8, 80.7)	95.0 (88.2, 98.0)
Africa	38.9 (27.1,52.2)	4.8 (0.2, 62.5)	10.1 (0.3, 78.3)	26.6 (11.4, 50.5)	63.4 (42.9, 80.0)	29.6 (22.8, 37.3)	16.7 (11.8, 23.1)	58.4 (44.1, 71.3)	40.0 (17.3, 67.9)	74.1 (59.7, 84.6)	97.4 (90.7, 99.3)
S. Asia	37.4 (31.6,43.6)	18.3 (9.0,33.8)	27.1 (8.7, 59.4)	-5.5 (-31.2, 20.2)	56.6 (36.8, 74.4)	19.3 (15.7, 23.4)	11.8 (8.9, 15.5)	37.7 (28.8, 47.4)	15.9 (4.0, 46.3)	58.7 (42.7, 73.1)	89.4 (76.9, 95.5)
China	35.9 (31.5,40.5)	18.0 (12.3, 25.4)	20.3 (7.5, 44.2)	5.7 (0.6, 38.3)	62.3 (50.4, 72.9)	221 (18.7, 26.0)	10.0 (8.3, 11.9)	5.5 (1.4, 19.4)	35.4 (24.6, 47.9)	43.8 (36.7, 51.2)	89.9 (84.3, 93.6)
S.E. Asia	36.0 (25.9,47.5)	11.2 (3.9, 28.4)	31.8 (16.3,52.8)	27.7 (15.6, 44.4)	69.9 (56.4, 80.7)	38.4 (33.3, 43.6)	21.0 (16.8, 25.9)	57.9 (49.1, 66.2)	26.7 (17.8, 38.1)	67.7 (52.0, 80.2)	93.7 (84.6, 97.5)
Australia/NZ	44.8 (33.0,57.2)	11.1 (3.8, 28.3)	23.8 (14.0,37.5)	18.6 (10.3, 31.4)	66.0 (54.3, 76.1)	22.6 (15.6, 31.6)	7.2 (3.9, 12.8)	61.3 (45.6, 74.9)	28.9 (10.0, 59.7)	43.4 (16.0, 75.6)	89.5 (68.1, 97.2)
S. America	38.3 (31.0,46.1)	6.6 (1.4, 25.6)	27.6 (14.7, 45.6)	-3.7 (-23.7, 16.3)	56.6 (41.3, 70.7)	32.7 (27.2, 38.8)	12.7 (9.4, 17.0)	45.5 (32.3, 59.5)	35.6 (17.2, 59.5)	47.6 (29.6, 66.2)	89.4 (78.2, 95.2)
N. America	26.1 (8.8,56.5)	19.8 (8.5, 39.6)	25.6 (11.3, 48.1)	25.5 (10.3, 50.6)	59.9 (37.8, 78.6)	19.0 (9.2, 35.2)	8.0 (2.9, 20.1)	59.5 (40.3, 76.2)	51.4 (24.0, 78.0)	50.5 (18.2, 82.4)	98.7 (83.9, 99.9)
Overall 1	36.4 (33.9,39.0)	12.9 (10.6, 16.6)	25.5 (20.1, 31.8)	13.9 (9.3, 20.2)	62.9 (58.5, 67.1)	23.4 (21.7, 25.1)	12.3 (11.2, 13.5)	33.7 (30.2, 37.4)	28.8 (22.6, 35.8)	54.1 (49.6, 58.6)	90.4* (88.1, 92.4)
Overall 2	35.7 (32.5,39.1)	13.7 (9.9, 18.6)	12.2 (5.5, 25.1)	6.7 (2.0, 20.2)	54.6 (47.6, 61.4)	17.9 (15.7, 20.4)	9.9 (8.5, 11.5)	20.1 (15.3, 26.0)	32.5 (25.1, 40.8)	49.2 (438, 54.5)	90.4* (88.1, 92.4)

The PAR estimates in women in some countries is based on small numbers and so it is less reliable.
Smoke=Smoking, FR=Fruits, Vg=Vegetables, Exer=Exercise, LS=Lifestyle, HTN=Hypertension, Abd Obes=Abdominal Obesity, PS=Psychosocial, W=Western, E/C=Eastern, Central, S=South, NZ=New Zealand, N=North, Overall 1=Only adjusted for age, sex, and smoking, Overall 2=Additionally adjusted for all other risk factors
SE Asia includes Japan
*Saturated model, no difference between adjusted and unadjusted models

Table 4a: PURE Pilot Study (n=first 7538) from Bangalore, India demonstrating marked differences in mechanization, diet, risk factors, obesity, diabetes, and CVD

	Urban	Rural
2.3 Level of Mechanization/automation	**2.4**	**2.5**
2.6 Motorised two-wheelers	**2.7 78.2 %**	**2.8 6.8%***
2.9 T.V. ownership	**2.10 98.3%**	**2.11 22.3%***
2.12 Washing machine	**2.13 41.5%**	**2.14 0%***
2.15 Kitchen mixer	**2.16 95.2%**	**2.17 3.7%***
2.18 Refrigerator	**2.19 68%**	**2.20 0.3%***
2.21	**2.22**	**2.23**
2.24 Physical Activity (MET.min/day)[b]	**2.25**	**2.26**
Occupational	454 ± 21.2	1022 ± 14.9 *
Household chores	393 ± 13.3	523 ± 9.3 *
Discretionary exercise	67.2 ± 2.6	1.3 ± 1.8 *
% Sedentary/mildly active	60.1	24.7*
2.27 Psychosocial Factors	**2.28**	**2.29**
2.30 % with 2 or more major stressful life event	**2.31 12.8%**	**2.32 21.1%***
2.33 % depressed	**2.34 18.1%**	**2.35 8.7%***
Risk Factor Prevalence:		
2.36 Anthropometric Indices	**2.37**	**2.38**
BMI (kg/m^2) [b]	25.5 ± 0.14	19.0 ± 0.10 *
% Overweight (BMI > 25)	52.4	5.5*
Waist (cm)	84.7 ± 0.41	67.1 ± 0.30*
Waist-hip ratio [b]	0.87 ± 0.003	0.80 ± 0.002*
Triceps skinfold (mm) [b]	17.6 ± 0.22	10.2 ± 0.16*
Lipids		
LDL cholesterol (mmol/L)	3.1 ± 0.04	2.57 ± 0.04*
HDL cholesterol (mmol/L)	1.06 ± 0.01	1.0 ± 0.01*
Tobacco use		
Men	43.5%	62.2%*

Women[§]	1.9%	18.3%*
Hypertension (history or measured BP >140/90 mm Hg)		
Females	29.1	9.6*
Males	32.1	12.0*
Diabetes (history or measured FBS >126 mg/dl)		
Females	15.2	1.6*
Males	20.4	3.2*
CHD (history or Q-waves on ECG)		
Females	8.2	3.8*
Male	10.5	6.2*

[b] adjusted for age and gender, * P < 0.0001 [§] tobacco use in women is mainly chewing

Table 4b: PURE study extended to 4 other centers in India. Data from 3 of these (n=5415) plus Bangalore (n=7538) indicate that the differences in overweight, hypertension, diabetes, and smoking between urban vs rural sites is variable.

Rates (%)

	Smoking*		BMI>25**		Diabetes**		Hypertension**	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Bangalore (n=7538)	17	37	50	5	17	2	31	10
Tamilnadu (N=1923)	18	28	43	25	23	12	32	27
Rajasthan (N=1859)	16	38	44	7	18	4	48	13
Kerala (N=1633)	20	23	35	30	20	18	28	36

***Adjusted for age and gender**
****Adjusted for age, gender and smoking**

Data from Chandigarh (Punjab) are modest and undergoing quality checks, and therefore are not included.

3.0 RATIONALE FOR A STUDY INVOLVING URBAN AND RURAL POPULATIONS IN DIFFERENT COUNTRIES AT DIFFERENT STAGES OF THE EPIDEMOLOGIC TRANSITION:

While much has been learnt about the causal risk factors for CVD from epidemiologic studies in Western countries, these studies have important limitations. **First**, they have included countries at, or after the peak rates of CVD, thereby providing little direct insights into the relationship of societal transitions to behavioural transitions and the evolution of the CVD epidemic. Therefore, while societal transitions are likely to have played a major role in the current epidemics of obesity, DM and CVD, this is difficult to study in populations that only involve HIC (as there would be few individuals without risk factors, e.g. in INTERHEART which consisted mainly of urban populations, less than 1% had no risk factors). This can be overcome by additionally studying urban-rural contrasts in LIC, MIC (the rural setting in LIC probably parallels the situation regarding causal risk factors in the early 1900's in Western countries at a time when CVD was extremely rare, the urban setting in LIC or MIC paralleling the situation in Western countries in the 1950's and 1960's) and HIC; and in these settings over time. **Second**, studies in HIC have tended to include "homogenous" populations whose population distribution of risk factors is likely to be "abnormal". For example, fat consumption, BMI, LDL-cholesterol and blood pressure are on average **moderately** higher in Western populations compared to urban populations in many LIC and MIC, which in turn is **much** higher than in their rural populations (Fig 3). Therefore, in studies confined to HIC, there may not be many individuals with "desirable" levels of fat intake, BMI, lipids or BP, so that exposure "contrasts" are reduced leading to reduced statistical power. For example, the mean BMI was about 27 kg/m^2 in controls from Western countries in INTERHEART, about 25 kg/m^2 in controls from urban India in INTERHEART, and in the pilot PURE study; and is much lower in rural India (19.6 kg/m^2), with >45% having values below 19 kg/m^2 (which is very rare [<2%] in healthy North Americans). Therefore studies done solely in Western populations would tend to substantially underestimate the strength of the relationship of risk factors to disease, and therefore even "low" (e.g. lowest one quarter) values derived from HIC alone may underestimate the potential for interventions to reduce CHD. For

example, the INTERHEART study showed that the ratio of ApoB/ApoA-1 had a graded log-linear relationship to MI globally across deciles (Fig 4). If we had confined our study to N. America and Western Europe, individuals in the lowest one-fifth from these regions, would have been in the middle of the global distribution, thereby providing a spurious impression of what is "desirable". Thus, under the reasonable assumption that most risk factors have a graded relationship with CVD, and that this relationship holds for most ethnic groups (indicated by INTERHEART), a study confined to a homogeneous population provides limited, or in some cases spurious answers (e.g. "threshold" for lipids claimed in the Framingham Heart Study, which is now widely accepted as being incorrect). **Third**, demonstration of the importance of various factors across different populations, but at different levels of exposures provides both internal replication and external validation. These considerations strongly suggest that an international study (as opposed to one conducted solely within Canada) is preferable in understanding the epidemics of diabetes, obesity, and CVD, globally and in Canada. The current proposal is an **evolution of our work over the last 15 years**. Of these, the most relevant are the INTERHEART study and the pilot results of the PURE study.

4.0 PREVIOUS WORK: INTER-HEART

INTERHEART utilized a case-control design with standardized methods and involved 27,500 subjects from 52 countries, representing every inhabited continent.[8] It included 6,323 Chinese, 4,758 South Asians, 1,945 other Asians, 2,795 Arabs, 2,985 South Americans, 7,052 individuals of European origin, 526 Black Africans and 649 coloured Africans. This study indicated that the association of risk factors to MI was similar in all parts of the world and in all ethnic groups; 54% of the PAR of AMI could be attributed to abnormal lipids (ApoB/ApoA-1), 37% to smoking, 12% to self-reported diabetes, 24% to self-reported hypertension, 34% to abdominal obesity, 13% to dietary factors, 25% to physical inactivity, and 29% to psychosocial factors. **After mutual adjustment, these simple measures accounted for over 90% of the risk of AMI globally and within most regions of the world (Table 3, Fig 4 to 10).** INTERHEART also demonstrated the graded relationship between risk factors (lipids, tobacco use and abdominal obesity) and MI. This implies that substantial modification of these risk factors globally could lead to large (e.g. more than halving) reductions in the rates of premature CVD. However, it would be impractical and unaffordable to lower the levels of these risk factors by individual pharmacologic or lifestyle interventions. Such a strategy confined to "high risk" individuals is also likely to be inadequate because it ignores the large population burden that derives from those with "average" risk factor levels, when the relationship between a risk factor and CVD is graded. Therefore, our future research efforts aim to identify the population level factors that drive the development of known risk factors, so that their distribution in the entire population can be shifted favorably by appropriate societal interventions **(primordial prevention)**. A combination of "treating" the "sick individual" with "elevated" risk factors (for which several intervention trials have been and are continuing to be conducted) along with the prevention of development of "sick populations" (for which there are few data on which to develop an evidence-based policy) is likely to provide **the most balanced, efficient, and effective strategy to reverse the global epidemics of obesity, DM and CVD**. The IH-9 risk factors which predict over 90% of the PAR for CVD, could be used as the "outcome" for assessing the relationship of societal factors in both populations and individuals. Further, the assessment of the relative contributions of individual behaviours and population factors will provide valuable information to guide the development of preventive strategies that incorporates both population and individual level approaches. Of the IH-9, alcohol had

the lowest PAR and promoting alcohol use would be controversial; and some aspects of the psychosocial factors require prospective validation. . Therefore, we will utilize the changes in the 8 risk factors (smoking, fruits and vegetable consumption, activity, lipids, elevated BP and glucose, and a psychosocial score based on life events and depression derived from INTERHEART) as both an outcome measure of the impact of societal factors that affect health transition, as well as a mediator of CVD. (Note that the INTERHEART score is derived from multiple ethnic groups and incorporates several risk factors that are not included in previous risk scores, such as the Framingham Score)(Details of the score are in Appendix 1-4a)

5.0 Main Objectives:

1). To assess the relative contributions of societal influences [as measured by each of the 4 domains of interest (built environment, nutrition, policy, psychosocial/socioeconomic factors and tobacco)] on individual lifestyle choices (diet, activity) and on risk factor levels (INTERHEART score) obesity, DM and CVD (cross-sectional component). **2).** To determine if societal changes in the above 4 domains over time affect behaviours, and risk factor levels. It is likely that over time many rural and urban communities will change their characteristics (types of employment, transportation, diet, attitudes, psychosocial factors and built environment), promoting adaptive or maladaptive behaviours (cohort and repeated cross-sectional components). Relating these societal changes to changes in rates of obesity and risk factors in a community will provide further evidence of causality.

