ABSTRACT

DESCRIPTION. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This descnption is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

The increased prevalence of type 2 diabetes among children is attributed to a simultaneous increase in childhood obesity. Most children are diagnosed during puberty. Ethnic minority children, such as Native Americans, African Americans, and Hispanics, are disproportionately affected. Asians represent a rapidly growing minority group in the United States that is poorly represented in epidemiologic research. Based on information from studies of school children in Japan and studies in Japanese-American adults, Japanese American children are also likely to be at high risk due to a tendency toward central adiposity.

The overall aim of this study is to better understand in children the metabolic changes that precede the development of type 2 diabetes, and the influence of Asian ethnicity on diabetes risk. The specific aims of this project are: 1) to describe the metabolic changes and adipose factors that are associated. with the insulin resistance metabolic syndrome in prepubertal children; 2) to describe the relationship between pancreatic islet B-cell function and family history of type 2 diabetes; 3) to describe changes in these factors as children progress through puberty; 4) to describe the relationship of diet and physical activity to the metabolic and adipose factors; and 5) to describe the relationship between Japanese ancestry and metabolic, adipose, and insulin secretion factors. To accomplish these goals, a longitudinal cohort study of 450 prepubertal (8-10 year old) nondiabetic Japanese-American and Caucasian children is proposed. Measurements at baseline and 2 year follow-up will include: lipids and LDL particle size, insulin, C-peptide, proinsulin, glucose tolerance and insulin secretion determined by an intravenous glucose tolerance test, plasminogen activator inhibitor-1, fibrinogen, C-reactive protein, insulin-like growth factor-1 and insulin-like growth factor binding protein-3, body composition by DEXA, and intra-abdominal fat by MRI.

This study will improve the understanding of how pubertal changes in metabolic and adipose factors affect diabetes risk in Asian and Caucasian children.

PERFORMANCE SITE(S) ***(organization, city, state)***

University of Washington (UW), Seattle, WA
Children's Hospital and Regional Medical Center (CHMC), Seattle, WA
Veteran's Administration Medical Center (VAMC), Seattle, WA

KEY PERSONNEL See instructions on Page 11. ***Use continuation pages as needed*** **to provide the required information in the format shown below.**

Name	Organization	Role on Project

A. SPECIFIC AIMS

The increased prevalence of type 2 diabetes among children is attributed to a simultaneous increase in childhood obesity. Many ethnic minority groups are known to be at increased risk for type 2 diabetes in adulthood, yet relatively little is known about the risk factors that precede this condition among ethnic minority youth. Asians represent a rapidly growing minority group in the United States that is poorly represented in epidemiologic research. Asian adults, despite having a low prevalence of obesity, appear to be at increased risk for diabetes due to a predisposition to accumulate visceral fat. While no data are available on type 2 diabetes among Asian-American children, in Japan type 2 diabetes prevalence among school children has increased markedly in recent years as obesity has become more prevalent. Rates are likely to be even higher among Asian children in the United States than children in Japan, since obesity is more prevalent in this country.

The **long-term aim** of this study is to better understand in children the metabolic changes that precede the development of type 2 diabetes, and the influence of Asian ethnicity on diabetes risk. This proposal extends the Japanese American Community Diabetes Study to create a separate, longitudinal study of prepubertal children of varying proportions of Japanese ancestry (ranging from 0 to 100%) who will be followed into and through puberty.

Specific Aim 1: To describe in prepubertal (8-10 years), nondiabetic children the metabolic and adipose factors that are associated with the insulin resistance metabolic syndrome. These include fasting plasma lipids (cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol), LDL particle size, plasminogen activator inhibitor-1 (PAI-1), fibrinogen, C-reactive protein, glucose, insulin, C-peptide, and proinsulin; glucose tolerance assessed as glucose disappearance rate constant (K_G) during an intravenous glucose tolerance test; body composition by DEXA; body fat distribution by MRI; and body mass index.

