



Published in final edited form as:

*Circ Res.* 2018 September 14; 123(7): 886–904. doi:10.1161/CIRCRESAHA.118.312806.

## Age-related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk

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### Abstract

Aging and diabetes are two well known risk factors for cardiovascular disease (CVD). Over the past 50 years there has been an dramatic increase in life expectancy with a simultaneous increase in the prevalence of diabetes in the older population. This large number of older individuals with diabetes is problematic, given that CVD risk associated with aging and diabetes. In this review, we summarize epidemiological data relating to diabetes and CVD, with an emphasis on the aging population. We then present data on hyperglycemia as a risk factor for CVD and review the current knowledge of age-related changes in glucose metabolism. Next, we review the role of obesity in the pathogenesis of age-related glucose dysregulation, followed by a summary of the results from major randomized controlled trials that focus on cardiovascular risk reduction through glycemic control, with a special emphasis on older adults. We then conclude with our proposed model of aging, that body composition changes and insulin resistance link possible dysregulation of physiological pathways leading to obesity and diabetes—both forms of accelerated aging—and risks for CVD.

### Keywords

aging; diabetes; obesity; cardiovascular risk

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Aging and diabetes are two well known risk factors for cardiovascular disease (CVD). Aging alone is associated with decline in physiological function leading to chronic diseases such as diabetes, CVD, cognitive impairment and physical disability. Diabetes is a recognized cause of accelerated aging and there is evidence that aging, and diabetes share common pathophysiological pathways. The mechanisms linking advancing age to metabolic dysregulation are multifactorial and complex. Asymptomatic hyperglycemia occurs in many people as they age and creates an insidious and progressive metabolic dysregulation that over many years increases the susceptibility to many age-related chronic diseases, including CVD as well as premature CVD mortality. Altering the trajectory of pathology by lowering mean blood glucose levels may not be possible owing to permanent vascular changes once

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### DISCLOSURES

None.

type 2 diabetes manifested clinically. Thus, there is active research in developing criteria for early diagnosis.

In this report, we summarize the statistics related to diabetes in the context of the current aging population. We review the current knowledge of age-related changes in glucose metabolism, present data on hyperglycemia as a risk factor for CVD, and we attempt to quantify the role of obesity in the pathogenesis of age-related glucose dysregulation. For the purpose of this review, the acronym CVD is meant to include diseases of the heart and blood vessels, with atherosclerosis being the predominant underlying cause, and the main risks factors for CVD, such as obesity, diabetes and aging, acting as prominent players. We conclude with a review of the results from major randomized controlled trials with focus on CVD risk reduction through glycemic control and address a long-time vexing question as to whether reducing blood glucose, independent of other risk factors, also reduces the burden of CVD, with special attention on older adults.

## EPIDEMIOLOGY OF AGING, DIABETES AND CARDIOVASCULAR DISEASE

Humans are living longer. Life expectancy has increased dramatically over the past century. In the United States, people older than age 65 increased from 3 million (4% of the population) in 1900 to 40 million (13% of the population) in 2010, and are projected to double to 83 million (21% of the population) in 2050.<sup>1</sup> This trend is occurring worldwide, and is even faster in developing countries. In 2015, an estimated 617 million people (8.5%) in the world were age 65 or older and is expected to more than double to about 1.6 billion (17%), in 2050.<sup>2</sup>

Aging is by far the strongest known risk factor of diabetes and CVD.<sup>3</sup> In 1958, 1.6 million Americans, or less than 1% of the US population were diagnosed with diabetes.<sup>4</sup> In 2015, an estimated 30.2 million Americans, or 12.2 % of the US population, had diabetes with 7.2 million, or 24%, of them unaware of their diabetes diagnosis.<sup>4</sup> Of the 30 million with diabetes, 12 million are over age 65 and represents over 25% of all individuals in this age group.<sup>5</sup> If current trend continues, it is projected that by 2050, out of 48 million people with diabetes, nearly 27 million, that is more than half of all people with diabetes, will be in the older than 65 age group. Similar trends are projected for the population worldwide with nearly 253 out of 629 million people with diabetes in the older than 65 age group in 2045.<sup>6</sup> Ninety percent of people with diabetes have Type 2 diabetes. For the rest of this review, the term diabetes will be used interchangeably with type 2 diabetes.

Every year, about 1 in 4 deaths in the US are directly due to CVD, with coronary artery disease being the most frequent type of heart disease that kills. In people aged 65–84 years, heart disease, the largest single cause of all deaths in this age group, accounts for at least 25% of all deaths while that number increases to 30% in people age 85 and older, with heart disease again being the single largest cause of death in this age group.<sup>7</sup> Viewed through the lens of diabetes, atherosclerotic CVD, defined as coronary heart disease, cerebrovascular disease, or peripheral artery disease, is the leading cause of morbidity and mortality in persons with diabetes.<sup>8</sup> In the Framingham study population the presence of diabetes doubled the risk for CVD in men and tripled it in women.<sup>9</sup> Similar findings were reported

from the Multiple Risk Factor Intervention Study.<sup>10</sup> Interestingly, illustrating the impact of circulating glucose levels *per se*, a study of more than 24,000 non-diabetic individuals found that elevated fasting blood glucose increased the risk of coronary artery disease in this group of apparently healthy adults, after adjusting for other known risk factors.<sup>11</sup> Furthermore, cardiologists consider diabetes a risk factor for CVD and suggest that the screening of diabetes should be an integral part of the CVD evaluation in their assessment guidelines (2013 American College of Cardiology (ACC)/American Heart Association (AHA) risk assessment guidelines).<sup>12</sup> However, there is still conflicting information/data about the role of glucose *per se* in CVD in persons who are not diabetic.

## DIABETES DIAGNOSTIC CRITERIA AND THE OLDER ADULTS

According to the latest guidelines by the American Diabetes Association and the World Health organization the diagnosis of diabetes is established according to the following criteria: (1) random plasma glucose  $\geq 200$  mg/dL (11.1 mmol/L); (2) fasting plasma glucose (FPG)  $\geq 126$  mg/dL (7 mmol/L); (3) 2hr plasma glucose (2hG) post-75gm oral glucose tolerance test (OGTT)  $\geq 200$  mg/dL (11.1 mmol/L); or (4) hemoglobin A1c (A1c)  $\geq 6.5\%$ .<sup>13–15</sup> OGTT is not routinely used in clinical settings for diagnosing diabetes because of the added burden and expense of performing OGTT but continues to be performed in many longitudinal studies, including our own Baltimore Longitudinal Study of Aging (BLSA).