6.0 Methods:

We will establish cohorts in multiple urban and rural communities in 15 countries to track changing environments, societal influences on lifestyle, risk factors and CVD, utilizing periodic standardized data collection (Table 5 & 6) involving a total of about 135,500 individuals. These countries are drawn from every major region of the world, and include HIC, MIC and LIC. This study will be conducted in three phases:

6.1 **A Vanguard phase** (12 months) during which all aspects of the protocol would be further refined and tailored to each country (in addition to a common core), final training and standardization of methods will occur, and at least 1000 subjects will be recruited from each country, plus additional 5000 from India. In parallel, we will fully develop our approaches to measuring societal level variables in each country. At the end of this phase, common societal factors across the 4 domains of interest would also be identified using cross sectional data from the first 30,000 individuals and an assessment will be made whether an integrated health transition index can be developed and whether it is superior to each individual component.

6.2 **Recruitment phase:** (3 yrs) during which an additional 110,000 individuals will be recruited and will undergo detailed baseline assessments (Tables 7 and 8). During this phase, the initial cross-sectional analysis using baseline data will be initiated.

6.3 **Follow-up phase:** (additional 8 to 12 yrs) consists of follow-up of all subjects every 3 years, for up to 12 years, to document clinical events (cohort component). In a random sample of all individuals (35 to 70 years) from each community (average of 20%, proportion from each community varies depends on

its size) additional data for the INTERHEART risk score, diet, physical activity, and a non-fasting blood sample and an ECG will be obtained at 6 and 12 years (cross sectional snapshots). At these times, community level parameters that reflect the societal domains of interest will also be documented.

Table 5: Target number of individuals to be recruited into PURE from each country

	National Leader/Investigators		Urban	Rural	Total
High Income Countries (HIC)	S Yusuf, K Teo, G Dagenais, S Lear, A Wielgosz	Canada[1]	7,343	3,120	10,463
	P Raina	Canada (CLSA)[3#]	25,000	25,000	50,000
	A Rosengren	Sweden[1]	3,251	902	4,153
	AH Yusufali	UAE[1]	995	504	1,499
	K AlHabib	Saudi Arabia[2]	3,000	1,500	4,500
		Total HIC	**39,589**	**31,026**	**70,615**
Middle Income Countries (MIC)	L Kruger, T Puoane	South Africa[1]	2,364	2,337	4,701
	A Avezum	Brazil[1]	3,958	2,123	6,081
	R Diaz	Argentina[1]	3,615	3,920	7,535
	P Lopez-Jaramillo	Colombia[1]	3,805	3,738	7,543
	F Lanas	Chile*[1]	2,820	748	3,568
	W Zatonski. A Szuba	Poland*[1]	9,418	5,790	15,208
	K Yusoff, NH Ismail	Malaysia[1]	6,945	8,883	15,828
	Li Wei. Liu Lisheng	China[1]	23,413	24,264	47,677
	R Kelishadi	Iran[1]	3,031	2,982	6,013
	A Oguz	Turkey[1]	2,766	1,468	4,234
	T Dans	Philippines[2]	2,500	2,500	5,000
	J Miranda	Peru[2]	2,919	700	3,619
	R Khatib	Palestine[2]	1,000	1,000	2,000
	S Boytsov, A Kontsevaya	Russia[2]	2,500	2,500	5,000
	K Davletov	Kazakhstan[2]	1,000	1,000	2,000
	E Mirrakhimov	Kyrgyzstan[2]	1,000	1,000	2,000
		Total MIC	**73,054**	**64,953**	**138,007**
Low Income Countries (LIC)	J Chifamba	Zimbabwe[1]	459	804	1,263
	P Mony, V Mohan, R Gupta, R Kumar, K Vijayakumar, R Kutty	India**[1]	14,286	15,012	29,298
	R Iqbal, K Kazmi	Pakistan**[1]	1,500	1,500	3,000
	O Rahman, R Yusuf	Bangladesh[1]	1,380	1,556	2,936
	K Yeates	Tanzania[2]	2,000	2,000	4,000
		Total LIC	**19,625**	**20,872**	**40,497**
		Grand Total	**132,268**	**116,851**	**249,119**

[1]Phase 1: Actual recruited numbers

[2]Phase 2: Projected recruitment numbers

[3#] Canadian Longitudinal Study of Aging (CLSA): Through collaboration (many data items are identical, and many are similar). We have experience is "mapping "out the data items across different studies to identify and harmonize key variables.

*****Chile and Poland, which were MIC at the beginning of the study are currently HIC as per World Bank
******India and Pakistan which were LIC at the beginning of the study are currently LMIC as per World Bank

Nos. of participants by region:

South East Asia = 20,828
China = 47,677
Middle East = 14,012
Europe = 23,595
South America = 28,346
South Asia = 35,234
Russia/Central Asia = 9,000
Africa = 31,964
Canada = 60,463

7.0 Selection of countries, communities, households and individuals:

7.1 **The goal of our sampling strategy is to choose countries, communities and individuals with diversity in lifestyles.** The choice and number of countries selected in PURE reflects a balance between involving a large number of communities in several countries at different economic levels with substantial heterogeneity in social and economic circumstances and policies, and the feasibility of centers to successfully follow-up the cohort long-term. (Table 5 lists countries divided by HIC, MIC, LIC). We expect to select multiple communities within each country [(e.g. an average of 5-10 urban locations, which can be further subdivided into smaller units (e.g. by postal codes) and 10-80 villages or rural locations in a single center)]. This means that overall we expect to include approximately 400-500 communities, with varying number of individuals (50 to 500) in each. No universally applicable definition for urban and rural regions exists as summarized by a National Academy of Sciences Review[40]. . PURE has therefore adopted existing country specific definitions, with additional consideration of population density, percent of the particular population employed in agricultural work and distance from major urban centers. Small variations in criteria used to select individual locations are unlikely to matter because in our approach the characteristics of a community are related to the levels of risk factors or CVD rates within a population and then regressed across communities. Further, we do not consider "urban" and "rural" communities as being totally distinct, but instead exhibiting a range of characteristics. Urban centers are generally where our collaborating investigators and their institutions are located and represent large and medium sized cities. Choice of the specific sampling frame at a center is based on both "representativeness" and feasibility of long-term follow-up. Our approach has been one where each center identifies neighborhoods or villages, based on broad study guidelines (Table 9), but have flexibility to identify a range of heterogeneous communities under this broad framework. However once a community has been identified, common and standardized approaches to enumeration of households, identification of

individuals, their recruitment and data collection are required. In each urban area we will attempt to enroll **all** eligible households from 3 sources: a) households of workers from large companies who usually live in "colonies", or institutions, representing some of the largest employers in that location, b) geographically defined middle class housing areas, and c) defined slum or shanty town dwellers. This will lead to inclusion of several different types of communities with varying environments and risk factor levels within each participating urban site. In rural areas, a number of villages of varying population sizes will be identified with door-to-door enumeration of all households.
All identified households in a community will be contacted (at least 3 attempts). When a household refuses to participate in the full study, simple demographics, risk factor and CHD history are recorded. This will assist in assessing whether there are any obvious selection biases (See Table 10 and Fig 11 for Bangalore pilot data). Once an eligible household has been identified, all residents are enumerated and data are collected on the census form. All individuals between 35 years and 70 years are invited to participate in the study, and those providing written informed consent are enrolled. A brief non-responder form is completed in those who refuse.

7.2 **Data Collection** occurs at four levels (national, community, household, and individual) incorporating 4 societal determinants (Built environment, Nutrition environment, Tobacco use, and Socioeconomic/psychosocial determinants) (Table 7 and 8, Fig 12). National level data will be obtained using similar sources where possible i.e. World Bank reports, National Census and Survey data, and will be collected at baseline and every three years. Community level data will be an aggregate of individual level data for some determinants (i.e. perception of built environment, and social capital) and local information on traffic and zoning laws, food pricing and availability, and tobacco advertising, etc. Household level data will be collected using a *Family* Census Questionnaire which records demographic information (i.e. number of individuals in the household, sex ratio, number of children and other relatives), tobacco use, average level of education and morbidities in all inhabitants of the household. A *Household* Questionnaire covers domains related to house structure, amenities, access to water and sanitation. In all consenting and eligible *individuals* detailed additional information is recorded: lifestyle factors using the IPAQ and NEWs to assess physical activity and a validated food frequency questionnaire to measure food patterns, personal and environmental tobacco exposure, socioeconomic factors including employment income, education, and psychosocial factors such as social capital, attitudes and beliefs, and the IH-9 risk factors. Physical measures are performed, bloods and urine are collected; and ECGs are obtained. These methods have been developed, standardized and tested in >20,000 people in 10 countries. The current version of the questionnaires is provided in Appendix 1-2. Some changes are expected, but the main elements will be retained. All physical exams and tests are done in a clinic setting, utilizing standard methods.

Blood samples are stored immediately in ice filled insulated containers and are centrifuged, aliquoted and frozen immediately. Each centre has been provided with all necessary supplies. Samples are shipped by courier from each site to the National Blood Storage Site where they are stored in liquid nitrogen canisters (-180^{o}C). Samples are then shipped in nitrogen vapor tanks to the Core Laboratory at the Hamilton Regional Laboratory Medicine Program Canada. The timing of all

local and international shipments is guided by a specified schedule that is determined by local blood storage capacity (e.g. blood samples will remain at -20 ^{o}C or -70 ^{o}C for a maximum of two or six months respectively). Laboratory specimens are being stored centrally in liquid nitrogen at the Core Laboratory where analyses will be conducted.

All blood and urine measurements will be analyzed blindly at the Core Laboratory in Canada.

7.3 **Assessment of Built Environment (Tables 7 and 8):** The built environment refers to all of the buildings, spaces, and products created or modified by people, e.g. buildings (housing, schools, workplaces); land use (industrial or residential); public resources (parks, museums); zoning regulations; and transportation system. Perception of the built environment will be assessed using the NEWs (Neighborhood Environment Walking Scale)[25]. This instrument has been developed to assess individual perceptions of how conducive a neighborhood is for walking and physical activity. It is constructed around the following domains: population density, land use mix, access to services, neighborhood esthetics, traffic safety, crime safety, and neighborhood satisfaction. The questionnaire's test-retest reliability, criterion validity and validity of predicting physical activity assessed by accelerometer and anthropometric measures have been established[25,41]. The responses from this questionnaire can be used as separate variables, or scores for each domain can be calculated. Together, domains are used to characterize community walkability. Occupational, leisure time and sedentary behaviors are collected using the IPAQ which is a questionnaire to measure physical activity and validated in multiple countries. Geographic Information Systems (GIS) mapping will be used to provide an objective measurement of the built environment at the community where available. The collection and standardization of GIS information will be overseen by Dr. Daniel Rodriguez, who is an expert in transportation. National indicators such as the degree of urbanization and expenditure on roads and transportation will be obtained from the World Bank reports.

7.4 **Measurement of nutrition environment (Tables 7 and 8)**:
Assessment of diet is complex, involving multiple trade-offs between different methods, while facing the challenge of international comparability. To assess the nutrition environment we will collect information at national, community, household, and individual level. The food frequency questionnaire will be used in each country to obtain information on macro and micronutrient intake. For some countries existing FFQs will be used but in most of the others the FFQ will be developed and validated (see Appendix 1-4b for an example for the Middle East). A master international nutrient databank being compiled at the Population Health Research Institute, will be used in the analysis of the FFQ. Food patterns will be generated using the FFQ data by the methods of Hu et al.[42] Food patterns will also be reported at the household level as well as the proportion of family income spent on food per month. This approach builds on the work of the European Union sponsored DAFNE project[43] that has used data from household budget surveys to assess food availability across Europe. The accumulated experience has led to the development of instruments that capture food consumption inside and outside the house, as well as food stocks. This will be supplemented with questions adapted from the US Department of Agriculture measures of food security[44] as well as other research examining food insecurity in low income

settings[45]. In addition, we will ask the head of each household of his or her perception about the extent to which cost and availability of selected food items influence the household's decisions to buy those items. The items we will include in this list are: fruits, vegetables, beans, lentils, cooking oil, soft drinks, meat, fish, poultry, and eggs. The degree of influence will be measured on an ordinal scale and a score will be calculated. Relative food pricing and availability, measurement of time/distance to nearest shop, will also be collected at the community level. At national level, subsidizations/ taxation specific foods and fruit and vegetable intake will be obtained from the WHO's FAO (http://faostat.fao.org), and using data from Food and Agriculture Food Balance Sheets, can be used to provide a proxy measure of annualized national intake of major dietary components.[46]

7.5 **Tobacco related variables (Table 7 and 8 and Appendix 1-4c):** At the individual level, active and passive smoking exposures will be obtained using standardized questionnaires. At the household level the number of members in the household who smoke and exposed environmental smoking will be calculated. At the community/national level information on tobacco policies (laws regarding smoking bans, advertising and promotion, packaging and labeling, health warnings, degree of enforcement, taxation, and other information that will allow calculation of the advertising restriction score (ADR), an internationally validated measure), cultural attitudes towards tobacco use, price, degree of adherence to the FCTC convention and tobacco related health awareness will be related to levels of tobacco use, both at the beginning of the study and at 6 and 12 years. These measures have been demonstrated to predict rates of tobacco use.

7.6 **Measurement of Socioeconomic and Psychosocial Determinants (Tables 7 and 8):** After extensive consultation with experts, the key domains include measures of income, employment and occupation at the individual level; household assets, and at the national level information on literacy, work force composition, and income inequality. Psychosocial factors measured at the individual level include perceived stress, role strain and social capital (trust), family structure, social networks, community club membership, levels of trust, and volunteerism. Depression[47] and perceived control at home and at work[48] – two measures for which the global literature[26] has most consistently supported an association with CVD will also be measured. Measures obtained at an individual level can be grouped by community to characterize the community. Gender specific influences on psychosocial factors (women's empowerment, household decision making and autonomy, as well as National indicators of women's presence and roles in government) will also be assessed.