Hypothesis 1: Features of the metabolic syndrome are evident in some prepubertal children.

Specific Aim 2: To assess variation in pancreatic islet ß-cell function by measuring fasting plasma insulin, C-peptide, proinsulin, and acute insulin response to glucose (AIR_g) by an intravenous glucose tolerance test.

Hypothesis 2: Glucose-stimulated insulin secretion is lower among children with a family history of type 2 diabetes.

Specific Aim 3: To describe the changes in these factors as children progress through and complete puberty. Tanner staging is used and plasma testosterone, estradiol, DHEA-S, IGF-1, and IGFBP-3 are measured.

Hypothesis 3: Puberty is associated with changes in body fat distribution and metabolic parameters in a direction consistent with higher risk of glucose intolerance and cardiovascular disease.

Specific Aim 4: To describe the relationship of lifestyle factors (diet and physical activity) to the metabolic and adipose factors, and changes therein.

Hypothesis 4: Diet and physical activity are important predictors of adiposity and metabolic changes in children.

Specific Aim 5: To describe the relationship of proportion of Japanese ancestry to the metabolic and adipose factors, and changes therein.

Hypothesis 5: A higher proportion of Japanese ancestry is associated with a greater predisposition to the metabolic syndrome and diminished insulin secretion.

B. BACKGROUND AND SIGNIFICANCE

B1. EPIDEMIOLOGY OF TYPE 2 DIABETES IN CHILDREN

· ***Increasing Incidence of Childhood Type 2 Diabetes.***

The natural history of type 2 diabetes is characterized by both insulin resistance and islet ß-cell dysfunction, and hyperglycemia usually develops gradually. Thus, it is relatively asymptomatic in its early stages. Type 2 diabetes is often associated with obesity. In contrast, the pathophysiology of type 1 diabetes is completely different. Type 1 diabetes results from insulin deficiency due to autoimmune islet ß-cell destruction, and is thus often associated with autoantibodies to islet ß-cell components and contents. Unlike type 2 diabetes, the onset of type 1 diabetes is often precipitous with prominent diabetic symptoms, often including ketoacidosis. The majority of children with diabetes have type 1. Prior to the 1990's, there were only a few reports of childhood type 2 diabetes, which has therefore been considered a disease of adults. However, although population-based data are sparse, there is consensus that the incidence of type 2 diabetes among children and adolescents has increased in recent years [1-4]. This trend is attributed to increasing rates of childhood obesity and physical inactivity.

In Cincinnati, where almost all children (ages 0 to 19) diagnosed with diabetes are referred to a single specialty clinic, the proportion of type 2 diabetes increased from ≤ 4% prior to 1992 to 16% in 1994 [5]. Of diabetic children aged 10 to 19, the proportion with type 2 diabetes increased from between 3-10% before 1992 to 33% in 1994. The incidence of clinically diagnosed type 2 diabetes among children aged 10 to 19 living in Cincinnati increased 10-fold between 1982 and 1994, from 0.7/100,000 to 7.2/100,000. An 8.5-fold increase in type 2 diabetes was reported by a tertiary pediatric endocrine center in Arkansas between 1988 and 1995 [6]. In Allegheny County, Pennsylvania, a 3-fold increase in newly diagnosed diabetes among African American adolescents was noted between the periods 1985-89 and 1990-94, although the type of diabetes was not confirmed [7]. Subsequent evaluation demonstrated that many diabetic African-American adolescents did not have antibodies associated with type 1 diabetes, suggesting that many of the excess cases were type 2 [8].