Using FPG only to diagnose diabetes misses a significant proportion of the population with diabetes, especially older adults.<sup>16–19</sup> In the Rancho Bernado Study of adults aged 50–89 years, 70% of women and 48% of men with previously undiagnosed diabetes had isolated post-OGTT hyperglycemia.<sup>17</sup> The Cardiovascular Health Study reported that 52% of participants newly diagnosed with diabetes had isolated post-OGTT hyperglycemia.<sup>19</sup> Results from the DECODE Study Group (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) showed that 35% of adults 60–79 years and newly diagnosed with diabetes had isolated post-OGTT hyperglycemia.<sup>16</sup> Using our own data collected from the BLSA, we selected all participants with data on A1c and OGTT that had no history of diabetes and were not on antidiabetic medication. Out of 1152 participants, we identified 131 individuals with newly diagnosed type 2 diabetes using either A1c, FPG or 2hG (Figure 1). This sub-cohort has an average age of 70 years. Using FPG alone identified 40 (31%) cases, 2hG alone identified 89 (68%) cases, and A1c alone identified 66 (50%) cases. Clearly, these three measures, FPG, 2hG, and A1c, are each identifying different groups of diabetic individuals. This is especially problematic in older individuals: if only FPG and A1c are used for diagnosis, more than one-third of those with diabetes are missed. The main mechanism underlying age-associated increases in 2hG is the progressive change of body composition due to excessive food consumption. The significance of post-prandial glucose, represented by 2hG in OGTT, in CVD risks will be discussed in detail in this review.

The DECODE Study Group reported that 2hG is a better predictor of deaths from all causes, CVD, coronary heart disease and stroke, than FPG.<sup>20, 21</sup> The Cardiovascular Health Study found that 2hG was associated with CVD and mortality independent of FPG.<sup>22</sup> In a study that combined data from NHANES III (1998–1994) and Continuous NHANES (1999–2004), participants greater than 65 years of age with undiagnosed diabetes and A1c  $> 6.5\%$

had a 30% greater risk of all-cause mortality compared to those without diabetes and A1c (5.0–5.6%).<sup>23</sup> A systematic review (74 published studies) and meta-analyses of (46 studies) reported that in non-diabetic individuals, A1c > 6.5 % has twice the risk of cardiovascular mortality compared to those without diabetes and A1c (5.0–6.0%).<sup>24</sup>

## HYPERGLYCEMIA AND CARDIOVASCULAR RISKS

The worldwide prevalence of impaired glucose tolerance and type 2 diabetes continue to rise, and increasing emphasis, resources, and amounts of GDP of nations are used in the services of lowering circulating plasma glucose levels to prevent CVD and other diabetes-related outcomes. Therefore, the causality of the relationships between elevated plasma glucose, type 2 diabetes and CVD are of great importance to global public health and cannot be overstated.

Despite data accumulated now over several decades, there is still some hesitation in firmly stating that elevated plasma glucose *per se* is causative for CVD and, if it is not, this would infer that the effects of glucose seen in observational studies of CVD are due to confounding factors, such as hypertension, hyperlipidemia and sleep apnea. There is strong rationale to believe that high glucose may be causative of CVD: (1) advanced glycated end product receptor activation may accelerate atherosclerotic plaque formation by causing increased intima-medial thickening and arterial stiffness; (2) hyperglycemia stimulates the differentiation of monocytes to macrophages leading to formation of foam cells and intracellular accumulation of oxidized lipids; (3) fluctuating glucose levels may promote oxidative stress and activation of protein kinase C and lead to fibrosis and dysfunction of endothelium. Some studies have also reported that variability in blood glucose, especially in the post prandial period, is more detrimental to CVD development than stably high glucose levels that may allow compensatory adaptation.<sup>25</sup> Of note, if glucose is causative of CVD, then we would expect that increasing glucose levels would be associated with progressively high risk of CVD and overall this does appear to be the case.

Elevated plasma glucose level, without diabetes, does appear to be associated with CVD. Impaired FPG and insulin resistance (without abnormal 2hG) are associated with an increased risk of CVD based on meta-analysis that included over 100 studies.<sup>26, 27</sup> Additionally, in a recent retrospective study performed in a Korean Cohort of more than 260,000 individuals free of diabetes or CVD at study entry, change in fasting serum glucose from normal, to impaired to diagnostic for type 2 diabetes resulted in a progressively higher risk of developing myocardial infarction, CVD, and all-cause mortality.<sup>28</sup>

In the BLSA study population, a combination of FPG and 2hG provides better evaluation of mortality risk than does the FPG alone.<sup>29, 30</sup> Categorization of risk based only upon FPG concentration suggests that risk for cardiovascular mortality among men with impaired FPG is intermediate between that of men with normal and diabetic FPG concentrations. Characterization of men by FPG and 2hG concentrations indicates that 1) there is no increased CVD mortality risk for men with impaired FPG who have a normal 2hG, and, 2) CVD risk increases progressively in men with impaired FPG as the 2hG glucose becomes progressively higher.<sup>31</sup>

Type 2 diabetes, with both diabetic FPG and 2hG levels, is associated with an increased risk of CVD by two to fourfold, according to two observational studies.<sup>27, 32</sup> This effect appears to be due to exposure to excessive amount of glucose levels since it is independent of other known type 2 diabetes-associated risk factors such as body mass index (BMI), low-density lipoprotein (LDL) cholesterol, blood pressure, and inflammatory markers and smoking. At least three studies (DECODE study, Japanese cohort study, and Mauritius/Fiji/Nauru study), cross-classified subjects by their FPG and 2hG levels and were followed to determine whether 2hG added to the predictive power of the FPG.<sup>33–35</sup> Subjects with normal or impaired FPG were combined into a single nondiabetic group. In the DECODE study, men, but not women, with nondiabetic FPG and a diabetic 2hG did worse than those men with nondiabetic FPG and 2hG concentrations.<sup>33</sup> A study performed in the population sampled from Mauritius, Fiji, and Nauru extended the same conclusions to both men and women.<sup>34</sup> The Japanese cohort study is difficult to compare to the other reports because participants received a 50-g OGTT, and the cut point for FPG considered normal was 140 mg/dl (7.8 mmol/l), while the cut-point for 2hG was 200 mg/dl (11.1 mmol/l). A more recent study of subjects with genetic variants known to be associated with diabetes suggested that even a small increase in elevated FPG levels in non-diabetic individuals is associated with a trend towards increased CVD risk,<sup>36</sup> illustrating that glucose is an important mediator linking rising circulating glucose levels with CVD pathogenesis. These results support the hypothesis that a relationship between circulating glucose and CVD is indeed causal and inextricably linked, and lead to the conclusion, which will be further discussed below, that long-term efforts to lower glucose levels and prevent type 2 diabetes should decrease CVD risk.

## AGE-RELATED CHANGES IN GLUCOSE METABOLISM

Glucose metabolism is central to normal physiological function. The liver is a major metabolic regulatory organ and provides 90–95% of the circulating glucose during the post-absorptive state, when the body is no longer absorbing nutrients from the gut. The brain takes up about 50% of the glucose during post-absorptive phase with skeletal muscle using about 15%. In healthy individuals, skeletal muscle is very sensitive to insulin and can increase its uptake up to 85% of circulating plasma glucose with sudden increases in plasma glucose. Insulin secretion from  $\beta$  cells is tightly coupled to the availability of glucose, allowing the fine tuning of blood glucose levels within a normal range.