7.7 **Data transfer and checks:** All data will be entered locally at each site into a computer utilizing a customized database developed and validated over the last year. Range and consistency checks are programmed. These data will be transmitted electronically to the Project Office in Hamilton every week, where further data checks will be done, and further quality control measures implemented.

7.8 **Standardization of data collection:** All data are collected by trained staff using standardized protocols. Key staff (principal investigator, coordinator, nutritionist) from each center attend an initial training session in each country/region. They in turn train local staff using centrally created manuals and training videos. These staff are trained, tested on "mock" subjects and certified. If the variation between the staff compared to the coordinator is unacceptable, the staff member is

retrained. A random 10% of all ECGs and all positive ECGs are checked by a second physician. Standard definitions for outcome events are being developed and all bloods are either shipped to Hamilton (most countries) or national core laboratories (India and China) which are being standardized. All staff convene every month at each site to review study progress and data quality. In addition, we plan to hold annual retraining meetings of key staff every year, during recruitment, and every 2 years during follow-up. Site visits will be conducted once a year during recruitment, and alternate years during follow-up.

7.9 **Follow-up:** By excluding households that plan to move in the next 5 years, and individuals <35 yrs who tend to be mobile, high follow-up rates [>96% over 6 years and >92% over 12 years, which is similar to a study conducted by R. Peto in China (personal communication), but lower than our experience in several long term trials where follow-up rates have been >99% over 5 years] are expected. Follow-up is conducted by local health care workers who are familiar with the community. All households will be contacted every three years. Deaths will be recorded, and their causes will be ascertained by the validated verbal autopsy method[49]. Morbidity will be ascertained by review of hospitalizations, new diagnoses and treatments, investigations and surgical procedures. These will be adjudicated, utilizing standardized definitions and without knowledge of the participant's residence. To further minimize differential rates of ascertainment for CVD between countries and settings, we will compare fatal CVD events, ECG changes of MI (e.g. new Q waves), and administer a standardized symptom questionnaire for angina. Weight, waist-hip ratio and blood pressure will also be measured every 6 years in all subjects. At every 6 year visit, in randomly selected individuals (i.e. approximately 20% of individuals overall, but varying the proportion randomly drawn from the entire population in each community depending on their size to provide periodic "representative" snapshots), assessments of the key risk factors from INTERHEART and a 5 ml blood sample will be obtained. This will provide an unbiased assessment of community level change in risk factors. Additionally, community level data to characterize the 4 societal domains will be collected at these times.

7.10 **(j) Power and Statistical Analysis Plan: Main Objective 1:** The contribution of each of the 4 societal domains on the *prevalence* of outcomes (INTERHEART risk score, its components, and CVD) at baseline will be analyzed using a multilevel generalized linear model, with hierarchical levels for the individual, the household, the community, and the country. Models will be built to examine prevalence of various outcomes, and will include covariates for the individual, household, and community prevalence (Table 11a). All models will include age and sex as covariates to adjust for imbalances across communities. Sex interactions for all putative determinants of CV risk factors will be examined. The model predicting the INTERHEART risk score will be a linear multilevel model with the predicted probability of CVD, using the logit transformation. All other outcomes will use logistic multilevel regression models. Power: The impact of each component of the 4 societal domains cannot be precisely calculated. However, power to detect differences in the rates of outcomes when communities are categorized into thirds based on levels of the societal domains can be calculated. Table 11a also shows the differences in INTERHEART risk score, metabolic syndrome (a subcomponent of the INTERHEART score), and CVD which can be detected from this analysis assuming different outcome rates and levels of inflation factor (IF). With 400 communities and an average sample size of 300 each, we would have 90% power to detect difference of 0.6% to 1.2% in prevalences of metabolic syndrome.

Sample sizes per communities will vary (50 to 500 subjects) and this may reduce power. However, given the currently estimated high power, we will still have high study power if such imbalances occur. Using information about each societal domain separately would likely increase the power for these same analyses. **Main Objective 2:** We will estimate the effect of *changes* in each societal domain and lifestyles (diet, physical activity, smoking), estimated from periodic random sampling from each community (6 and 12 years). In order to estimate the characteristics of each community within an acceptable margin of error, an adequate sample will be collected for each community. Since the purpose is to observe time trends across communities, it is not necessary for trends within individual communities to be measured with a great degree of accuracy. Sample size will also be increased for communities where greater heterogeneity is noted at baseline. The exact sample size will be dependent on the sampling fraction or ratio of sample size to community size.[50] For example, given a baseline prevalence of smoking of 25%, a sample size of between 30 (for a community of 50 individuals) to 63 individuals (for a community of 500 individuals) will be taken to estimate this rate in follow within ±5%, 95% of the time. To estimate a 10% incidence within a margin of error of ±4%, between 43 and 183 individuals would need to be sampled, for communities of 50 and 500 respectively.

Since the rates of outcomes within a community will be directly related to its age distribution, we will age-standardize these rates as well as the mean values or incidences of the explanatory factors. Direct standardization will be used with weights based on the age distribution of the entire study cohort. As suggested by Breslow et al[51] these standardized rates will be analysed using a multiplicative model where the observed rate for each community is the product of the expected rate given its age distribution and the exponentiated regression coefficients for the explanatory factors. The number of individuals observed with a risk factor within communities is assumed to follow a Poisson distribution. Possible heterogeneity of rates between communities, beyond what is expected from a Poisson distribution or "overdispersion" will be allowed for in the models. The natural logarithm of these standardized rates will be modeled using generalized linear models with overdispersion. For modeling the mean INTERHEART risk score, linear regression will be used. For both the Poisson and linear regressions, the explanatory factors are not fixed by design but each has their associated measurement error, so we will not use simple linear regression but instead use a weighted linear regression to avoid bias[52]. The weights used will be given by the variance estimates of each outcome and explanatory factors. For analysis of change in rates over time, the change in standardized rates will be regressed on the change in explanatory factors**.**

Power: Power calculations for incidence would require knowledge of the standard deviations of changes in outcomes rates and change in societal domains. For the purpose of these calculations we assume that communities are classified into those with small, medium and large changes in the above factors. Since the variance of change in rates of metabolic syndrome and other outcomes is not known, we are using variance estimates for the baseline prevalence estimates, which would assume a correlation of 0.5 between rates over time[53]. See Table 11c for effect sizes that could be detected for mean INTERHEART risk score, metabolic syndrome and CVD prevalence and incidence. The former calculation is from an ANOVA and the latter two assume a normal approximation for a Poisson distribution. The natural log of the standardized rates will be normally distributed with estimated variance of inverse of the outcome count. The

prevalence rates are used for estimates of these variances. Using this assumption and with approximately 400 communities, we should have 90% power to detect differences between communities with low, middle and high changes in societal domains of approximately 0.0342 for the INTERHEART risk score. This would mean that, for a prevalence INTERHEART Score of 32.5%, an effect size of 0.0342 would be equivalent to group changes in means of 0%, 0.5%, 1% in low, medium and high HIC countries. **Main Objective 3:** We will assess the relative contribution of lifestyle changes in individuals and community level changes in societal domains on the risk factors and their separate and joint effects. As for objective 1, we will use a multilevel generalized linear model, with hierarchical levels for the individual, the household, the community, and the country. Models will be built to examine incidence of outcomes and will include covariates for the individual, household, community over time, adjusted for age and sex.

Table 6: PURE Recruitment Sites

Country	Urban Site	Slum/Shanty Town	Rural Site
Canada - Ontario	Hamilton	N/A	Southwest rural Ontario*
Canada - Quebec	Quebec City	N/A	Northern Quebec*
Canada – BC	Vancouver	N/A	Rural BC*
UAE	Dubai City – Mumzar District	Dubai City – Hatta District	N/A
Argentina	Rosario City	Within Rosario City	Salta Region
Sweden	Göteborg	N/A	Northern Sweden
South Africa	Potchefstroom Ikageng, Top city & sonder water	To be Determined	Vryburg Ganesysa & Tklagameng
Chile	Temuco	Within Temuco	Vulcun Region
Poland	Wroclaw	N/A	Wegry
Brazil	Sao Paulo City – Employees of Volkswagen	Sao Paulo City – Favela Paraisopolis	Angatuba Region
Thailand	Bangkok - Employees of Bangkok University	Within Bangkok	Rocet Region
Iran	Tehran Isfahan	N/A	Regions of Isfahan
Colombia	Bucaramanga	Within Bucaramanga	Regions of: Barichara, Guane, Aratoca, Curiti, Palmas del Socorro
China	Cities in Beijing, Jiangsu, Shaanxi, Shandong and Shanxi Provinces	Community within Beijing, Jiangsu, Shaanxi, Shandong and Shanxi Provinces	Regions of: Jiangsu, Shaanxi, Shandong and Shanxi
Zimbabwe	Harare – Mount Pleasant, Mabel region, Mbare	Harare - Epworth	Chidamoyo region
India – Karnataka	Bangalore – Employees of HAL, residents of Koramangala district	Within Bangalore	Regions of Palamaner
India – Kerala	Trivandrum – Peyad Resident's Association Colony	Within Trivandrum	Varkala ICDS Block

India – Tamil Nadu	Chennai- Asiad Colony, Annai Sathia Nagar, Bharathi Nagar, Venkatapuram	Within Chennai	Mahuramangalam Region
India – Rajasthan	Jaipur – Lal Kothi, Civil Lines, Murlipura	Within Jaipur	Rural Bikaner –Sujana Rural Jaipur – Naila & Goner
India – Punjab	Chandigarh – Sectors 12 & 38	Within Chandigarh	Raipur Rani CHC, Panchkula District, Haryana State
Turkey	Istanbul (Marmara Region) Ankara (Mid-Anatolia Region), Adama (South Region), Samsun (North Region), Erzurum (Northeast Region)	N/A	Areas within 50-100 kms from the Urban selected regions

*Agriculture based communities will be assessed
NA=Not applicable

Table 7: Components measured at the country, community, household and individual levels (country level and some of the community level information will be obtained from existing databases)

Societal Determinants	Components Measured at National Level	Components Measured at Community Level	Components Measured at Household Level	Components Measured at Individual Level	CV Risk Factor Influenced
Built Environment	Population density [a] Car ownership [a] Degree of urbanization [a] Government expenditure on roads and transportation [a] GIS data [b]	GIS in some communities [b] Policy (laws regarding zoning, traffic speeds, etc.) Summary score of NEWS for community [c]	Perception of Built Environment using NEWS [c]	Perception of Built Environment using NEWS [c] Utilitarian Physical activity in METS [d]	Physical Activity Body Mass Index Abdominal Obesity Lipids BP Glucose
Nutrition Environment/ Nutrition Policy	Subsidies/taxes on specific food products [e] Annualized national intake of major dietary components [e] Per capita food supplies available for human consumption (calories, fruits and vegetables by year and country)[f]	Relative Food Pricing and Availability of Common foods [g] Price/availability of alcohol [g]	Food Patterns,[h] Proportion of Family income spent on food/month[i]	Food Patterns using country – specific FFQ [h]	Body Mass Index Abdominal Obesity Lipids
Tobacco Use	National Tobacco Control Policies [j] Compliance with FCTC [k] Advertising Restriction Score[l] National Prevalence of smoking [m]	Tobacco Pricing and Availability [n] Compliance and enforcement of FCTC and National Control Policies [o] Focused awareness programs [p] Alternative forms of tobacco control programs [p]	Number of users of Tobacco per household [i]	Tobacco Exposure [q] Cost of tobacco product as proportion of daily income [q]	Tobacco Use Body Mass Index
Socio-economic	Literacy [r] Employment [r] Workforce composition [r] GDP [r] Income inequality [r] Governmental expenditures (i.e. on health) [r] Unionization [r]	Aggregate of Asset Index [s]	Asset Index [s] Household Income [i] Economic Decision Making [i]	Employment [t] Education level [t] Occupation [t] Marital Status [t]	Lifestyle Behaviors such as Dietary intake, Physical activity, Depression Perceived Stress Blood Pressure Tobacco Use
Psychosocial	Social trust [u] Ethnic composition [v] Level of democracy in government [a,z]	Aggregate of Social Capital measured at Individual level [x]	Family Structure [i] Sex Ratio of Household [i]	Social Capital [x] Perceived Level of Stress [y] Role Strain [y]	Depression Locus of Control Blood Pressure Dietary and Physical Activity Patterns

	Cultural Norms (i.e. gender roles) [w,u] Women's Empowerment in household decision making and autonomy [w,u] Number of women in federal government [a]		Decision Making/Autonomy of Women [i]		