• ***Lifestyle and Childhood Obesity***

The prevalence of childhood obesity in the United States is increasing [9], probably as a result of lifestyle changes. Obese children report a higher proportion of total caloric intake as fat than non-obese children [10]. In Japan, increased prevalence of childhood obesity is associated with increased consumption of animal fat and protein [11]. Obese adolescents with type 2 diabetes also report high fat consumption and no regular exercise [12]. A greater amount of time spent in sedentary activities, such as television viewing, is also associated with childhood obesity [13]. Furthermore, obese children who reduce time spent in sedentary activities lose weight, even in the setting of stable food intake [14]. Physical activity is inversely related to obesity [15], and an exercise program has been shown to reduce fat mass and intra-abdominal fat accumulation in obese children [16]. Intra-abdominal fat is strongly associated with the metabolic sequelae of obesity (see section B2c.), yet little is known about the effect of lifestyle on longitudinal changes in intra-abdominal fat accumulation in non-obese children.

· ***Risk Factors for Childhood Type 2 Diabetes***

In general, the risk factors for type 2 diabetes among children are similar to those reported for adults. Adolescents are affected more often than younger children, with an average age at diagnosis of about 13.5 years [2]. This suggests that body composition and/or metabolic changes during puberty play an important role in the onset of diabetes. About 95% of affected children are ≥ 85th age- and sex-specific percentile for body mass index (BMI), and most have a family history of type 2 diabetes [2, 5]. A strong association between acanthosis nigricans and childhood type 2 diabetes has also been reported [2, 5, 6]. As with adults, Hispanic [17, 18], African-American [5, 6], and Native-American [19, 20] children appear to be disproportionately affected. Several studies have shown a gender discrepancy, with more girls affected than boys [2], an observation that is consistent with the earlier onset of puberty in girls.

· ***Lack of Information on Asian-American Children Despite Increased Risk in Asian Adults.***

There are little population-based health data available on Asian Americans, and this is especially true for Asian-American children. Yet Asians are the fastest growing ethnic minority population in the United States [21]. Despite having a lower average BMI than Caucasians, South Asian adults living in the United Kingdom are 4 times as likely to have diabetes [22]. The prevalence of self-reported, physician diagnosed diabetes in residents of Hawaii is lowest in Caucasians (2.7%), highest in Japanese Americans (6.4%), and intermediate in those of Chinese (3.5%), Filipino (4.6%), and Native Hawaiian (4.7%) ancestry [23]. The increased risk of diabetes among Asians has been associated with a propensity for central or visceral adiposity [24-26]. Thus, there is reason to suspect that Asian-American children, particularly those who have adopted a western lifestyle, are at increased risk for diabetes.

The only published data on the incidence of type 2 diabetes in Asian children comes from Japan [11]. In a population-based study from Tokyo, asymptomatic schoolchildren were periodically screened for glucosuria, and an oral glucose tolerance test was performed on those who screened positive. Among primary school children, diabetes incidence increased tenfold from 0.2/100,000 in 1976-1980 to 2.0/100,000 in 1991-1995. Among junior high school children, diabetes prevalence increased from 7.3/100,000 to 13.9/100, 000 during the same years. Diabetes trends mirrored upward trends in body mass index and consumption of animal fats. Thus, it appears that vulnerability to diabetes among Asians begins in childhood. It is likely that the problem is even greater in the United States, where the prevalence of childhood obesity exceeds 20% [9].

B2. PATHOPHYSIOLOGY OF TYPE 2 DIABETES

The pathophysiology of hyperglycemia in type 2 diabetes includes both abnormalities in islet ß-cell function and development of insulin resistance. The latter is associated with overall obesity as well as with increased accumulation of body fat centrally.

B2a. ß-cell Dysfunction

· ***Abnormal Glucose-Stimulated Insulin Secretion***

It is well established that even with obesity and insulin resistance, euglycemia is maintained in the presence of normal ß-cells, although at the expense of hyperinsulinemia. As is true for adults, normoglycemic obese children and adolescents are insulin resistant and hypersecrete insulin [27-30]. Japanese adults with impaired glucose tolerance demonstrate both impaired insulin sensitivity and hypersecretion of insulin, particularly if they are obese [31]. Despite hypersecretion of insulin, however, individuals with impaired glucose tolerance exhibit reduced glucose-stimulated insulin secretion relative to the degree of insulin resistance. Furthermore, the defect is even greater in persons who have type 2 diabetes. Thus, in the setting of insulin resistance, plasma glucose levels are more likely to reach values diagnostic of diabetes among individuals with abnormal ß-cell function who are unable to maintain adequate insulin secretion to compensate for insulin resistance. Although there is evidence that insulin resistance precedes the decline in insulin secretion among some individuals at high risk for type 2 diabetes [32], other reports suggest that impaired insulin secretion precedes or accompanies the development of insulin resistance [33]. In Japanese adults with impaired glucose tolerance, low insulin secretion predicts progression to diabetes [34, 35].