With age, some people progressively lose the ability to regulate glucose levels as they did when they were younger.<sup>37–43</sup> The 2-hr OGTT data from the BLSA, published in the 1990s, illustrated nicely such positive correlation of age with worsening glucose tolerance in over 770 healthy men and women.<sup>44, 45</sup> The decline in glucose tolerance from young (17–39 years) to middle age (40–59 years) is globally explained by the secondary influences of body fat and physical fitness.<sup>44</sup> However, changes in glucose tolerance that occur between 60–92 years were significant and remained unexplained when body composition and physical activity were accounted for in the analysis.<sup>44</sup> These findings were replicated three decades later with updated OGTT data from over 2700 non-diabetic BLSA participants (Figure 2 top panel). With advancing age, plasma glucose levels in response to OGTT were progressively higher for every decade of age until they peaked at the 7<sup>th</sup> decade. Men and women have

similar patterns, but levels of glucose were higher in men than in women. In individuals without diabetes, FPG levels were progressively 0.7–1.1 mg/dL higher per age decade and 2hG levels were progressively 5.6–6.6 mg/dL higher per age decade. This phenomenon has been observed in other population studies.<sup>46–48</sup>

After adjusting for BMI, the difference in glucose levels with age is drastically attenuated to 0.01–0.3 mg/dL per age decade for FPG and 0.03–0.5 mg/dL per age decade for 2hG but remains significantly correlated with age (Figure 2 bottom panel). Although BMI is a relatively rough measure of body composition, body fat plays a major role in glucose metabolism which we will discuss in more details later in this review. Other population studies also attributed the decline in glucose tolerance in large part to factors such as obesity, physical activity, dietary habits, and others.<sup>46–48</sup>

The use of epidemiology data to understand aging and glucose metabolism has limitations. First, some measures may not be precisely assessed. For example, body fat is usually estimated by BMI, and physical activity and diet are assessed by questionnaires that are notoriously biased. Second, the use of statistical methods involves making certain assumptions regarding confounders, linearity of relationships, and interactions among variables that may not be real. To better understand the relationship between aging and glucose metabolism, detailed physiology studies were performed during the 1970s and 1980s period, a large portion of them at the National Institute on Aging (NIA) and used the glucose clamp technique. Box 1 gives a brief description of the two most common clamp procedures, hyperinsulinemic-euglycemic clamp and hyperglycemic clamp. This information may help readers interpreting the clamp results detailed below from different studies.

### Effect of age on hepatic glucose production

The European Group for the Study of Insulin Resistance (EGIR) used the hyperinsulinemic-euglycemic clamp (1mU/kg/min) to study hepatic glucose output in 344 non-diabetic subjects (212 men and 132 women) with age ranged from 18–95 years and BMI from 15–55 kg/m<sup>2</sup>.<sup>49</sup> The basal hepatic glucose output was highly variable; highly correlated with lean body mass and % body fat; 23% higher in men than in women; and was progressively lower at older ages, although the age association was not significant (p=0.10). All these associations disappeared when hepatic glucose output was normalized for lean body mass. Therefore, the variability of basal hepatic glucose output appears to be due to differences of lean body mass which also explains the difference of hepatic glucose output due to age, sex and BMI.

The effect of age on the ability of insulin to suppress hepatic glucose output was also investigated by several other researchers. The EGIR study showed that at insulin levels of 73 uU/mL (510 pmol/L), hepatic glucose output was mostly suppressed in nondiabetic subjects.<sup>49</sup> Similarly, two other groups showed that hepatic glucose output was suppressed similarly in nondiabetic young and old persons (~95%) at higher insulin levels (100–110uU/mL).<sup>50, 51</sup> Hepatic glucose output at lower physiological plasma insulin levels were also investigated. At insulin levels of 33uU/mL in the young and 61 uU/mL in the old, hepatic glucose output was suppressed more in the young (89%) compare to the old participants (77%).<sup>50</sup> However,

another group showed that hepatic glucose production was suppressed more rapidly in older individuals at physiologic insulin levels (25–40 uU/mL) with no difference in either the basal hepatic glucose output or in the dose-response curve of its suppression by insulin between young and old individuals.<sup>52</sup> The differences in results shown by these studies might be due to small sample size and sex imbalance in their young and old groups. Overall, the available evidence suggests that hepatic glucose output is not affected by age *per se* but rather by differences in body composition.

### Effect of age on peripheral glucose uptake

Glucose uptake occurs by two mechanisms: insulin-mediated glucose uptake (IMGU) and noninsulin-mediated glucose uptake (NIMGU). IMGU occurs primarily in muscle and fat tissues while NIMGU occurs in brain and splanchnic organs. In the fasting state, glucose utilization occurs mostly in NIMGU tissues, specifically brain ~50%, splanchnic organs ~20%, skeletal muscle ~15%, adipose tissue ~5%, blood cells ~5%, and other tissues ~5%.<sup>53, 54</sup> In post-prandial state, glucose uptake occurs in IMGU tissue with skeletal muscle accounting for the majority of uptake.

**Insulin-mediated glucose uptake**—Whether age *per se* causes insulin resistance is controversial. Studies using a variety of techniques (hyperinsulinemic-euglycemic clamp, forearm perfusion, minimal model) have shown decreases in insulin action with advancing age.<sup>50, 51, 55, 56</sup> However, reports from some other studies also could not detect any significant association.<sup>57–62</sup> Unfortunately, true comparability between studies is difficult since they used different methods, had relatively small number of subjects and studied mostly men. In addition, some studies included subjects with impaired glucose tolerance, that as explained above, could mean the hepatic glucose output was not fully suppressed while other studies did not take into account body composition.

One of the most comprehensive study of insulin sensitivity was conducted by the EGIR using hyperinsulinemic-euglycemic clamp (1mU/kg/min dose) in 1146 healthy subjects (766 men, 380 women) with normal glucose tolerance and age ranging from 18 to 85 years and BMI ranging from 15 to 55 kg/m<sup>2</sup>. Insulin sensitivity for the whole group declined slightly with age but this effect of age was no longer significant when differences in BMI were accounted for. Subgroup analysis showed that after adjusting for BMI, insulin sensitivity was lower at older age only in lean women.<sup>63</sup>

The limitation of the EGIR study is that only a single dose of insulin was tested. It was therefore argued that complete dose-response curves are needed to comprehensively evaluate the effect of age on insulin resistance. Hyperinsulinemic-euglycemic clamps using 3 different insulin doses (20, 80, 200 mU/m<sup>2</sup>/min) were performed to examine the effect of age on peripheral glucose uptake in 17 young and 10 old nondiabetic non-obese subjects.<sup>55</sup> An age-associated decrease in glucose utilization was demonstrated with no change in maximal tissue responsiveness or maximal glucose uptake. In another words, the insulin-glucose dose-response curve shifts to the right with age (Figure 3 left panel). The half-maximal glucose uptake occurred when plasma insulin levels were 54 uU/mL in the young and 113 uU/mL in the old. This association held significant after accounting for lean body

mass (Figure 3 right panel). These findings were replicated by other studies that used several insulin doses during hyperinsulinemic-euglycemic clamps.<sup>50, 55</sup>