[a] Population Density, Car Ownership, Degrees of Urbanization, Government Expenditure on Roads and Transportation, Level of Democracy in government, Number of women in federal government: World Bank Database: www.worldbank.org
[b] Country and Community Specific Census Data GIS Data: To be collected on a country and community basis under the supervision of Dr. Daniel Rodriguez
[c] Perception of Built Environment Assessment using the NEWS: Neighborhood Environment Walking Scale
[d] Physical Activity at work and in leisure time, including sedentary behaviors using IPAQ: International Physical Activity Questionnaire
[e] Subsidies/taxes on specific food products: Collected on a Country by Country basis for major food products; Annualized national intake of major dietary components using Food and Agriculture Food Balance Sheet[46f] Per capita food supplies available for human consumption (calories, fruits and vegetables by year and country): Food and Agriculture Organization data, http://faostat.fao.org/faostat/collections/nutrition
[g] Relative Food Pricing and Availability of Common foods: Based on DAFNE Project[43] and US Department of Agriculture measures of food security[44,45]
[h] Food Patterns using country –specific FFQ and the methods of Hu et al to calculate food patterns[42]
[i] Family income spent on food/month, Number of users of Tobacco per household, Household Income, Economic Decision Making, Family Structure, Sex Ratio of Household, Decision Making/Autonomy of Women: Direct Questioning on Household Questionnaire
[j] Government Policy Review: http://www.who.int/tobacco/resources/publications/en/
[k] Framework Convention on Tobacco Control of World Health Organization
[l] Advertising Restriction Score: range of 0-10 obtained for each country and year as per (Laugesen M, Meads C. Tobacco advertising restrictions, price, income and tobacco consumption in OECD countries, 1960-1986. Br J Addict. 1991;86:1343-1354.)
[m] National Prevalence of smoking: Country Specific Census Data or Health survey whichever is most recent
[n] Tobacco Pricing and Availability
[o] Compliance and enforcement of FCTC and National Control Policies: World Health Organization
[P] Focused awareness programs, Alternative forms of tobacco use programs: Collected per Community using Community Census
[q] Smoking history, types and quantity of tobacco products used, exposure to ETS, Proportion of income spent on tobacco on Individual Questionnaire
[r] Literacy: UNESCO Institute for Statistics http://www.uis.unesco.org/ev.php; Employment and Workforce Composition: Country Specific Census Data or Health survey data whichever is most recent; income inequality: www.globalpolicy.org/socecon/inequal/indexinq.htm ; GDP: World Bank Statistics: www.worldbank.org/ . International Labour Organization: http://www.ilo.org/public/english/bureau/stat/portal/topics.htm
[s] Household Asset Index derived from Demographic and Family Health Survey: http://www.measuredhs.com/sitemap.cfm
[t] Income, Employment, Occupation gathered on the Individual Questionnaire
[u] World Values Survey: http://www.worldvaluessurvey.org/services/index.html
v: Ethnic composition: National Statistics or Survey Data
w: Women's Empowerment: decision making and autonomy : Demographic and Family Health Survey: http://www.measuredhs.com/sitemap.cfm
[x] Social Capital measured using Trust, civic engagement, and community involvement: *(Social Capital Initiative Working Paper No.1, The World Bank, April 1998: http://www1.worldbank.org/prem/poverty/scapital/SChowmeas1.htm*
[y]: Perceived level of Stress and Role Strain Questions derived from the Life Experiences Survey (LES), and the Perceived Stress Scale (PSS): www.ncbi.nlm.nih.gov/entrez/query.fcgi, and Stockholm Marital Stress Scale: Orth-Gomer KJAMA 2000.
[Z]:Freedom House: http://www.freedomhousel.org/ratings/index.htm

Note: A compendium of National and Community Level Data will be collected using standardized sources by graduate students in Year 1 of this project and updated every 3 years:

Note: Use of standardized core questions allows for comparisons across different countries

Legend: DFS: Demographic and Family Health Survey, GIS: Geographic Information System , FCTC: Framework Convention on Tobacco Control of World Health Organization; GDP: Gross Domestic Product; DAFNE

8.0 PURE Pilot Study:

Over the last 4 years, we evaluated urban vs rural differences in societal structure, automation, physical activity, nutrition, risk factors and CVD rates initially in Bangalore, India.[21] This allowed testing the feasibility of our protocol. Preliminary results on the first 7500 subjects (Table 4 and Fig 11), demonstrate markedly increased automation at home and in transportation in urban compared to rural settings, leading to three fold reduced utilitarian physical activity levels. Additionally, there was a 30% increase in energy in the urban setting, along with higher fat consumption (which is much lower than in Western countries). Consequently, there were large differences in body weight (BMI of 25.5 kg/m^2 urban vs 19.0 kg/m^2 rural), abdominal obesity (waist to hip ratios of 0.87 vs 0.80), diabetes (14.0% vs 1.7%), hypertension (18.4% vs 3.7%), and LDL levels (3.05 vs 2.57 mmol/L). Depression was more common (18% vs 9%) and tobacco use less frequent in urban populations (44% vs 62% in men and 2.0% and 18% in women). CHD prevalence was twofold higher in urban (2.5%) compared to rural (0.7%) populations. These data strongly support our hypothesis that CVD and its risk factors are affected substantially by the nature of societies. The study has been expanded to 4 other centers in India (preliminary data in 4b) and approximately 20,000 subjects have been recruited. The first cycle for the 3 year follow-up has just been initiated in Bangalore. We have also adapted and translated the protocol and forms for use in 12 new countries, and have conducted pilot studies (100 to 200 subjects per location) to assess feasibility in 7 (Argentina, Brazil, Colombia, Zimbabwe, S. Africa, China, and UAE) with ongoing pilots in Chile, Thailand, Sweden and Iran. We will establish urban and rural cohorts in five locations in Canada (Newfoundland, Nova Scotia, Ontario, Quebec, and British Columbia; n=8,000) and Sweden as examples of **"reverse" transition in an HIC,** where we expect more obesity, higher risk factors and higher CVD rates in the rural populations. Although we have not implemented a specific pilot study for PURE in Canada, the SHARE[54,55] cross-sectional study (1300 subjects in 4 locations), and the Quebec Cardiovascular Cohort Study conducted by G. Dagenais on 2000 subjects[56], establishes feasibility.

9.0 International Consultations:

In order to broaden the expertise needed to conduct the study, we have held two dedicated meetings in Hamilton over the last 6 months of international experts in the area of social epidemiology (G. Davey-Smith, Bristol, UK; S.V. Subramanian, Harvard; S. Elliott, McMaster; J. Lynch, McGill), built environment (A. Forsyth, Minneapolis; D. Rodriguez, Chapel Hill, NC; P. Kanaroglou, McMaster), societal transitions (M. McKee, London, UK), tobacco policies (L.Waverley, Ottawa), nutrition policies (W. Zatonski), statistics of population studies (H. Shannon, McMaster) followed by extensive correspondence. These have strengthened the societal component of our study. All the above individuals are collaborators/co-investigators and will play significant roles in developing specific substudies. Thus, this expanded group of experts complements our core strengths in epidemiology, cardiology, metabolism, statistics, and experience of research in developing countries.

10.0 Investigators And Their Experience (Tables 12a, 12b and 12c):

The principal investigator (S. Yusuf) and several co-investigators (S. Anand, K. Teo, H. Gerstein) have played leadership roles in several large trials and epidemiologic studies collectively involving over 300,000 subjects from >60 countries requiring long term follow-up. A. Merchant is a nutrition epidemiologist who has been working on the PURE pilot studies for the last 2 years, and has supervised the development and validation of the FFQ and creation of a nutrient database for several countries. In addition, several

experts in social epidemiology/social sciences, health transitions, built environment, tobacco policy, food policy and nutrition, complex statistics, obesity, and genetics are co-investigators or collaborators, and have provided substantial input into specific areas (see above and Table 12b). The global infrastructure and network has been built over the last 20 years, and most investigators have previously collaborated successfully. The Project Office processes over 1.5 million data forms every year, and extensive computing and statistical support exists. The core laboratory has processed about 1 million aliquots on 75,000 subjects from 40 countries over the last decade and has standardized core labs in China and in India. A number of special working groups on each of the societal domains (tobacco, psychosocial, nutrition and built environment), genetics, statistical methodology, outcome validation and standardization of methods, etc have been formed (Fig 13). Therefore, we have assembled an experienced team with the necessary broad expertise to successfully complete this project.

11.0 Timelines: (See Table 13)

12.0 IMPORTANCE OF THE STUDY:

PURE follows in the footsteps of a few previous international epidemiologic studies (e.g. Seven Countries studies[57] INTER-SALT[58], INTERHEART[8], NI-HON-SAN Study[59], CINDI[60] CARMEN[61], MONICA[62]) which have considerably advanced knowledge. However, none explicitly studied the impact of societal influences on lifestyles and their interactions with genetic factors. Thus, PURE will extend current knowledge by addressing the complex relationships between societal factors on individual lifestyles, and potential interactions with genes (for which a separate proposal will be developed) that lead to the development of risk factors and CVD.

This global study will provide important and novel information on the causes of the major health burden, due to obesity, diabetes, and CVD globally. Its size, scope (both societal and individual influences) and inclusion of individuals from a diverse group of countries, enables it to both discern truly universal risk factors, and importantly their contextual variations and contingencies, and their impact on CVD, and this avoids the limitations inherent in studying more homogeneous groups. Its focus on societal changes, affecting individual lifestyles and biologic markers is novel and will provide valuable and new information that governments and international organizations could utilize to develop policies that are “actionable” and could mitigate the adverse health effects of rapid societal changes so that evidence based societal level interventions can be developed (See Table 14). At a scientific level, it will provide valuable insights regarding interactions between societal, household, individual, and genetic determinants of disease for decades to come, which will influence CVD prevention, within Canada and globally.

Table 8: Levels of Data Collection* and areas covered during the house to house survey

Data-Level	Questionnaire**	Areas Covered
Community	**Rural Profile** **Urban Profile**	• Access to Major Roads • Connectedness to urban centers • Presence of schools/health clinics/various types of stores • Electrification/water sources/access to TV
Community/ Household	**Transition Profile**	• Characterization of the built environment: 1) Perception 2) Objective using GIS or geomapping (in a substudy, to be developed) • Affordability of prudent nutrition • Legislation and enforcement: nutrition and tobacco • Social capital
Household/Family	**Family Census**	• Enumeration of all family members • Basic demographics and disease prevalence
Household/Family	**Household Questionnaire**	• Structure of the house • Household income/food expenditure • Household possessions • Dependency Ratio • Family/household relationship • Agriculture
Individual	**Adult Questionnaire**	• SES (education, occupation, income) • Social Support (group membership, network of friends, family structure) • Tobacco and alcohol consumption • Medical history and medications • Family medical history • Sex specific questions • Stress and locus of control • Anthropometry (height, weight, waist and hip circumferences, mid-arm and calf circumference, triceps and calf skinfold)^ • Spirometry*** (FEV1, FVC, PEFR) ^ • Blood pressure, heart rate, ECG • Blood and urine samples #
Individual	**Physical Activity Questionnaire**	• Detailed assessment of energy expenditure related to: work, chores and leisure time activity (outcome of METs)
Individual	**Semi-Quantitative Food Frequency Questionnaire**	• Culture specific foods • Carbohydrate refinement • Vitamin supplementation

Table 9: Guidelines and approach to inclusion of communities and individuals (Selection of countries to ensure that each region of the world involving LIC, MIC and HIC are included)

	Urban	**Rural**
1) Definition	National definition of urban	National definition of rural + OECD definition of < 150 people/sq km.
2) Location	Cities	In addition to above definition, rural centres must be reasonably isolated/ separate (distance of >50 km or lack easy access to transportation) from urban centers. Note most rural sites are >100 km from the urban site.
3) Facilitating Organization	Large employers (government and private), insurance companies, clubs, religious organizations, neighbourhoods and slums, clinic or hospital service regions	Local authorities such as priests or community elders, hospital or clinic, village leader or local politician
4) Sampling Frame:	Catchment area of clinics, lists from insurance plans and employers, neighbourhood census data, etc.	All villages that are within a 45-minute drive to a medical centre or appropriate facility (to enable all bloods to be processed within 2-hours).
a) Feasibility For Long-Term Follow-Up	Choose employers or sites that have a stable population. Consider approaches such as cluster-sampling to improve efficiency	Choose villages that have a stable population. Avoid communities that may be susceptible to large migration to urban centres.
b) Choosing the Sampling Site	From each strata, sites are chosen at random. Other factors pertaining to feasibility must be considered. Specific sites may have to be included to cover full SES spectrum	Ideally, lists of small, medium and large villages are compiled and from each, random locations are selected for sampling. Other local factors pertaining to feasibility must be considered (e.g. distance to blood processing facility).
5) Approaches to Recruitment	Door to door contact or through employer	Door to door recruitment of all households in selected village.

Table 10: Comparison of key demographic characteristics between Responders and Non-Responders in the PURE pilot study in Bangalore (based on the first 5000)

	Urban		Rural	
	Responders	**Non Responders**	**Responders**	**Non Responders**
4.0 Sample	2,078	1,484	2,955	321
Gender (M/F)	1,209 / 869	876 / 608	1,538 / 1,417	126 / 195
Age (yrs)	47.0	46.2	47.0	48.8
Current tobacco use (%)	16.3	16.9	45.8	50.8
History of Diabetes (%)	16.1	12.0	1.8	0.6
History of Hypertension (%)	20.1	20.1	4.1	0.6
History of CHD (%)	3.1	4.5	0.9	0

There were few major differences in demographic variables between responders and non-responder within the urban and rural populations. Note that these differences are much smaller than the differences between urban and rural populations. Rural non-responders may have lower morbidity levels than responders because they were more likely to be employed in vigorous agricultural work (healthy worker effect). Accounting for this effect would likely further increase the differences in risk factor and disease prevalences between the urban and rural populations. Collection of similar data at each site will ascertain the impact of any potential biases due to non-response. NOTE: We will have the above key data in practically all (>95%) individuals in the households surveyed. An analyses using this information, as well as the more detailed information can both be performed as a "sensitivity" analysis, to assess the impact of any potential biases.

Table 11a: Detectable difference between communities with low and medium levels of societal factors, or medium and high levels of societal factors at 90% power and type I error rate of 0.05 for prevalence. Assumes the number of communities is 400 with an average of 300 individuals per community*

INTERHEART Risk Score				**Metabolic Syndrome[c]**				**CVD[d]**			
Baseline Prevalence	25%	33%[a]	40%	Baseline Prevalence	10%	15%	20%	Baseline Prevalence	1%	3%	5%
Inflation Factor				Inflation Factor				Inflation Factor			
40	1.26%	1.48%	1.61%	3	0.6%	0.7%	0.8%	3	0.20%	0.35%	0.45%
50[b]	1.41%	1.64%	1.80%	5	0.8%	0.9%	1.0%	5	0.25%	0.45%	0.55%
60	1.54%	1.81%	1.98%	7	1.0%	1.1%	1.2%	7	0.30%	0.55%	0.70%

Inflation factor (IF) is the quantity that the sample size needs to be multiplied by to preserve power for cluster sampling, compared to independent sampling.

In INTERHEART, the average control subject is predicted to have a 33% probability (SD=17%) of having an MI based on their risk factor profile.

Based upon the intra-country variability among controls in INTERHEART, an inflation factor of 50 would be required to preserve power for cluster sampling. In reality, our sampling strategy would be based on smaller, but more homogeneous communities and therefore the IF would be substantially lower, and hence power much greater. Nevertheless, even with the current inflation factor; we have 90% power to detect differences in predicted risk of 1.64% between adjacent thirds of communities divided by societal factors. Power with varying IF (40 and 60) and baseline predicted probabilities of MI (25% and 40%) are also provided. A detectable difference of 1.5% represents an effect size that is 9% of one SD. In reality, because we expect lower IF, smaller effect sizes would be detected at >90% power.