The causes of impaired glucose-stimulated insulin secretion are not fully understood. Among adults, aging is associated with a gradual decline in insulin secretion, and may explain the increased incidence of type 2 diabetes in the elderly [27, 36]. Insulin secretion capacity may also be genetically determined. For example, insulin secretion is 65% lower among nondiabetic individuals who have an identical twin with type 2 diabetes, compared to other nondiabetic individuals [37]. Other studies have demonstrated reduced insulin secretion among first-degree relatives of patients with type 2 diabetes

compared to individuals of similar age and BMI without a family history of diabetes [38]. Thus, it is plausible that ethnic variation in diabetes prevalence may be partly explained by genetic determinants of insulin secretion.

· ***Abnormal Processing of Proinsulin to Insulin***

Another measure of islet ß-cell dysfunction is incomplete processing of proinsulin to insulin. Within the secretory granules of the ß-cell, two enzymes (prohormone convertases 2 and 3) process proinsulin to intermediate proinsulin split products and then to insulin plus C-peptide [39]. If this process is abnormal, increased amounts of proinsulin and intermediate split products are present in plasma. Depending on the assay used to measure proinsulin, this increase may be measured as the plasma concentration of either proinsulin or of proinsulin plus intermediates. Individuals with type 2 diabetes secrete excess proinsulin [40, 41]. Both the concentration of proinsulin and the proportion of immunoreactive insulin attributable to proinsulin are increased. Moreover, the magnitude of the proinsulin to insulin ratio is inversely correlated with insulin secretion in patients with type 2 diabetes [42]. Since the orderly cleavage of proinsulin appears intact in type 2 diabetes, the excess release of incompletely processed proinsulin seems to be the result of either slower conversion or reduced storage time in the ß-cell [40].

This abnormality of proinsulin secretion precedes the diabetic state. Individuals with impaired glucose tolerance have an elevated proinsulin to insulin ratio compared to normoglycemic individuals [43], and fasting proinsulin levels predict the development of diabetes [44-46]. Among normoglycemic individuals, the proinsulin level and the proinsulin to insulin ratio are inversely correlated with insulin secretion, independent of age, gender, body mass index, waist to hip ratio, and insulin sensitivity [47]. Although it has been reported that proinsulin levels increase following hemipancreatectomy, suggesting that this may be a response to increased ß-cell demand [48], insulin resistance induced by administration of nicotinic acid is not accompanied by a disproportionate increase of proinsulin [49, 50]. Thus elevated proinsulin levels found with type 2 diabetes appear not to be simply a response of the ß-cell to insulin resistance, but probably represents an intrinsic abnormality of the ß-cells.

B2b. <u>Obesity and Insulin Resistance</u>

Increased adiposity, as measured by BMI, triceps skinfold thickness, and dual-energy x-ray absorptiometry (DEXA), is associated with increased fasting insulin levels in prepubertal and postpubertal children [29, 51-53]. As mentioned previously, normoglycemic obese children and adolescents are insulin resistant and hypersecrete insulin [27-30]. Thus, the association between obesity and insulin resistance seems to be well established in children.