**Noninsulin mediated glucose uptake**—The effect of age on noninsulin-mediated glucose uptake (NIMGU) has been relatively understudied. Using a minimal model approach, older subjects were shown to be insulin resistant but *glucose effectiveness*, a measure of the increase in fractional disappearance of glucose per unit increase in glucose or insulin-independent glucose utilization, was unchanged with aging.<sup>56</sup> Another group reported similar results using hyperinsulinemic clamp at four different glucose concentrations.<sup>64</sup> In one study, somatostatin infusion was used to suppress endogenous insulin secretion with simultaneous dextrose infusion to evaluate NIMGU. At older ages, NIMGU was impaired at normal FPG levels (~100 mg/dL) but unchanged at physiological hyperglycemia (~200 mg/dL).<sup>65</sup>

It is clear that besides age, other factors such as body composition (fat mass, fat distribution, lean body mass), physical fitness, dietary composition and genetic factors, influence peripheral insulin sensitivity. When these factors are taken into account, it is not clear that age in and of itself still has an independent effect on peripheral glucose uptake, regardless of whether NIMGU or IMGU are considered.

### Effect of age on $\beta$ -cell function

The balance between insulin secretion from  $\beta$  cells and peripheral insulin sensitivity is what maintains normal glucose homeostasis. Diabetes develops when the ability for  $\beta$  cells to secrete adequate amounts of insulin to maintain normal glycemia falters. Whether aging *per se* impacts  $\beta$ -cell function has been controversial. With age,  $\beta$ -cell function has been reported to be either unchanged<sup>39, 59, 66, 67</sup>, or decreased.<sup>56, 68, 69</sup> Again, these studies used different quantification methods, had relatively small number of subjects of mostly men, some studies included subjects with impaired glucose tolerance, and did not take into account body composition.

Perhaps the most robust study on the effect of age on  $\beta$ -cell function was conducted in the BLSA where 230 hyperglycemic clamps were performed in 85 young, 47 middle age and 98 old participants. Four hyperglycemic plateaus were maintained for two hours at 54, 98, 142, 230 mg/dL above basal glucose levels.<sup>70</sup> During hyperglycemic clamps, there are several distinct features in the resulting endogenous insulin response. The insulin response to square-wave-induced hyperglycemia is bimodal: there is a first-phase insulin secretion that occurs during the first 10 min with a peak response at approximately 4 min; then, plasma insulin level falls reaching a nadir at approximately 10 min; next, the second insulin secretion phase begins and insulin levels increase linearly until a plateau is reached.<sup>71, 72</sup> Figure 4 shows the bimodal insulin response to the four levels of hyperglycemia. Within each age group, higher glucose doses corresponded with statistically higher insulin responses at all levels but differences of insulin responses among the age groups were not significantly different. Similar results were shown in another study using hyperglycemic clamps performed at 125 mg/dL above basal glucose levels.<sup>51</sup> In a study of  $\beta$ -cell function



of 42 highly trained women athlete aged 18–69 years, insulin secretion did not differ according to age.<sup>73</sup>

These dose-response hyperglycemic clamps examined the  $\beta$ -cell response to continuous glucose stimulation at elevated and even supraphysiologic levels for up to two hours. The second phase insulin response has been shown to rise linearly for this two-hour duration, but does this rise ever plateau? And does the continuous glucose stimulation on  $\beta$  cells result in  $\beta$ -cell “fatigue”? Ten-hour hyperglycemic clamps at 98 mg/dL above basal glucose levels were performed in 10 young and 10 old subjects with normal glucose tolerance.<sup>74</sup> The second phase insulin responses do reach a plateau during hyperglycemic clamps, around 300 min for the young and 120–150 min for the old group.

### Effects of aging on pulsatile insulin secretion

Like most, if not all, hormones, insulin is secreted in a pulsatile fashion. There are two different insulin pulse patterns: the rapid, low amplitude pulses that occur every 8–15 min and the large amplitude ultradian pulses with a periodicity of 60–140 min.<sup>75, 76</sup> These two types of pulses have different functions in glucose metabolism: the rapid low amplitude pulses are important in inhibiting hepatic glucose production and preventing insulin receptor downregulation;<sup>77–79</sup> while the ultradian pulses are important in stimulating peripheral glucose disposal.<sup>80</sup> These insulin pulses are disturbed in individuals with glucose intolerance, obesity, and type 2 diabetes.<sup>81–83</sup> In healthy non-obese individuals, aging is associated with a decrease in the amplitude and mass of rapid insulin pulses and a reduction in the frequency of ultradian pulses during the fasted state.<sup>84</sup> With stimulation by sustained hyperglycemia for 10 hours, a reduction in mass and amplitude of rapid pulses and a decrease in frequency, amplitude and regularity of ultradian pulses were noted in the old group.<sup>74</sup> Whether these age-related changes in insulin pulsatility have any clinically relevant consequences is not known.

### The effect of aging on incretins

Incretins are enteroendocrine hormones which augment insulin response from  $\beta$  cells beyond the amount attributed to glucose alone. There are two incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Their release from enteroendocrine cells, K cells for GIP and L cells for GLP-1, is stimulated by the presence of nutrients in the gut. Different nutrients have various degree of potency in stimulating incretin secretion: GIP (fatty acids > carbohydrates > amino acids); GLP-1 (carbohydrates > fatty acids > amino acids).<sup>85, 86</sup>

GIP has been shown to be elevated in type 2 diabetes and obesity in both fasting state and during glucose stimulation.<sup>87–89</sup> Baseline GLP-1 is not affected by type 2 diabetes but its secretion is lower during oral glucose stimulation in obesity.<sup>87–89</sup> Fasting GIP levels have been found to be unchanged with aging.<sup>90</sup> Compared to young healthy controls, GIP and GLP-1 levels, both fasting and in response to oral glucose have been found to be unchanged in nonobese older adults,<sup>89–91</sup> and GIP is increased in obese healthy old people.<sup>92</sup>

The effect of age on the sensitivity of  $\beta$  cells to endogenous GIP was examined using hyperglycemic clamp in combination with OGTT. Relative to basal state, GIP levels rose by

179% in the young subjects and 265% in the old subjects in response to oral glucose while the rise in insulin levels were comparable between young and old individuals, indicating that  $\beta$ -cell sensitivity to GIP is reduced in advancing age.<sup>89</sup> Dose-response of exogenous GIP on insulin secretion was also studied using hyperglycemic clamp at two different glucose levels (198 mg/dL or 330 mg/dL plasma glucose levels) and two different GIP doses (2 or 4 pmol/kg/min). The  $\beta$ -cell sensitivity to exogenous GIP was significantly decreased in the old compared to the young at the lower glycemic levels (198 mg/dL). However, the insulin response to GIP was similar in both young and old at the higher glycemic levels (330 mg/dL).<sup>93</sup> The insulinotropic effect of GIP decreases with aging, however, the defect in  $\beta$  cell response to GIP disappear when plasma glucose is increased to higher levels. Taken together, the findings of these studies suggest that the age-related impairment in response to GIP may be an important cause of the glucose intolerance of aging.