The IF for metabolic syndrome was calculated from the PURE pilot study from 8 locations in India, and was 3. The average prevalence of metabolic syndrome across these 8 communities is 13.5%. This indicates high power to detect differences as small as about 1% between thirds of communities in rates of MS.
The IF for CVD was calculated from 8 centres in the PURE pilot, and prevalence was derived from the Global Burden of Disease study. For example, if prevalence of CVD is 3% in a community, there is high power to detect differences versus another community which has a rate of 3.5%.
*Target sample size is 135,000, however an approximate sample size of 120,000 was used to be conservative.

Table 11b: Detectable differences in specific outcomes (INTERHEART score, metabolic syndrome, CVD rates) comparing 3 groups with varying degrees of changes in each societal domain in the community at 90% power and type I error rate of 0.05.

	INTERHEART Risk Score			Metabolic Syndrome			CVD		
Baseline Prevalence	25%	32.5%	40%	10%	15%	10%	1%	3%	5%
Effect size	0.0324	0.0342	0.0356	0.0107	0.0106	0.0107	0.0113	0.0116	0.0120

The effect size is the variance of the means divided by the within group variance. The effect size is an index of the separation expected among the observed means or proportions using a logit scale for the INTERHEART risk score, and a log scale for metabolic syndrome and CVD. For example, an effect size of 0.0342 could be found if the mean INTERHEART risk score increased by an average of 0.0%, 0.5% and 1.0% in the low, medium and high change HTI groups, respectively. For CVD, an effect size of 0.0116 could represent increases of 0.0%, 0.4%, and 0.9% in the same three groups, from a baseline rate of 3.0%. Similar considerations indicate high power to detect even small differences in each risk factor for CVD (e.g. BP, lipids, obesity, etc).

Table 12a: Names, expertise and roles of Canadian Investigators

Investigator name	Study Role	Expertise	Study Responsibility
S. Yusuf	Principal Investigator (PI)	Cardiology, Epidemiology	Will be responsible for overseeing all aspects of the study both at the Project Office and worldwide.
S. Anand	Co-Investigator	Ethnicity, Nutrition, Psychosocial, Gender/Sex Issues	Input into specific areas plus assisting PI and A. Merchant in supervision of the project
K. Teo	Co-Investigator	Cardiology, Epidemiology	Oversee recruitment of subjects in Canada & at Hamilton site, co-chair of events & adjudication committee
M. McQueen	Co-Investigator	Laboratory Medicine	Supervision of blood storage, all biochemical analyses and standardization of laboratories.
G. Dagenais*	Co-Investigator (Laval)	Cardiology, Epidemiology	Oversee recruitment of subjects in Quebec, co-chair of events and adjudication committee
H. Gerstein	Co-Investigator	Diabetes, Cardiology	Expert in diabetes & dysglycemia
C Chow	Co-Investigator	Cardiology, Epidemiology	Overseeing all aspects of EPOCH
S.A. Lear§	Co-Investigator (UBC/Simon Fraser) (Simon Fraser)	Cardiology, Lipids, Epidemiology, Community Health	Oversee recruitment of subjects in BC, input into lipids, hypertension, activity, built environment
P. Raina	Co-Investigators	Epidemiology/ Population Health	Links to Canadian Longitudinal Study of Aging

*At University of Laval, Quebec. All others are at McMaster University
§At University of British Columbia

Table 12b: Names and Roles of Additional Special Experts who will facilitate key aspects of the study

Working Groups	Names/Institutions	Expertise
1. Psychosocial working group	George Davey Smith, U of Bristol, UK	Social Epidemiology
	S.V. Subramanian, Harvard, US	Psychosocial factors, social geography
	John Lynch, McGill, Montreal	Social Epidemiology
2. Built Environment	Ann Forsyth, U of Minneapolis, Minn, USA	Urban Planning, architecture, and health
	Daniel Rodriguez, U of North Carolina, Chapel Hill*	Urban Planning, City development with expertise in developing countries
3. Tobacco policy	Martin McKee, London School of Tropical Medicine, UK	Tobacco Policy
4. Genetics	Daniel Gaudet, U of Montreal	Population Genomics
	Tom Hudson, McGill University¶	
	Bernard Keavney, Univ of Newcastle upon Tyne, UK¶	
5. Statistics	Harry Shannon, McMaster University¶	Advanced Statistical Issues
	Janet Wittes, Washington, DC¶	

¶Collaborator

Table 12c: Names, Roles and Expertise of International Collaborators

	Study Role	Expertise
AFRICA		
SOUTH AFRICA		
Lanthe Kruger	National Coordinator and Principal Investigator	Epidemiology, Nutrition
Thandi Puoane	Investigator	Epidemiology
TANZANIA		
Karen Yeates	National Coordinator and Principal Investigator	Epidemiology, Nephrology
Kemi Tibazarwa	Investigator	Epidemiology
NORTH SUDAN		
Ahmed ElSayed	National Coordinator and Principal Investigator	Cardiology
ZIMBABWE		
Jephat Chifamba	National Coordinator and Principal Investigator	Physiology, Tobacco surveillance
ASIA		
BANGLADESH		
Omar Rahman	National Coordinator and Principal Investigator	Epidemiology, Public Health
Rita Yusuf	Investigator	Epidemiology
CHINA		
Liu Lisheng	National Coordinator and Principal Investigator	Cardiology, Hypertension
Li Wei	National Coordinator and Principal Investigator	Statistics
INDIA, Bangalore		
Prem Mony	National Coordinator and Principal Investigator	Epidemiology
INDIA, Chennai		
Mohan. V	Investigator	Diabetes
INDIA, Trivandrum		
Raman Kutty	Investigator	Public Health
K Vijayakumar	Investigator	Public Health
Sanjeev Nair	Investigator	Respiratory Health
INDIA, Jaipur		
Rajeev Gupta	Investigator	Cardiology
INDIA, Chandigarh		
Rajesh Kumar	Investigator	Community Medicine
Kazakhstan		
Nurlan Abdikaliyev/Kairat Davletov	National Coordinator and Principal Investigator	Epidemiology
KYRGYZSTAN		
Erkin Mirrakhimov	National Coordinator and Principal Investigator	Epidemiology
MALAYSIA		
Khalid Yusoff	National Coordinator and Principal Investigator	Epidemiology, Diabetes
Noor Hassim	Investigator	Community Medicine
PAKISTAN		
Romaina Iqbal	National Coordinator and Principal Investigator	Epidemiology
Khawar Kazmi	Investigator	Cardiology
PHILIPPINES		
Tony Dans	National Coordinator and Principal Investigator	Epidemiology, Cardiology
RUSSIAN FEDERATION		
Sergey Boytsov/Anna Kontsevaya	National Coordinator and Principal Investigator	Epidemiology

EUROPE		
POLAND		
Witold Zatonski/ Andrej Szuba	National Coordinator and Principal Investigator	Epidemiology Cancer Prevention
SWEDEN		
Annika. Rosengren	National Coordinator and Principal Investigator	Preventive Cardiology Psychosocial Factors
TURKEY		
Aytekin Oguz	National Coordinator and Principal Investigator	Endocrinology, Metabolism
MIDDLE EAST		
IRAN		
Roya Kelishadi	National Coordinator and Principal Investigator	Cardiovascular Epidemiology & Prevention
PALESTINE		
Rasha Khatib	National Coordinator and Principal Investigator	Epidemiology, Public Health
SAUDI ARABIA		
Khalid Alhabib	National Coordinator and Principal Investigator	Cardiology
UAE, Dubai		
A.M. Yusufali	National Coordinator and Principal Investigator	Preventive Cardiology, Clinical Trials
SOUTH AMERICA		
ARGENTINA		
Rafael Diaz	National Coordinator and Principal Investigator	Preventive Cardiology, Clinical Trials
BRAZIL		
Alvaro Avezum	National Coordinator and Principal Investigator	Preventive Cardiology, Clinical Trials
CHILE		
Fernando Lanas	National Coordinator and Principal Investigator	Preventive Cardiology, Epidemiology
COLOMBIA		
Patricio Lopez Jaramillo	National Coordinator and Principal Investigator	Pharmacology
PERU		
Jaime Miranda	National Coordinator and Principal Investigator	Epidemiology

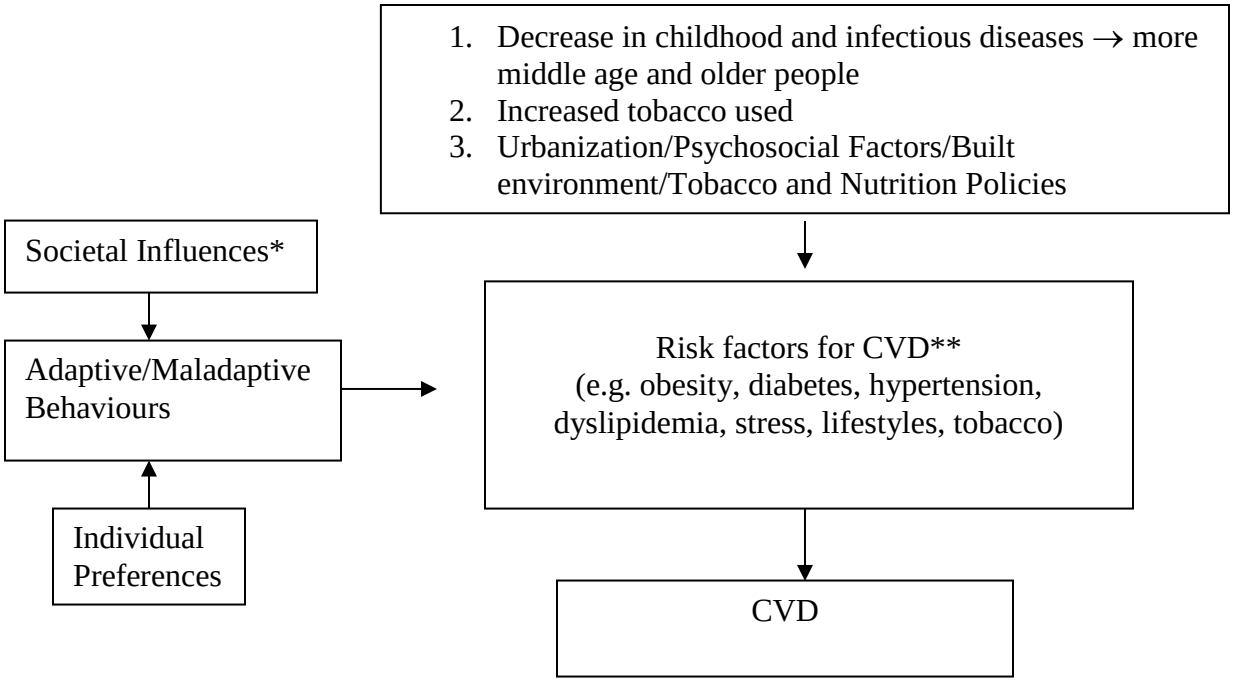
Table 13: PURE Study Development and Future Timelines

Activity		Status
July 2000	Initial Conceptualization of Project and "seed" funding from Aventis	Done
Jan 2001	Data collection starts in Bangalore and Palamner	Done
December 2001	2500th subject recruited	Done
June 2001	PURE-India Meeting – 3 other sites in India (Kerala, Rajasthan, Tamil Nadu) join the study	Done
December 2002	5000th subject recruited in Bangalore, other sites start recruiting	Done
May 2002	*1st PURE International Steering Committee Meeting, Sydney Australia.* Representatives from Canada, Chile, UAE, Kuwait, Zimbabwe, Kenya, China, Thailand, Russia and India.	Done
June 2002	Chandigarh identified as 5th site within India	Done
April 2003	*2nd PURE International Steering Committee Meeting, Marbella Spain.* New Countries in attendance: Colombia, Argentina, Brazil, S. Africa	Done
July 2003	Received CIHR pilot funding through the *Global Health Research Program Development and Planning Grant*	Done
August 2003	Setup and Recruitment for an international PURE Pilot begins in 11 countries outside of India	Done
October 2003	PURE nutrition development grant submitted to CIHR (under review)	Done
December 2003	10,000th subject recruited in India	Done
December 2003	*3rd PURE International Steering Committee Meeting, New Delhi, India.*	Done
February 2004	PURE India second staff training meeting	Done
March 2004	Initial application submitted to CIHR (not accepted)	Done
April/May 2004	Contacts with additional pharmaceutical companies	Initiated
April/May 2004	Grants submitted in China, India, Colombia and Chile	Done
July 2004	Funding commitment from Boehringer Ingelheim	Done
July 2004	South Africa receives funding from Sanpad	Done
August 2004	15,000th patient recruited from India	Done
Aug/Sept 2004	Grant resubmitted to CIHR (not accepted)	Done
September 2004	Database programming in advanced stage	Done
September 2004	FFQ developed for most countries	Done
December 2004	*4th PURE International Steering Committee Meeting*	Done
December 2004	Completion of core protocol related development work by all working groups	Done
April, May, June 2005	Several 1 to 2 day consultations with experts in social epidemiology, social sciences, built environment, nutrition and tobacco policy and health transitions leading to considerable revisions of the protocol	Done
August 2005	Additional funding commitment from Sanofi-Aventis, Servier, and AstraZeneca obtained	Done
September 2005	**Grant resubmission to CIHR**	**Done**
October 2005	Initial cycle of follow-up commences in India	Done
January 2006	Final global training meeting and initiation of vanguard recruitment in all countries	Done
July 2006	Completion of 25,000 subjects from India + 10,000 (500-1000/country) from other countries (35,000 subjects from 100 communities)	Done
August 2006	Initiation of full scale recruitment	Done
September 2008	Recruitment of 140,000 subjects to be completed	Done
2007 onwards	2-year follow-up for events begins in centers outside India, plus average of random 20% of each community to characterize community profile	Done
2007 onwards	Manuscripts based on baseline data from 140,000 subjects and methodology papers to be submitted	Done
2010 to 2013	6-year follow-up begins	Done
2010 onwards	Manuscripts from 3-year follow-up to be submitted.	Done
2013 to 2016	2-yearly follow-up continues	Initiated
2016 onwards	Manuscripts from 6 year follow-up to be submitted	To be done
2016 to 2019	12-year follow-up begins	To be done
2019 onwards	Manuscripts from 12-year follow-up to be submitted.	To be done

Table 14: Examples of Potential Interventions at a Societal level for which supportive evidence can be gathered from PURE.

a. Will taxation or subsidies of goods other than tobacco have an impact on their use (e.g. on bad or good foods)? • This could affect economic policies • This could affect food policies
b. How can changes in the built environment influence activity? • This could affect transportation policies: - walking, cycling paths - community design - zoning laws regarding land mix - traffic patterns - building architecture
c. What factors affect the consumption of healthy foods? • This could affect food policies, food choices and urban and rural planning that promotes access to low cost fresh foods: - advertising - pricing - food labeling - access-local store vs supermarkets - availability and seasonal supplies
d. What factors affect psychosocial factors and stress? • This could affect social services as well as policies that promote specific social and family values and structures: - social capital - networking - family structures
e. Impact of work place policies on activity, exposure to tobacco and nutrition (activity breaks, design of workplaces to decrease sedantariness, decreasing unhealthy foods in cafeteria, subsidizing healthy choices). Impact on national policies and their enforcement related to tobacco use (smoke free zones, restriction of sales to minors, enforcement of policies)
f. Health education about activity, nutrition, and avoidance of tobacco to facilitate more healthy choices which can be sustained (by making the environment conducive to such behaviours).