· ***Effect of Pubertal Stage***

A recent study demonstrated transient insulin resistance (measured by euglycemic clamp) during early puberty (Tanner stages 2 to 3), returning to prepubertal levels by late puberty [51]. Girls were more insulin resistant than boys regardless of pubertal stage in this study. These findings are consistent with prior studies demonstrating lower insulin levels in prepubertal children compared to midpubertal children [54, 55]. Both sex steroids and growth hormone (and peptides related to growth hormone action) have been implicated as causing insulin resistance during puberty since both rise during puberty [56-62]. Growth hormone effects are now more commonly assessed by measurements of insulin-like growth factor-1 (IGF-1) [63], the peripheral hormone that mediates many of the effects of growth hormone, and insulin-like growth factor binding protein-3 (IGFBP-3) [64].

· ***Effect of Ethnicity***

The effect of ethnicity has been most extensively studied in African-American and Caucasian children. In prepubertal children, insulin sensitivity (determined by a tolbutamide-modified frequently sampled intravenous glucose tolerance test with minimal modeling) was 42% lower among African-American children compared to Caucasian children [52]. This same group reported higher fasting insulin

levels in African American prepubertal children [53]. African-American adolescent girls have higher fasting insulin levels and decreased hepatic insulin clearance compared to Caucasians [65]. Arslanian and colleagues also showed decreased insulin sensitivity and increased insulin secretion among African-American adolescents compared to Caucasians using a 2-hour hyperglycemic clamp [66]. In contrast, others reported that insulin resistance (measured by euglycemic clamp) was greater in pubertal Caucasian than African-American boys, but did not differ by ethnicity in pubertal girls [51]. It remains unclear if these discrepant findings are due to differences in methodology or pubertal stage of the subjects.

B2c. Visceral Adiposity and Features of the Insulin Resistance Syndrome.

· Adults

The terms insulin resistance syndrome, metabolic syndrome, and syndrome X refer to a constellation of metabolic findings associated with increased cardiovascular disease risk in adults [67-69]. These metabolic factors include hyperinsulinemia, insulin resistance, hypertension, dyslipidemia (elevated triglycerides, low HDL cholesterol, and increased amounts of small, dense LDL), and obesity. While not part of the original description, increases in hemostatic factors [70-72] and inflammatory markers such as C-reactive protein [73-75] are also associated with the insulin resistance syndrome. In adults, the insulin resistance syndrome is more strongly associated with central adiposity (particularly visceral or intra-abdominal fat) than total body adiposity or subcutaneous fat [76-85]. Since intra-abdominal fat deposition is influenced by gender and menopausal status [86-88], it is presumed that sex hormones are involved in body fat distribution. Thus, puberty may be an important milestone in determining body fat distribution.

· Prepubertal Children

A few research groups have studied the metabolic effects of intra-abdominal (visceral) fat in prepubertal children. Visceral adiposity is associated with elevated fasting insulin and triglycerides in prepubertal children [52, 89, 90]. Incremental 30-minute insulin measured during an oral glucose tolerance test is associated with visceral fat in Caucasian, but not African-American children [53]. Insulin sensitivity (measured by a tolbutamide-modified, frequently sampled intravenous glucose tolerance test with minimal modeling), however, is associated with total fat mass but not visceral fat [52]. The ratio of visceral to subcutaneous abdominal fat does not differ by gender prior to puberty, but is higher in Caucasian than African-American children [91]. One longitudinal study showed that before puberty, visceral fat was associated with total and LDL cholesterol, but not with fasting insulin, insulin area during an oral glucose tolerance test, or HDL cholesterol [92]. However, after puberty, visceral fat was associated with elevated insulin and low HDL cholesterol levels.

Only one study has examined hemostatic factors in relation to visceral adiposity in children. Fibrinogen and D-dimers were associated with percent body fat, subcutaneous fat mass, total fat mass, and BMI, whereas plasminogen activator inhibitor 1 (PAI-1) was associated with visceral fat and fat-free mass in children aged 7 to 11 [93].

· Pubertal Children

Studies of pubertal children show results similar to those seen in adults, with a correlation between increased visceral adiposity and hyperinsulinemia, insulin resistance, dyslipidemia, and elevated blood pressure [92, 94, 95].