The insulinotropic effect of GIP and GLP-1, separately and in combination, were examined during euglycemic or hyperglycemic (198mg/dL) clamps, in healthy subjects and subjects with type 2 diabetes.<sup>94</sup> They reported that: 1) GLP-1 is a more potent insulinotropic hormones than GIP; (2) the insulinotropic effect of GIP and GLP-1 are additive; (3) GLP-1 has a potent insulinotropic effect while GIP has lost its insulinotropic effect in subjects with type 2 diabetes.<sup>94, 95</sup> We, in collaboration with other investigators, studied the insulinotropic effect of exogenous GLP-1 in older type 2 diabetic subjects. We found that GLP-1 not only augments insulin release but also enhances both IMGU and NIMGU in these older type 2 diabetic subjects.<sup>96-98</sup> Furthermore, we reported that three months of continuous GLP-1 infusion in the same population was as effective as standard hypoglycemic medications in glycemic control but with a tighter glycemic excursion and less hypoglycemic events.<sup>99</sup> To our knowledge, there are no data from humans on the efficacy of GLP-1 as a function of age alone in subjects free of diabetes.

## MECHANISMS OF GLUCOSE DYSREGULATION

The detailed mechanistic studies reviewed above clearly show that processes driving metabolic dysregulation in aging are multifaceted, with changes in body composition leading to insulin resistance playing a major role. Insulin resistance is not a disease *per se* but rather it is a condition where higher insulin levels are required to maintain glucose homeostasis and adequate fatty acid utilization in liver, skeletal muscle, and adipose tissue. With increasing insulin resistance, pancreatic  $\beta$  cells upregulate insulin production and increase secretion to maintain normal glycemia. As long as  $\beta$  cells are able to produce sufficient insulin to overcome the insulin resistance, that is, keep shifting the curve of glucose-insulin secretion to the right, absolute circulating blood glucose concentration stays within a narrow normal range.

Over time, insulin resistance can lead to type 2 diabetes when  $\beta$  cells fail to keep up with the body's increased need for insulin. It is important to bear in mind that metabolic changes begin to occur many years prior to the diagnosis of diabetes. Two studies have found that 10–13 years prior to diagnosis of diabetes, FPG, 2hG, insulin sensitivity and insulin secretion were already significant different between those who were diagnosed with diabetes and those who were not.<sup>100, 101</sup> The evolution of the metabolic characteristics of the groups

who eventually developed diabetes compared to the group who remain healthy have never been reported. What causes insulin resistance and ultimately diabetes?

There are many etiologies for insulin resistance – genetic factors, sedentary behaviors, increasing chronological age, medications – but obesity, especially its distribution and content, is by far the single biggest risk factor. As mentioned earlier, the prevalence of diabetes in the US population has increased dramatically over the past half a century.<sup>4, 102</sup> It is impossible not to underline that such increase in prevalence of diabetes is in synchrony with that of obesity, and has generally been attributed to increased food consumption, lack of physical activity and sedentary lifestyles that are facilitated by the proliferation of electronic gadgets and aids. Using data from the US Department of Agriculture (USDA), the daily food consumption for an average American has increased from 2024 calories in 1970 to 2476 calories in 2010, with more than 20% increase in calories.<sup>103</sup> Of note, it has been calculated that an extra 500 calories per day roughly translate to one extra pound of weight gain a week! Figure 5 illustrates the prevalence of diagnosed diabetes and obesity together with average daily food consumption per capita over the past 50 years using data from the CDC and USDA.<sup>4, 102, 103</sup> It is not at all surprising that the increase prevalence of obesity and diabetes track that of increases in food consumption. This is even more problematic in older persons because aging *per se* is associated with increased body fatness and declines in lean body mass.

In addition to over-consumption of food, older individuals may be more likely than younger individuals to gain weight for many reasons including but not limiting to the following: (1) gradual loss of muscle mass and gain in fat mass starting the 4<sup>th</sup> decade of life; (2) menopause in women and decline in testosterone with age in men that compound the loss of muscle mass and gain of adipose tissue; and (3) catecholamine-induced lipolysis in adipose tissue blunting in older individuals.<sup>104</sup>

## **OBESITY, GLUCOSE DYSREGULATION AND ACCELERATED AGING**

Clinically, the definition of obesity is based on crude measure of BMI or waist circumference to gauge total body fat. In prospective studies, increased visceral fat, using waist circumference as a proxy, is an independent risk factor for coronary artery disease and death.<sup>105–107</sup> Obesity shares numerous biological similarities with adipose tissue dysfunction of aging such as chronic inflammation, cellular senescence and immune dysfunction. Accordingly, understanding the interplay between accelerated aging related to obesity and adipose tissue dysfunction of aging is critical to gain insight into the aging process and the pathophysiology of obesity resulting in insulin resistance.

### **Adipose Tissue Dysfunction**

Adipose tissue, once considered an inert, energy storage depot, is now proven to be an active dynamic endocrine organ. It is responsible for energy homeostasis through storing and releasing fat in response to energy-balance needs, synthesizing and secreting peptide hormones by adipocytes to meet energy needs, generating proinflammatory response to bacterial antigens, and other functions.<sup>108, 109</sup> Studies have shown that not all adipose tissue is alike and intra-abdominal fat or visceral fat and subcutaneous fat in the upper body and

lower extremities may have different functions.<sup>110</sup> Furthermore, health risk is associated with location as well as the amount of body fat in which visceral obesity is associated with increased risk for diabetes, hypertension, atherosclerosis, dyslipidemia, cancers, and mortality compared to peripheral obesity.<sup>111, 112</sup> Even humans of a normal weight with a high ratio of central-to peripheral fat have an increased likelihood of being insulin resistant.<sup>113</sup>

Mechanisms of adipose tissue dysfunction in obesity differ from aging in important ways.<sup>114</sup> Adipose tissue is composed of primarily adipocytes, preadipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells such as adipose tissue macrophages. Preadipocytes, a progenitor cell, accounts for 15–50% of cells in adipose tissue, and can differentiate into new adipocytes, macrophages, and other cell types.<sup>110</sup> The main role of preadipocytes is to give rise to new adipocytes. In addition, preadipocytes secrete cytokines and can undergo cellular senescence, particularly with aging and obesity.<sup>114, 115</sup> *In vitro*, human preadipocytes obtained from abdominal subcutaneous adipose tissue are capable of more extensive replication than those obtained from visceral adipose tissue.<sup>116–118</sup>

During weight gain, visceral fat generally enlarges through increases in fat cell size, whereas subcutaneous fat enlarges by increasing both fat cell size or number.<sup>119, 120</sup> In severe obesity, preadipocytes are driven to become new adipocytes with increase replication cycles, especially those preadipocytes in the subcutaneous adipose tissue, resulting in subcutaneous adipose tissue dysfunction. Subcutaneous adipose tissue dysfunction is associated with: (1) restricted capacity to store excess free fatty acid as triglyceride, thus resulting in dysregulated release of free fatty acids from subcutaneous fat instead of visceral fat; (2) excess free fatty acids from subcutaneous fat contributes to visceral fat enlargement, ectopic lipid accumulation, lipotoxicity resulting in secretion of proinflammatory cytokines and chemokines, immune cell infiltration, accumulation of senescent cells, an increase in senescence-associated secretory phenotype (SASP) components, and insulin resistance.<sup>110, 114, 121, 122</sup> With aging, there is a decline in preadipocyte replication, decrease in adipogenesis, increase in susceptibility to lipotoxicity, and increase in pro-inflammatory cytokine and chemokines.<sup>114</sup> Furthermore, adipocytes and preadipocytes, instead of macrophages, become the main sources of inflammatory cytokines and chemokines.<sup>123–125</sup> All these pathologies associated with adipose tissue dysfunction contribute to insulin resistance.