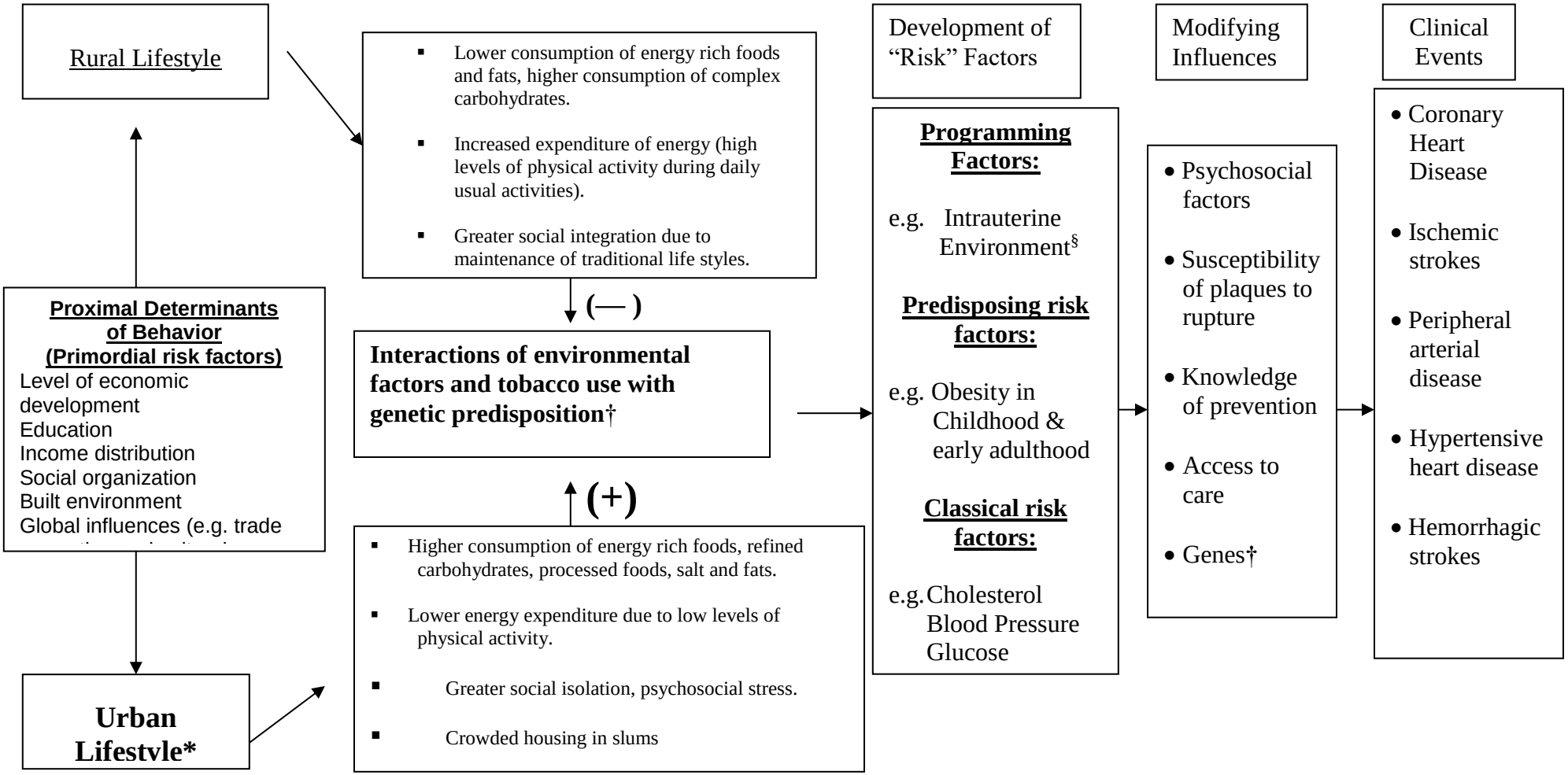
Figure 1: Hypothesis for the Global Increase in CVD



*Very little is directly known about the societal causes of CVD.

**The nine INTERHEART risk factors account for >90% of the population attributable risk for acute myocardial infarction globally and in every region of the world

Figure 2: Schema for societal level "Pathway" for the development of atherothrombotic cardiovascular diseases



*Footnote: The contrasting lifestyles between urban and rural settings are typical for countries in transition. However, for countries that are already urbanized for a few decades, lifestyles in rural settings have been altered due to mechanization and various social influences so that they may be similar to urban settings. Consumption of inappropriate diets, higher rates of tobacco use and decreases in physical activity even in rural settings, increase risk factors and lead to higher CVD rates. These changes are already evident in North America. †Genes likely play a role in both the development of risk factors, and in modulating the effect of known risk factors to CVD. §A separate study (funded by CIHR) is evaluating this

Figure 3: Distribution of BMI, LDL-cholesterol, and systolic blood pressure in urban Canadians of European and South Asian origin, urban Indians in Bangalore and rural Indians in Palamner. Note the marked differences in distribution of all risk factors between the rural group and three urban groups (which appear to be more similar). Therefore, inclusion of rural and urban populations in countries at different levels of health transition will provide an opportunity to assess the importance of a risk factor to CVD across a much broader range of values, than studies confined to a single MIC such as Canada.

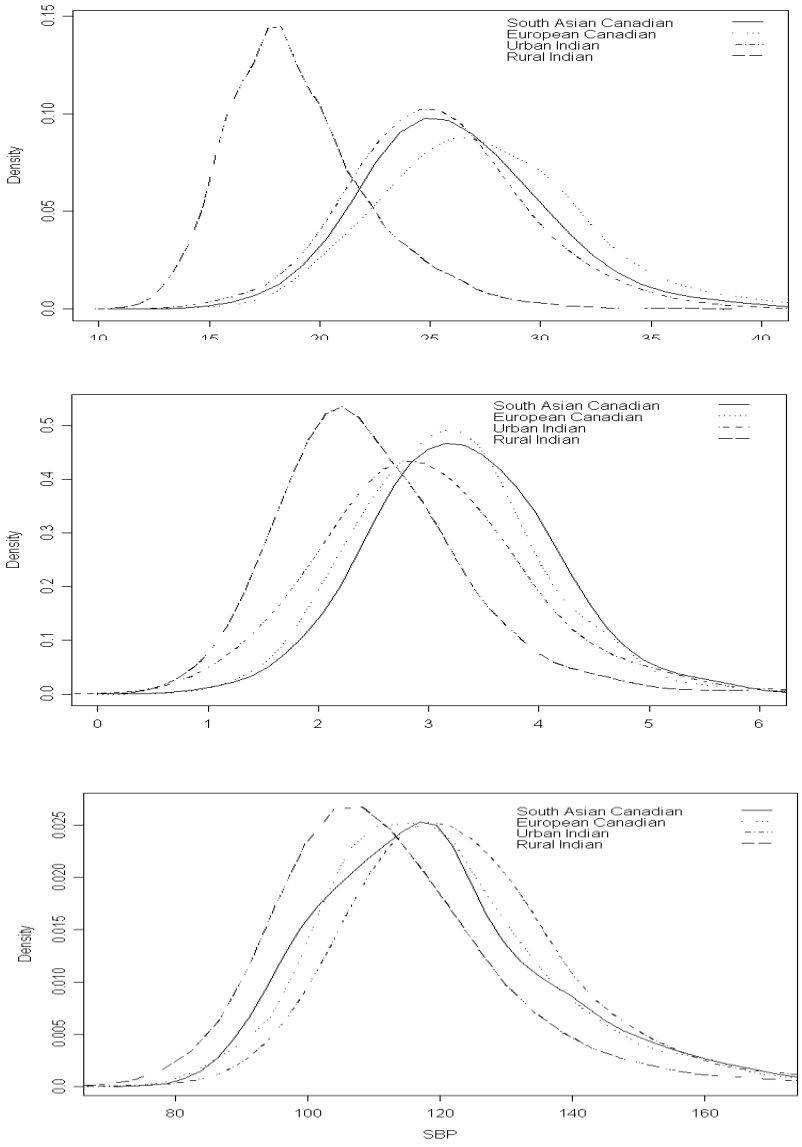


Figure 4: ApoB/ApoA-1 Ratio

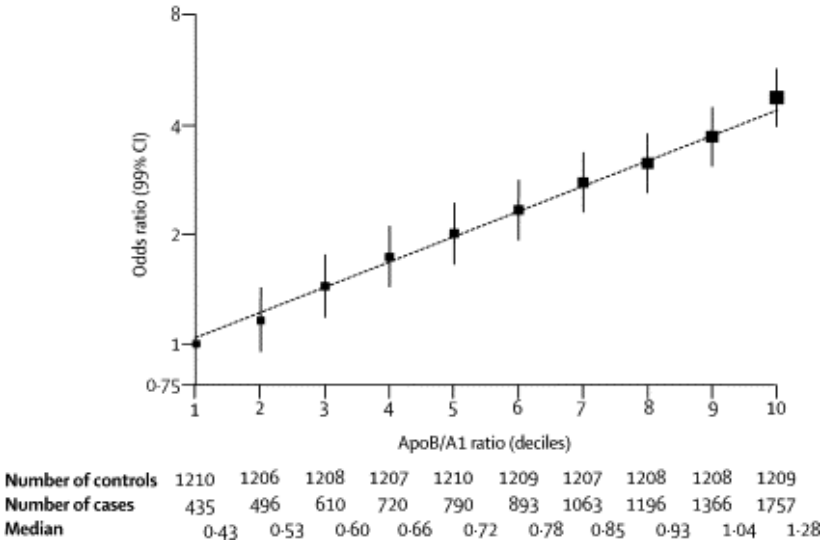


Figure 5: Risk of AMI associated with the composite <u>Psychosocial</u> Index overall and by region. Note the similarity of effects in all regions, except one. This might be due to the play of chance in light of the large number of subgroups analyzed.

Region	n	Odds ratio (99%CI)	PAR (99% CI)
Overall	24767	2·51 (2·15–2·93)	28·8% (22·6–35·8)
W Eur	1375	3·92 (1·95–7·91)	38·9% (20·2–61·5)
CE Eur	3473	1·14 (0·73–1·78)	4·9% (0·0–96·0)
MEC	2892	2·77 (1·78–4·29)	41·6% (25·2–60·1)
Africa	1259	3·17 (1·59–6·33)	40·0% (17·3–67·9)
S Asia	3300	2·15 (1·45–3·17)	15·9% (4·0–46·3)
China/HK	5894	6·00 (4·05–8·89)	35·4% (24·6–47·9)
SE Asia	1921	1·85 (1·02–3·37)	26·7% (17·8–38·1)
ANZ	1255	1·99 (1·02–3·87)	28·9% (10·0–59·7)
S Am	2783	2·08 (1·29–3·36)	35·6% (17·2–59·5)
N Am	615	4·16 (1·50–11·53)	51·4% (24·0–78·0)

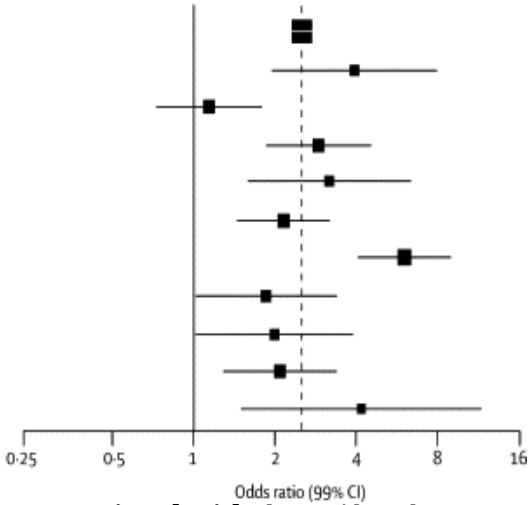


Figure 6: Risk of acute myocardial infarction associated with current or former <u>smoking,</u> overall and by region after adjustment for age and sex. Note the similarity of the odds ratios in all regions

Region	n	Control (%)	Case (%)	Odds ratio (99% CI)	PAR (99% CI)
Overall	26527	47·9	65·6	2·27 (2·11–2·44)	36·4% (33·9–39·0)
W Eur	1403	55·0	73·3	1·96 (1·47–2·62)	29·3% (17·8–44·1)
CE Eur	3624	54·2	69·8	1·92 (1·60–2·30)	30·2% (23·0–38·6)
MEC	3301	45·4	64·5	2·64 (2·19–3·19)	45·5% (39·3–51·9)
Afr	1339	53·8	70·1	2·18 (1·60–2·96)	38·9% (27·1–52·2)
S Asia	3706	41·0	60·3	2·43 (2·03–2·89)	37·4% (31·6–43·6)
China/HK	6062	42·7	62·2	2·30 (2·00–2·65)	35·9% (31·5–40·5)
SE Asia	2131	57·1	70·5	1·96 (1·54–2·49)	36·0% (25·9–47·5)
ANZ	1267	54·2	77·4	2·80 (2·03–3·86)	44·8% (33·0–57·2)
S Am	3068	48·9	69·4	2·35 (1·92–2·87)	38·3% (31·0–46·1)
N Am	626	64·6	76·8	1·82 (1·14–2·88)	26·1% (8·8–56·5)