Relatively little is known about how visceral fat depots change during puberty in normal children. In 16 obese, Italian children followed for 4 years, total fat mass increased significantly after puberty compared to prepubertal levels, whereas visceral fat was unchanged [92]. Testosterone levels are positively correlated with visceral fat in girls at the time of menarche, independent of estrogen, LH, and total body fat [96].

B3. RATIONALE FOR STUDYING JAPANESE-AMERICAN CHILDREN

Asian Americans are a diverse population. Japanese Americans are the third most populous Asian subgroup in King County, Washington (see Table C1). Unlike other Asian subgroups, the vast majority of Japanese Americans living in this region are U.S. born and their families have resided here for several generations. This distinction is highly relevant to this study. Dietary habits are associated with duration of time in the United States [97]. The rise in diabetes among Japanese children coincides with the adoption of a "westernized" lifestyle [11]. Thus, in order to understand diabetes risk in Asian-American children, it is preferable to study children whose lifestyle is typically American. Findings in children of recent immigrants may vary with time since immigration, and may not be generalizable to subsequent generations. Furthermore, follow-up is likely to be enhanced by geographic and economic stability, and English proficiency will facilitate recruitment of participants.

Another important reason to focus on Japanese Americans is their history of participation in similar local studies. We have conducted the Japanese American Community Diabetes Study in adults since 1983, with superb participation and cooperation by the Japanese-American community, and this will provide an excellent basis for recruitment of children for this study. Because of this history of participation in research, recruitment of Asians for the Diabetes Prevention Program Seattle clinical site was most successful in the Japanese-American community (personal communication, S. Kahn, Principal Investigator).

- ***Summary of Findings from the Japanese American Community Diabetes Study***

Japanese Americans have experienced a higher prevalence of type 2 diabetes than in Japan, suggesting that factors associated with "westernization" play a role in bringing out underlying susceptibility to diabetes [26]. Despite similar degrees of hyperglycemia, diabetic Seattle Japanese American men had significantly higher insulin levels than diabetic Tokyo men [98]. This suggested that diabetic men in Seattle were more insulin resistant than in Tokyo. Since insulin resistance is related to body weight, it was not surprising that diabetic men in Seattle had significantly higher levels of body mass index (BMI) than diabetic men in Tokyo. After adjusting for BMI, however, fasting insulin levels were still significantly higher in Seattle than in Tokyo. We postulated that the higher prevalence of diabetes in Japanese Americans might be explained by the superimposition of insulin resistance upon a genetic background of reduced β-cell reserve but that BMI could not account fully for this difference. Subsequent research has shown the importance of the pattern of body fat distribution in conferring risk for diabetes [99]. Diabetic Japanese Americans had significantly more intra-abdominal fat by CT scan than those persons with normal glucose tolerance [100]. The importance of intra-abdominal fat as a risk factor for diabetes was further confirmed by prospective studies [101]. Greater amounts of intra-abdominal fat were present prior to the development of diabetes. Other measures of adiposity such as BMI and skinfolds were not significant risk factors.

We have found a very close relationship between intra-abdominal fat and a number of metabolic features of the insulin resistance syndrome, including the insulin sensitivity index of Bergman (Si). The relationship of intra-abdominal fat with these variables was significantly positive for triglycerides and fatty acids and significantly negative for LDL flotation, HDL, and Si [102].

Intra-abdominal fat has also been associated with increased risk for coronary heart disease. Japanese American men with coronary heart disease had more intra-abdominal fat than individuals without coronary heart disease [103]. We also found that intra-abdominal fat was an independent risk factor for incident coronary heart disease [104]. Moreover, it is noteworthy that insulin levels were not independently related to incident coronary heart disease.