### Cellular senescence

Cellular senescence is a complex process characterized by arrest in replication that is thought to be intrinsically connected with aging. Accumulation of senescent cells occurs during aging and increase in the number of senescent cells are also seen in diseases associated with accelerated aging phenotypes such as obesity and diabetes. Senescent cells are identified by molecular markers such as  $\beta$ -galactosidase (SA-Bgal) and p16<sup>INK4a</sup>, and other features such as permanent growth and replication arrest and secretion of SASP components.<sup>126, 127</sup> SASP components include a large number of cytokines, chemokines, growth factors, and proteases. The physiological role of SASP is not clear, but there is some evidence that SASP is implicated in the mild pro-inflammatory phenotype of aging.

The number of senescent cells in adipose tissue increases with both obesity and aging. Senescent preadipocyte number is higher in obese subjects compared to lean age-matched controls.<sup>114</sup> Extremely obese subjects (BMI ~83 kg/m<sup>2</sup>) have up to 31-fold more senescent preadipocytes than nonobese subjects.<sup>114</sup> Higher levels of p53 protein, p21 mRNA expression and SA  $\beta$ -gal activity are found in the adipose tissue from subjects with diabetes.<sup>115</sup> Obesity-associated senescent cells may promote chronic, low-grade sterile inflammation through secretion of SASP components thus contributing to development and progression of insulin resistance and type 2 diabetes, although empirical evidence for this association in humans is lacking.<sup>128, 129</sup> In a vicious cycle, metabolic and signaling changes seen in diabetes, such as high plasma glucose levels and lipotoxicity, further promote senescent cell formation. Therefore, in diabetes and obesity, senescent cells may be both a cause and consequence of metabolic changes and tissue damage.

There is strong evidence that senescent cells accumulate with aging and, either directly or indirectly through SASPs, contribute to CVD and other chronic diseases. In the context of obesity, the major contribution can probably be ascribed to preadipocytes that acquire senescent attributes, as well as true senescent cells whose origin is still poorly understood. Studies from animal models also suggest that senescence plays a role in atherogenesis, from macrophages with senescent features that transport cholesterol in the arterial intima, to the processing of the intercellular matrix by metalloproteinases, to the fragmentation of the fibrotic cap that destabilizes plaque. However, evidence in humans is sparse. In the context of this review, we have focused more on senescent preadipocytes because their presence in the visceral fat has been associated with insulin resistance, while there is little current evidence that senescent endothelial cells drive this phenotype.

What role, if any, senescent cells play in islets is unclear.  $\beta$ -cell senescence induced by SA  $\beta$ -gal activity in mice fed a high-fat diet was associated with significant decrease in insulin secretion and diet-induced diabetes.<sup>130</sup> In another study, levels of p27<sup>Kip1</sup>, a cell cycle inhibitor and marker of senescence, were reported to be increased in  $\beta$  cells of genetic mouse models of type 2 diabetes. Deletion of p27<sup>Kip1</sup> seemed to enhance insulin secretion because of increased number of  $\beta$  cell proliferation.<sup>131</sup> However, a recent study showed that in healthy old mice and healthy humans, p16<sup>Ink4a</sup>-induced senescence of  $\beta$  cells resulted in enhanced insulin secretion compared to  $\beta$  cells from young animals in response to high glucose concentration.<sup>132</sup> Clearly, much more research is needed in this area to understand the role of senescent cells as they relate to aging, diabetes, and CVD.

## Inflammation

Another crucial factor that intertwine with hyperglycemia and aging is inflammation. Aging is associated with increased in proinflammatory cytokines including interleukin (IL)-1, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-8, IL-13, IL-18, C-reactive protein (CRP), interferons (IFN)  $\alpha$  and  $\beta$ , transforming growth factor  $\beta$  (TGF- $\beta$ ), tumor necrosis factor (TNF)- $\alpha$ , its soluble receptors (TNF-R1 and TNF-R2), and serum amyloid. Adipose tissue can produce proinflammatory and chemotactic compounds, such as IL-6, IL-1 $\beta$ , TNF- $\alpha$  and MCP-1, as well as hormones that modulate inflammation, such as adiponectin and leptin.<sup>133</sup> Both aging and obesity promote immune cell infiltration into adipose tissue with macrophage

infiltration more prominent in obesity and T-regulatory cells more prominent in aging.<sup>134, 135</sup> Adipose tissue dysfunction associated with aging or obesity are also linked to increase in number of senescent cells which secrete SASP components including cytokines and chemokines.<sup>114</sup> Therefore, chronic inflammation associated with adipose tissue dysfunction, SASPs components, are all intertwined and adding fuel to insulin resistance and accelerated aging phenotype of obesity and diabetes.

A number of clinical trials have clearly demonstrated that treatment with monoclonal antibodies, mostly directed against IL-1 $\beta$  and TNF- $\alpha$ , significantly reduces the risk of developing cardiovascular events. Targeted anti-inflammatory therapy has been suggested for both preventing and treating diabetes and this topic has been extensively reviewed.<sup>136</sup> The effects of TNF- $\alpha$  signaling antagonists are still controversial, and the best results have been obtained in patients with overt inflammatory diseases, such as rheumatoid arthritis. The positive effects of anti-IL-1 $\beta$ —including IL-1 receptor antagonist (anakinra) and IL-1 $\beta$ -specific antibody (gevokizumab, canakizumab, and LY21891020)—have been demonstrated in multiple trials, both in terms of reducing inflammation, as well as improving carbohydrate metabolism, with a relatively safe profile. Chronic administration of salsalate, a prodrug of salicylate with fewer adverse reactions than aspirin, has beneficial effects on glycemia and insulin sensitivity, probably by inhibiting the NF- $\kappa$ B pathway. Other anti-inflammatory drugs such as Diacerein (a treatment for arthritis) and chloroquine/hydroxychloroquine (an antimalaric) have also demonstrated benefits in improving glycemia.