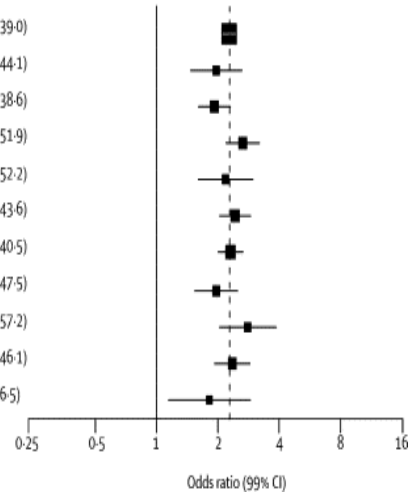


Figure 7: Risk of acute myocardial infarction associated with ApoB/ApoA1 ratio (top vs lowest quintiles), overall and by region after adjustment for age, sex, and smoking. Note the similarity of the odds ratios in all ages

Region	n	Control (%)	Case (%)	Odds ratio (99% CI)	PAR (99% CI)
Overall	21408	20·0	33·5	3·87 (3·39–4·42)	54·1% (49·6–58·6)
W Eur	1047	13·8	29·4	3·76 (2·10–6·74)	44·6% (23·5–67·8)
CE Eur	2618	20·3	29·4	2·20 (1·52–3·18)	35·0% (19·2–54·9)
MEC	3291	29·9	49·6	5·33 (3·48–8·18)	70·5% (57·8–80·7)
Afr	1037	18·0	39·0	7·93 (4·32–14·58)	74·1% (59·7–84·6)
S Asia	2820	27·7	42·7	3·81 (2·49–5·83)	58·7% (42·7–73·1)
China/HK	5400	7·3	14·3	3·43 (2·61–4·51)	43·8% (36·7–51·2)
SE Asia	1858	22·7	48·0	6·22 (3·71–10·41)	67·7% (52·0–80·2)
ANZ	487	13·8	26·8	3·97 (1·71–9·22)	43·4% (16·0–75·6)
S Am	2644	27·1	40·6	2·79 (1·85–4·23)	47·6% (29·6–66·2)
N Am	206	12·4	28·8	4·75 (1·34–16·86)	50·5% (18·2–82·4)

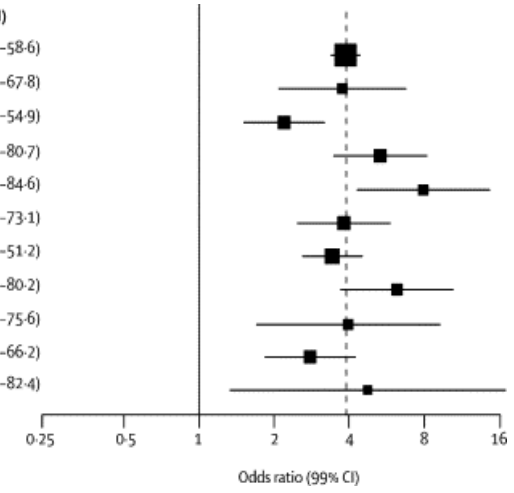
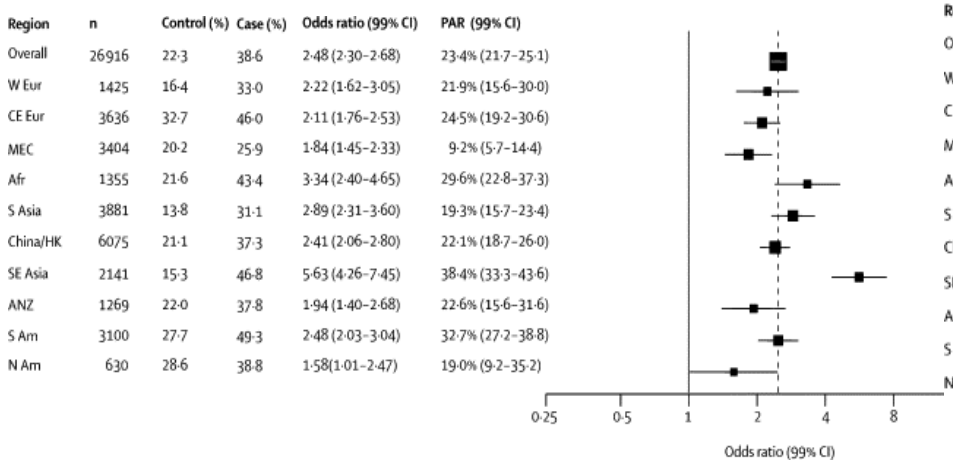
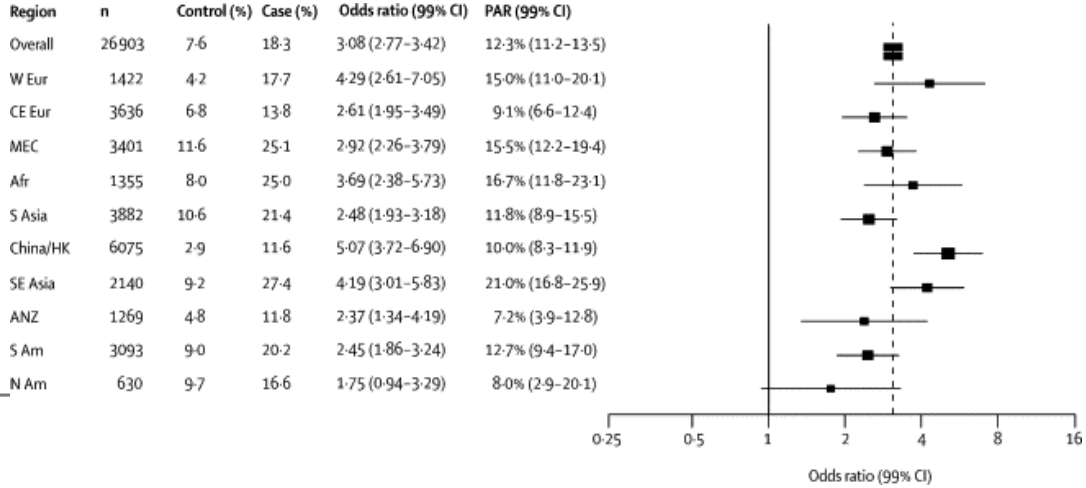


Figure 8: Risk of AMI associated with self-reported <u>hypertension</u> overall and by region after adjustment for age, gender, and smoking. Note the similarity of the odds ratio in every region.



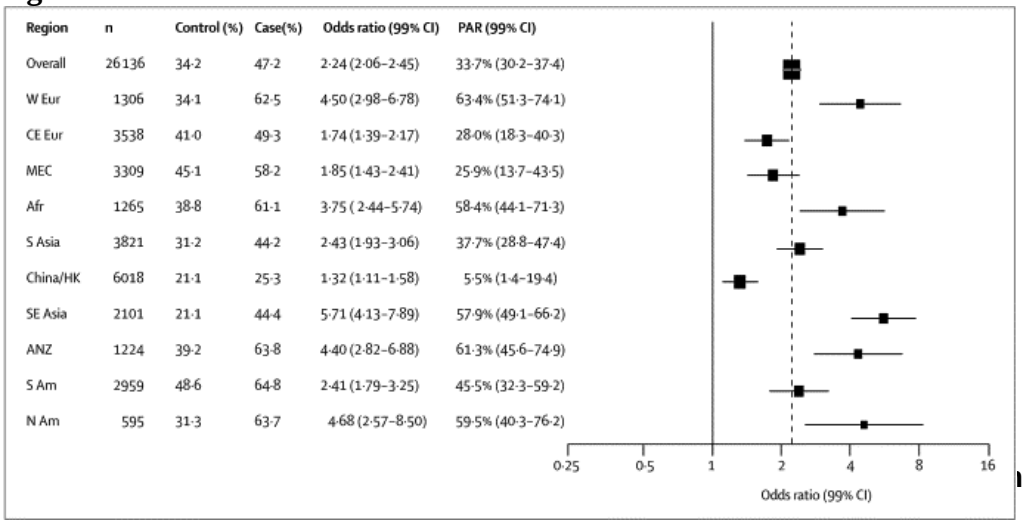
Region	n	Control (%)	Case (%)	Odds ratio (99% CI)	PAR (99% CI)
Overall	26916	22·3	38·6	2·48 (2·30–2·68)	23·4% (21·7–25·1)
W Eur	1425	16·4	33·0	2·22 (1·62–3·05)	21·9% (15·6–30·0)
CE Eur	3636	32·7	46·0	2·11 (1·76–2·53)	24·5% (19·2–30·6)
MEC	3404	20·2	25·9	1·84 (1·45–2·33)	9·2% (5·7–14·4)
Afr	1355	21·6	43·4	3·34 (2·40–4·65)	29·6% (22·8–37·3)
S Asia	3881	13·8	31·1	2·89 (2·31–3·60)	19·3% (15·7–23·4)
China/HK	6075	21·1	37·3	2·41 (2·06–2·80)	22·1% (18·7–26·0)
SE Asia	2141	15·3	46·8	5·63 (4·26–7·45)	38·4% (33·3–43·6)
ANZ	1269	22·0	37·8	1·94 (1·40–2·68)	22·6% (15·6–31·6)
S Am	3100	27·7	49·3	2·48 (2·03–3·04)	32·7% (27·2–38·8)
N Am	630	28·6	38·8	1·58(1·01–2·47)	19·0% (9·2–35·2)

Figure 9: Risk of AMI associated with self-reported <u>diabetes</u> overall and by region after adjusting for age, gender and smoking. Note the similarity of effects in all regions



Region	n	Control (%)	Case (%)	Odds ratio (99% CI)	PAR (99% CI)
Overall	26903	7·6	18·3	3·08 (2·77–3·42)	12·3% (11·2–13·5)
W Eur	1422	4·2	17·7	4·29 (2·61–7·05)	15·0% (11·0–20·1)
CE Eur	3636	6·8	13·8	2·61 (1·95–3·49)	9·1% (6·6–12·4)
MEC	3401	11·6	25·1	2·92 (2·26–3·79)	15·5% (12·2–19·4)
Afr	1355	8·0	25·0	3·69 (2·38–5·73)	16·7% (11·8–23·1)
S Asia	3882	10·6	21·4	2·48 (1·93–3·18)	11·8% (8·9–15·5)
China/HK	6075	2·9	11·6	5·07 (3·72–6·90)	10·0% (8·3–11·9)
SE Asia	2140	9·2	27·4	4·19 (3·01–5·83)	21·0% (16·8–25·9)
ANZ	1269	4·8	11·8	2·37 (1·34–4·19)	7·2% (3·9–12·8)
S Am	3093	9·0	20·2	2·45 (1·86–3·24)	12·7% (9·4–17·0)
N Am	630	9·7	16·6	1·75 (0·94–3·29)	8·0% (2·9–20·1)

Figure 10: Risk of AMI associated with abdominal <u>obesity</u> measured as waist to hip ratio (upper third vs lowest third) overall and by region, after adjusting for age, gender and smoking. Note the similarity of effect in all regions.

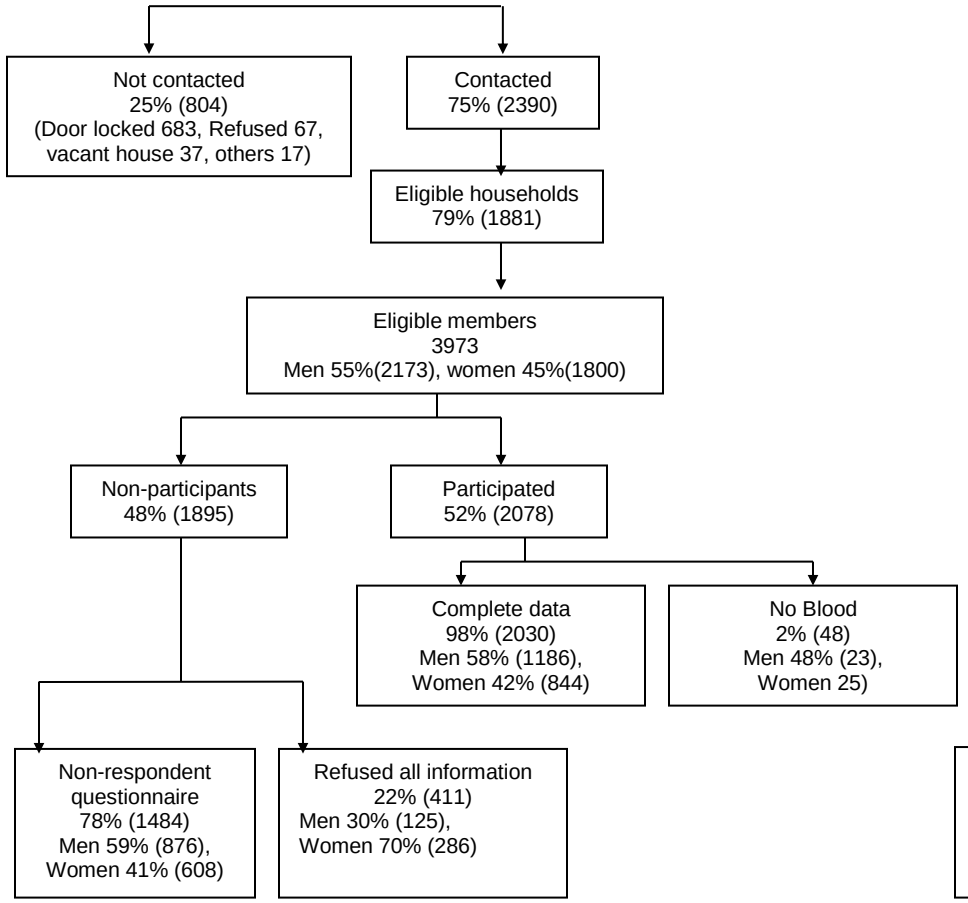


Region	n	Control (%)	Case(%)	Odds ratio (99% CI)	PAR (99% CI)
Overall	26136	34·2	47·2	2·24 (2·06–2·45)	33·7% (30·2–37·4)
W Eur	1306	34·1	62·5	4·50 (2·98–6·78)	63·4% (51·3–74·1)
CE Eur	3538	41·0	49·3	1·74 (1·39–2·17)	28·0% (18·3–40·3)
MEC	3309	45·1	58·2	1·85 (1·43–2·41)	25·9% (13·7–43·5)
Afr	1265	38·8	61·1	3·75 (2·44–5·74)	58·4% (44·1–71·3)
S Asia	3821	31·2	44·2	2·43 (1·93–3·06)	37·7% (28·8–47·4)
China/HK	6018	21·1	25·3	1·32 (1·11–1·58)	5·5% (1·4–19·4)
SE Asia	2101	21·1	44·4	5·71 (4·13–7·89)	57·9% (49·1–66·2)
ANZ	1224	39·2	63·8	4·40 (2·82–6·88)	61·3% (45·6–74·9)
S Am	2959	48·6	64·8	2·41 (1·79–3·25)	45·5% (32·3–59·2)
N Am	595	31·3	63·7	4·68 (2·57–8·50)	59·5% (40·3–76·2)