We have also reported that during a 75-g oral glucose tolerance test, insulin secretion in response to glucose was delayed as glucose tolerance deteriorated from normal to impaired to diabetic [105]. This was demonstrated by a lower amount of insulin secreted at 30 minutes following the oral glucose load consistent with an impairment in glucose-stimulated insulin secretion. In addition, a defect in the

processing of proinsulin accompanied type 2 diabetes in Japanese Americans. Importantly, abnormal glucose-stimulated insulin secretion [106] and elevated proinsulin levels [45] were present at baseline in Japanese Americans who subsequently developed diabetes. Hence both of these are risk factors for incident diabetes. Moreover, we have shown that the insulin secretory defect is present before the increase in visceral fat [33]. These observations were made in men who were lean, had normal amounts of visceral fat, and were nondiabetic at baseline, and were followed for 5 years.

The development of diabetes involves the interaction of genetic risk for the disorder with environmental (lifestyle) factors. Two such factors are diet and physical activity. We found that a diet higher in animal fat and protein was being consumed by Japanese-American men with diabetes than those who did not have diabetes [107]. Total energy intake was similar. Subsequently, we found that in those Japanese-American men with impaired glucose tolerance and a family history of diabetes, significantly higher 2-hr plasma glucose levels were present at 5 years in those men who were consuming higher amounts of animal fat and were less physically active at baseline [108]. Furthermore, animal fat intake was significantly correlated with subsequent intra-abdominal fat gain.

Thus lifestyle factors interacting with an underlying genetic risk appear to underlie the high prevalence of diabetes in Japanese Americans. Preceding the appearance of diabetes appears to be the development of central (visceral) adiposity, insulin resistance, and other features associated with this insulin resistance metabolic syndrome, such as dyslipidemia (high triglycerides, low HDL-cholesterol, and small and dense LDL particles), hypertension, and coronary heart disease. We have postulated that the superimposition of these metabolic changes upon a genetic background of reduced β-cell reserve results in hyperglycemia and diabetes in Japanese Americans. It is highly likely that these changes have their beginnings during childhood.

B4. SIGNIFICANCE

The risks of diabetic complications, such as renal failure, lower extremity amputation, blindness, and cardiovascular disease, increase with duration of diabetes. Thus, the increased prevalence of type 2 diabetes in children is especially concerning. Based upon observation from studies among adults, Asian-American children are probably at increased risk for type 2 diabetes, yet they are an under-studied group. The proposed study will provide important information on the metabolic features of the insulin resistance syndrome in Japanese-American children as they progress through puberty, and will also provide an opportunity to better understand the effect of Japanese ancestry on metabolic risk. These studies will probably be relevant to other Asian populations in the United States.

E. HUMAN SUBJECTS

E1. DESCRIPTION OF STUDY SUBJECTS

A total of 450 children, aged 8-10, will be studied. This age range was chosen so that children will be prepubertal at baseline and approximately half will enter or pass through puberty by the 2-year follow-up (see section D5c). Since this study focuses on Japanese Americans, we will initially recruit 300 children with any proportion of Japanese ancestry. We anticipate many of these children will be of mixed Japanese/Caucasion ancestry (see section C2). To allow study of the influence of Japanese ethnicity while minimizing the confounding effects of ethnic heterogeneity, 150 Caucasian children will also be studied.

E2. SOURCES OF RESEARCH MATERIAL

Information about medical and family history, diet and exercise habits will be obtained by questionnaire from children and a parent. Anthropometric measurements and sexual maturity will be

ascertained by physical examination. Blood specimens will be obtained. Magnetic resonance imaging and dual-energy x-ray absorptiometry (DEXA) will be performed.