### Endocannabinoids

Over the last few decades it has become apparent that the endocannabinoid system (ECS) plays a role in the development of obesity, modifies insulin receptor activity in liver, fat and muscle, and is involved in lipid metabolism – all risk factors for CVD. In abdominal obesity the ECS is upregulated both centrally and peripherally.<sup>137</sup> The endocannabinoids, anandamide and 2-arachidonoylglycerol, are lipid mediators, whose synthesis is directly influenced by dietary fats and their composition.<sup>138</sup> A hyperactive ECS can then, in a vicious cycle, lead to even greater fat accumulation by increasing food intake through activation of cannabinoid 1 receptors (CB1Rs) in brain and favoring reduced energy expenditure in peripheral tissues, such as muscle. In liver, activation of CB1Rs lead to downregulation of insulin receptor activity and worsening hepatic steatosis and steatitis in animals on a high fat diet.<sup>139–141</sup> Furthermore, CB1R activation causes endoplasmic reticulum stress that leads to increased ROS and ceramide synthesis and further insulin receptor down-regulation.<sup>142</sup> Moreover, islets of Langerhans contain an autonomous ECS system, complete with the enzyme machinery for synthesis and secretion of endocannabinoids and the presence of CB1Rs on  $\beta$  cells.<sup>139</sup> Moreover, an overstimulated ECS in islets of Langerhans leads to activation of proapoptotic proteins in  $\beta$  cells and serves as a brake on insulin secretion after  $\beta$  cell depolarization.<sup>139, 143, 144</sup> In-depth review of the relationship between the ECS and islets is available.<sup>145</sup> Data is now emerging concerning the roles of the ECS directly in cardiovascular functions. Activation of CB1Rs on macrophages enhances their recruitment and their recruitment in turn of other inflammatory-type immune cells to atherosclerotic plaques and sites of injury in the heart and vascular

epithelium, and to smooth muscle cells. An activated CB1R also promotes profibrotic pathways in myofibroblasts.<sup>146, 147</sup> Research in ECS and aging is lacking however.

### Intestinal permeability

In humans, the intestinal epithelium covers a surface of about 32 m<sup>2</sup>, which is approximately half the size of a badminton court.<sup>148</sup> Such vast surface is the site for interactions with ingested substances, and it serves two important functions: a barrier preventing entry of antigens, toxins and microorganisms into the body; a selective filter facilitating absorption of nutrients, electrolytes, and water; and exchange of molecules between host and environment. Age-dependent intestinal barrier dysfunction or increase in intestinal permeability is associated with metabolic dysfunction, inflammation and death in *Drosophila*.<sup>149</sup> A more permeable gut, in theory, would result in increased entry of antigens such as lipopolysaccharide (LPS), an endotoxin found in the outer membrane of gram-negative bacteria. LPS has been used as a surrogate measure of gut permeability.<sup>150</sup>

Proinflammatory responses of adipose tissue, primarily preadipocytes, to LPS may be combined with high local concentrations of fatty acids during ensuing cytokine-induced lipolysis to mitigate infection.<sup>150</sup> In a population study of 7169 participants, higher endotoxin activity was associated with prevalence and incident of diabetes.<sup>151</sup> Plasma LPS concentration was also significantly higher in healthy lean subjects after consuming high-fat, high-carbohydrate meals compared to high-fiber and fruit meal.<sup>152</sup> Clearly, much more research is needed in this area to investigate the association between diet (both the amount of calorie consumed and diet quality), gut microbiota, gut permeability and inflammation with aging and age-related diseases.

### Mitochondrial dysfunction

The decline of mitochondrial function with age is well described but the relationship between mitochondrial dysfunction and longevity is complex and is an area of active research.<sup>153, 154</sup> Mitochondrial dysfunction is associated with decrease in insulin sensitivity and  $\beta$ -cell death in type 2 diabetes.<sup>155, 156</sup> Mitochondrial defects of various causes such as mtDNA deletion, and decrease in gene expression of PGC-1 $\alpha$  (master control in the regulation of mitochondrial biogenesis) and nuclear respiratory factor 1 (NRF1), have been found in skeletal muscle of diabetic patients.<sup>157, 158</sup> Impaired oxidative phosphorylation and fatty acid  $\beta$ -oxidation in skeletal muscle was found in insulin-resistant offspring of patients with type 2 diabetes and older adults with insulin resistance.<sup>159, 160</sup> Reduced mitochondrial oxidative capacity measured by <sup>31</sup>P-magnetic resonance spectroscopy was associated with the prevalence and duration of insulin resistance in 248 non-diabetic participants in the BLSA.<sup>161</sup> Mitochondrial dysfunction observed in obesity is associated with chronic accumulation of nonesterified fatty acids due to excessive caloric intake.<sup>162</sup> Excess substrates load such as high-fat diet and hyperglycemia increase reactive oxygen species production in mouse adipocytes.<sup>163, 164</sup> In a vicious cycle, reactive oxygen species then reduce oxygen consumption in adipocytes, block fatty acid oxidation, and result in more lipid accumulation.<sup>165</sup> Dysfunctional mitochondria that cannot be recycled by mitophagy cause the release of damage-associated molecular species that trigger an inflammatory

response by the inflammasome. Both the accumulation of fatty acids and inflammation contribute to insulin resistance.

Altered myocardial mitochondrial metabolism has been observed in aging, as well as in diabetes and obesity. Aging is associated with a decrease in myocardial fatty acid utilization and oxidation, as well as a relative increase in myocardial glucose utilization at rest.<sup>166, 167</sup> In response to dobutamine, both younger and older hearts increase myocardial fatty acid metabolism; however, only the younger hearts had increased myocardial glucose utilization.<sup>168</sup> In obesity and diabetes, an increase in myocardial fatty acid uptake and oxidation has been observed in addition to a decrease in myocardial glucose metabolism. This increase in fatty acid uptake is coupled to enhanced myocardial oxygen consumption but without a concomitant increase in contractility, thus resulting in reduced cardiac efficiency.<sup>169, 170</sup> This age-, obesity-, and diabetes-related mitochondrial dysfunction contributes to the inability of the aging heart to withstand stressors, leading to increased incidence of cardiac failure with aging.

It is apparent that all these factors described above – adipose tissue dysfunction, cellular senescence, inflammation, endocannabinoids, intestinal permeability, and mitochondrial dysfunction – are all interconnected and work in a feedback loop of worsening metabolic control leading to obesity, insulin resistance, diabetes, CVD and associated morbidity and mortality. However, as we started earlier, the biggest single factor contributing to declining whole-body insulin sensitivity with age, is obesity especially when fat is accumulated outside the natural subcutaneous compartment. Researchers at the Mayo Clinic recently published data collected from 116 men and women ages 19–78 and attempted to account for the many factors that we mentioned above, which are supposed to impair insulin action with age. They used hyperinsulinemic-euglycemic clamps to measure M (rate of whole body glucose metabolism; see Box 1), delivered isotopes to measure hepatic glucose suppression, performed magnetic imaging and spectroscopy to quantify subcutaneous, visceral fat deposits, and intrahepatic/intramyocellular lipid amounts, while also using DEXA to measure total fat mass, fat-free mass and % body fat. They also performed muscle biopsies for measuring mitochondrial respiration capacity and reactive oxygen species. In summary the study was, all things considered, a very comprehensive study of liver, muscle, fat depots, and mitochondrial function using state-of-the-art techniques across a spectrum of ages. The relevant findings were: (1) total-body insulin sensitivity negatively correlated with visceral fat and hepatic lipid accumulation (no surprise); (2) hepatic insulin sensitivity was negatively correlated with total body and visceral fat (again, no surprise); (3) however, skeletal muscle mitochondrial function, measured in the biopsy from vastus lateralis muscles, was not a predictor of peripheral insulin sensitivity; and (4) age itself, at least up to age 78, did not appear to be an independent risk factor for reduced insulin sensitivity.<sup>171</sup> Another intriguing finding from the study was that intramyocellular lipid amounts, measured in the anterior tibialis muscle, were actually *positively* predictive of peripheral insulin sensitivity. One can hypothesize that this may be because the fat is fated to go towards  $\beta$ -oxidation, which may cause increasing mitochondrial turnover and capacity. However, this theory remains to be confirmed. Additionally, the findings from the Mayo group may not be outlier findings because there are hints from other investigators that actual lipid droplets in muscle are neutral or even positive with respect to insulin sensitivity.<sup>172, 173</sup>