India: Bangalore (Urban), Palamner (Rural)

PURE

URBAN (Bangalore)
(Total households = 3194)



RURAL (Palamner)
(Total households = 2734)

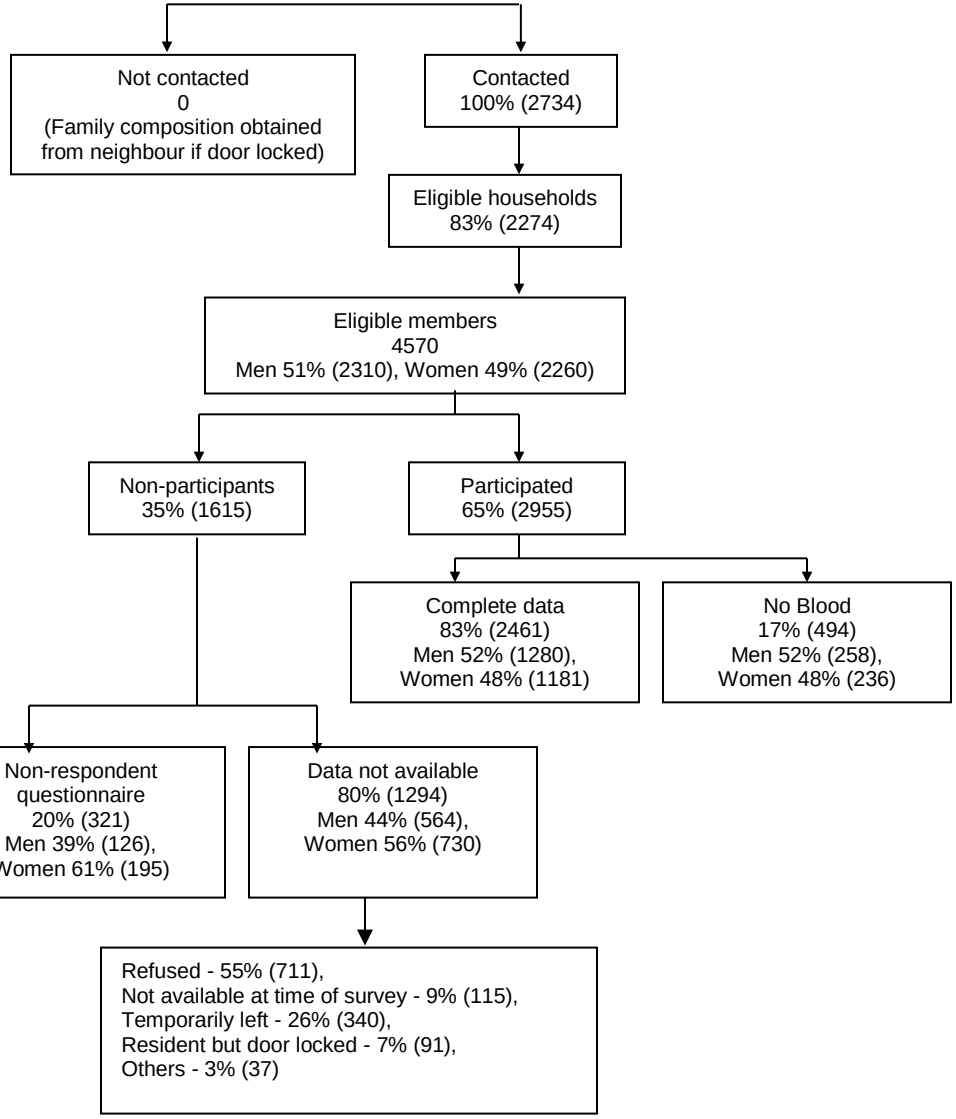
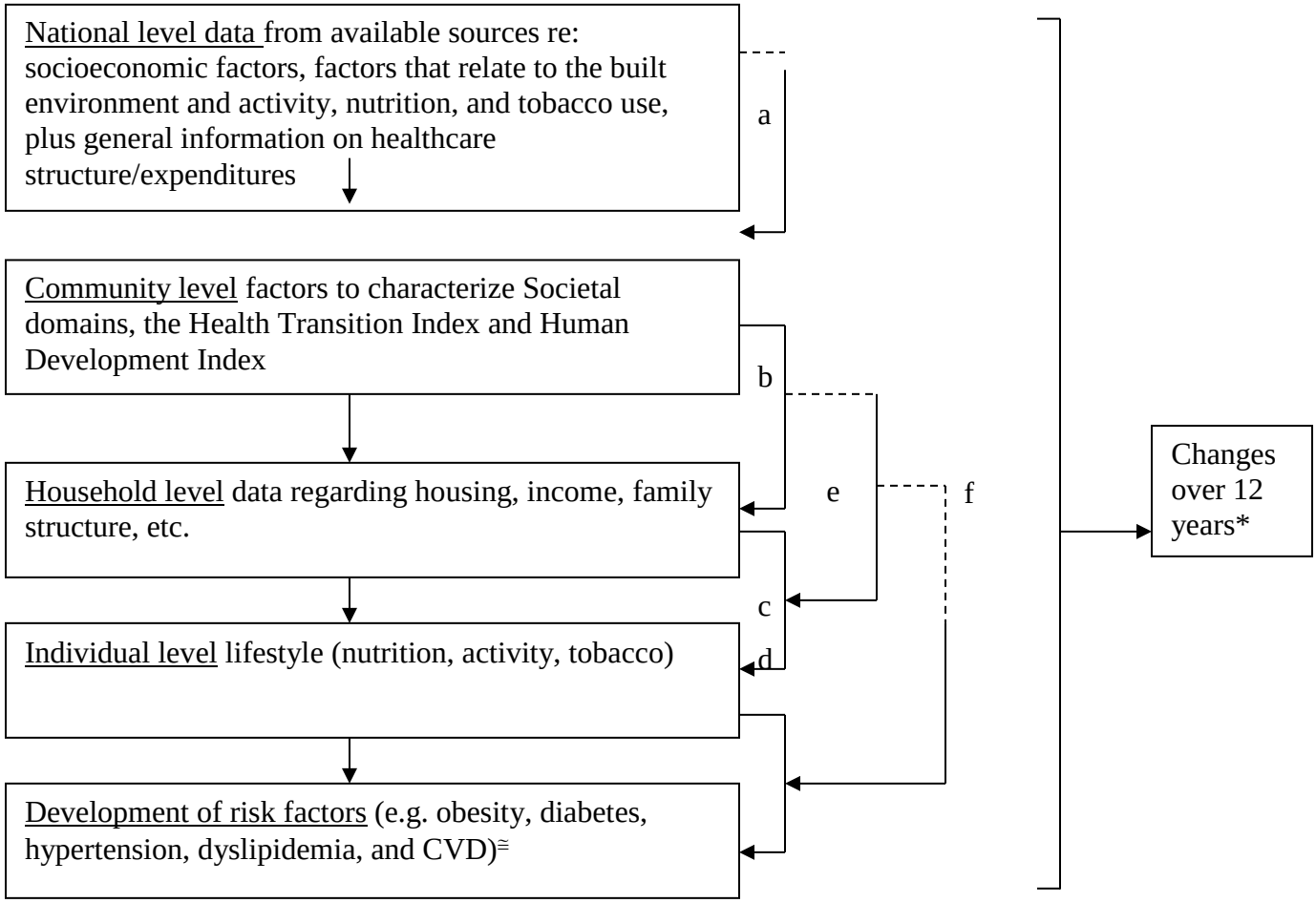


Figure 12: Schema for Levels of Data Collection and Potential Analyses

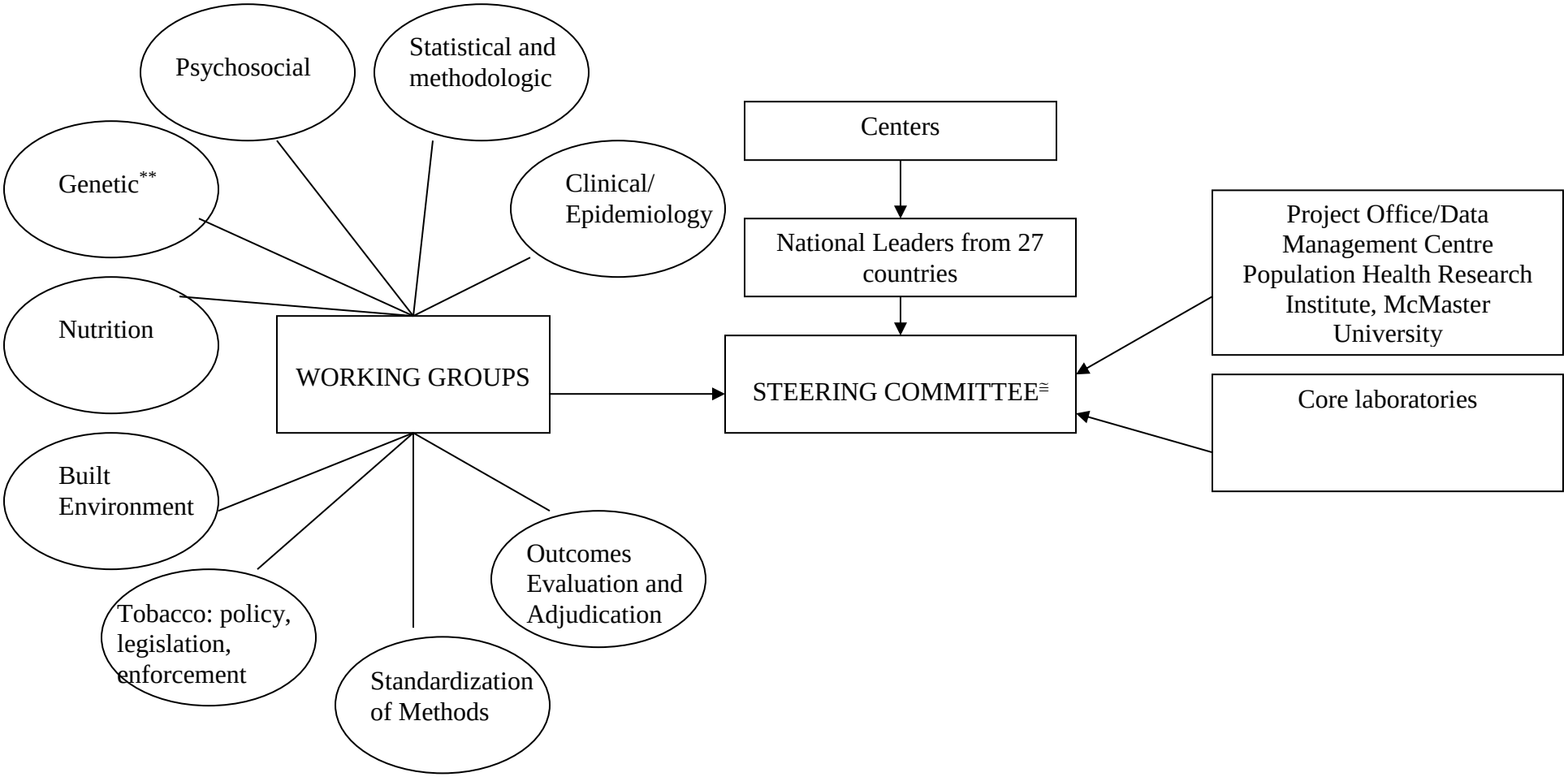


Data will be collected at 4 levels. Initial analyses will explore the impact of societal and community factors on household level parameters (b), the impact of the combination of household and societal community level factors on individual lifestyles (c and e) and the impact of community, household and individual level factors/data on individual level risk of developing risk factors or CVD (d and f)

≅The unit of measurement could be both the community level of risk factors, as well as individual levels of risk factors

* Changes in risk factors will be documented both at individual (cohort component) and community ("Snap shots" of a random sample at 6 and 12 years)

Figure 13: Structure/Organization of the PURE Study



≅The Steering Committee will include all National Leaders, 4 members from the Project Office, the Chair of each working group and representatives of all major funders.

Membership of various committees: a) Clinical/Epidemiology: S. Yusuf, A. Merchant, S. Anand, K. Teo, G. Dagenais, S. Lear, A. Chockalingam, H. Gerstein b) Statistical methodology: J. Pogue, J. Wittes, H. Shannon c) Psychosocial: S. Anand, G. Davey-Smith, H. Arthur, S. Elliott, J. Lynch, SV Subramanian d) Genetics: T. Hudson, S. Anand, D. Gaudet, B. Keavney e) Nutrition: A. Merchant, S. Anand, W. Zatonski, M. Vaz f) Built environment: A. Forsyth, D. Rodriguez, P. Kanaroglou, A. Merchant g) Tobacco policy, legislation and enforcement: L. Waverley, K. Teo, M. McKee h) Standardization of methods: K. Teo, G. Dagenais, M. McQueen, S. Lear i) Outcomes: G. Dagenais + one person from each country

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