E3. RECRUITMENT OF SUBJECTS

This is a critically important aspect of this research and we will depend heavily upon the experience we have gained and the extensive networking we have established over the past two decades in performing the Japanese American Community Diabetes Study. We will recruit children of Japanese ancestry through a variety of techniques that have proven to be successful in the past. Letters will be sent to the approximately 600 living adult participants in our Japanese American Community Diabetes Study, residing in King County, Washington, informing them about the expansion of our study to include children and asking them to contact us if they know of eligible children who may be interested. Our website (http://depts.washington.edu/jacds/) will include recruitment information. We will also carry out community-wide publicity through community events and activities as arranged through our Community Advisory Board (such as writing articles for the *Tayori* newsletter and participating in church bazaars). A draft of an article that will appear in the next issue of the *Tayori* is included in the Appendix. We will send information and recruitment letters to Japanese households in King County using a comprehensive mailing list. In addition, we will target private Japanese language schools and other activities in which Japanese-American children are likely to participate. We will also ask parents whose participant children are of Japanese/Caucasian ancestry for permission to recruit Caucasian cousins. Initial publicity has already begun about the growing epidemic of type 2 diabetes in youth, and our intention to study this in Japanese-American children. This includes discussions with our Community Advisory Board (see Appendix for letter of support).

Participants will be offered compensation for time and discomfort in the form of gift certificates. Twenty-five dollar gift certificates will be provided for each evaluation. Children will be allowed to select from a wide range of gift certificates, such as movie theaters, video rental, sporting events, activities (such as miniature golf or bowling), and toy stores. Parking vouchers will be provided to parents.

E4. RISKS AND DISCOMFORT

Serious potential risks from this study are extremely unlikely. Possible minor risks include discomfort, ecchymoses, or inflammation from venipunctures. Blood will be drawn through an intravenous catheter to minimize these risks. Entertainment (such as electronic games, television, and videos) will be available to distract children during venipunctures. There is also risk of pain and inflammation if glucose is injected extravascularly. While examination for sexual maturity is essential to interpretation of the study results, efforts will be made to minimize embarrassment. Children may choose to be examined in the presence of their parents, and all exams will be performed by an experienced clinician with a chaperone present. Moreover, since assessment of sexual maturation is a standard component of a routine pediatric examination, most children will already be familiar with this procedure. The radiation exposure associated with DEXA is less than 0.1 microGy (10 mrem) [138], which is at the lower end of the exposure range for diagnostic radiographs. This represents about 3% of the average annual exposure from natural background radiation in the United States. Magnetic resonance imaging does not involve radiation exposure. No investigational drugs will be used.

E5. CONFIDENTIALITY AND MINIMIZING RISK

Although it is considered unlikely that a menarcheal girl might be pregnant at the time of her scheduled follow-up visit, urine sample will be obtained from all menarcheal girls for pregnancy tests, and any girl found to have a positive test will not undergo tests scheduled for the follow-up visit and will instead be referred back to their primary care physician for further follow-up. Hematocrits will be done on all participants, and any child with a hematocrit <35 will not be studied.

Results which may be relevant to the participant's medical care (e.g. height, weight, blood pressure, hematocrit, glucose, cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) will be sent by mail to the child and consenting parent. If the parent provides written consent, these results will also be provided to the child's physician. All other study information, including genetic information, will be completely confidential and will be used for research purposes only.

Each study participant will be assigned a study code number. All data collected and stored on each participant will be identified by this code. A master sheet linking participant identification and their study code will be stored in a secured location. All information will remain strictly confidential and will be stored in a locked file cabinet and on a password-protected computer file. No data containing participant identifiers will be accessible to individuals who are not investigators or staff. Any information used for research presentations or publications will be reported in statistical format only.

· ***Institutional Review Board.***

The protocol for this study is currently under review by the University of Washington Institutional Review Board (Human Subjects Review Committee). Approval will be obtained and submitted within 60 days. Written, informed consent to participate will be obtained from each child and a parent by a member of the investigating team.

E6. BENEFITS

Potential risks are very unlikely, and are far outweighed by potential benefits to society. Clinically relevant information about each child will be reported to the child and their parents, and to the child's physician with written permission from the child's parents. While most children are expected to be healthy, a beneficial effect on the medical care of some children (e.g. those with hyperlipidemia) is possible. All participants may derive future benefit from the increased knowledge about type 2 diabetes and associated conditions expected to be gained from this study.

F. VERTEBRATE ANIMALS

Not Applicable

G. LITERATURE CITED

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