## BRIEF SUMMARY OF INTERVENTION TRIALS OF GLUCOSE LOWERING THERAPIES IN CVD PREVENTION

Despite the evidence linking exposure high glucose to CVD, randomized control trials (RCTs) of glucose lowering therapies in patients with type 2 diabetes have not been successful in CVD prevention or mitigation, though studies using the new classes medications, GLP-1 receptor agonists and sodium/glucose cotransporter 2 (SGLT2) inhibitors, appear to be more promising in this direction. The search for therapeutic strategies for preventing CVD by lowering blood glucose has been ongoing for more than 50 years. The University Group Diabetes Program (UGDP), a multicenter randomized controlled trial funded by the National Institutes of Health (NIH) in the 1960s, first tested the hypothesis that lowering glucose would prevent CVD in type 2 diabetic patients.<sup>174</sup> One thousand and twenty-seven participants (mean age of 53 years; age $\geq$ 55 years (45%)) were randomized to 1 of 5 treatment arms: (1) tolbutamide sodium, a first-generation sulfonylurea that was the most frequently prescribed glucose-lowering drug in the US at that time, (2) phenformin hydrochloride, a first-generation biguanide that was another of the most frequently prescribed glucose-lowering drugs at the time, (3) a variable amount of insulin aimed at keeping the blood glucose level close to 'normal', (4) a small, fixed dose of insulin based on body size, or (5) a placebo.<sup>175</sup>

Surprisingly, compared to placebo the UGDP found a 2.9 times higher death rate from CVD in the tolbutamide group ( $P = 0.005$  vs placebo) and a 4 times higher death rate from CVD in the phenformin group ( $P = 0.04$  vs insulin plus placebo); while no differences were seen in microvascular complications compared with placebo in these 2 groups.<sup>176–182</sup> The tolbutamide and phenformin arms of the trial were stopped after 8 years because of the increased death rates.<sup>176, 178</sup> The study was continued in the two insulin groups because their macrovascular or microvascular complications and mortality were no different from placebo, however results remained the same after 13 years of follow-up with no obvious benefit to insulin treatment in either insulin groups.<sup>181, 182</sup>

In the 2000s thiazolidinediones, a new class of glucose lowering agents, became available. The major glucose-lowering action of this new class was to increase insulin sensitivity resulting in a lowering of A1c on average by at least 1%. The expectation would be that lowering insulin resistance would prove beneficial in CVD. However, troglitazone, the first-in-class to become widely used, was withdrawn because of liver toxicity and, in many cases, resulting deaths. In 2007, a meta-analysis of 42 eligible studies (mean age of 56 years) of rosiglitazone compared to placebo/other glucose-lowering agents in treating type 2 diabetes, with pre-specified end points of heart attacks and deaths from cardiovascular disease. They found that rosiglitazone was actually associated with a significant increase in heart attacks and borderline increase in deaths from CVD.<sup>183</sup> With these negative effects of two thiazolidinediones in a row, things did not look good for this class of compounds especially with regards to CVD. However, the third drug in this class, pioglitazone, based on a very recent meta-analysis of nine trials with about 12,000 participants (mean age 62 years), seems to have cardiovascular benefits with regards to major cardiovascular events in people with insulin resistance, glucose impairment and type 2 diabetes, but it increases the risk of heart

failure, without increasing CVD deaths, likely because of its side-effect of fluid retention that is common to drugs in this class.<sup>184</sup> A meta-analysis of 19 RCTs that involved pioglitazone versus placebo/other glucose-lowering agents in the treatment of type 2 diabetes reported similar results.<sup>185</sup> The differences in the two compounds, rosiglitazone and pioglitazone, with regards to CVD are likely independent of any glucose-lowering effect but rather due, at least in part, to differences in lipid handling, with pioglitazone having favorable effects on the LDL particle size in particular.

More recent RCTs have reported inconclusive results on the effect of intensive glucose-lowering therapies regardless of the method, including increasing amounts of insulin to achieve as close to A1c of 7% or below, in the prevention of CVD in patients with type 2 diabetes. Four large-scale RCTs, three of which enrolled over 10,000 patients (mean age ranges from 60 to 66 years), shown no benefit of intensive glucose-lowering therapy, including in subgroup analysis of those older than 65 years of age. Indeed, one of these studies was stopped prematurely because of increased mortality in the treatment arm.<sup>186–189</sup>

In spite of the negative results of previous trials, a recent meta-analysis of five RCTs of intensive glucose-lowering therapy in type 2 diabetic patients (33, 040 patients, mean age 62 years), indicated a modest benefit to glucose lowering on CVD outcomes.<sup>190</sup> Furthermore, a follow-up observational study of patients who had been enrolled in the ACCORD RCT showed some evidence of benefit of intensive glucose-lowering therapy on cardiovascular outcomes.<sup>186</sup> And, of great interest, after mining the data from the UKPDS trial, early lowering of blood glucose, by any means, seemed tied into lower CVD at later times, in what is termed the legacy effect.<sup>191</sup> However, further follow-up for 2.5 years of the ORIGIN TRIAL participants to look for a legacy effect turned out to be CVD neutral.<sup>192</sup>

A class of compounds with glucose-lowering effects and minimal systemic or off-target effects, the alpha-glucosidase inhibitors, has fallen out of use despite very favorable CVD outcomes. As mentioned earlier, the DECODE study generated evidence that the 2hG has a strong link to cardiovascular and all-cause mortality: the higher the 2hG glucose, the greater the risk of CVD mortality.<sup>193, 194</sup> Acarbose is an alpha glucosidase inhibitor that reduces post-prandial elevations in glucose by reducing absorption of carbohydrates from the small bowel, and, unlike tolbutamide, it carries no risk of hypoglycemia. The STOP-NIDDM study (1,429 participants, mean age 54 years) investigated the effects of acarbose versus placebo on the development of diabetes over 5 years in patients with impaired glucose tolerance. The study also assessed the effect of acarbose on development of major CVD events. The risk of progression to diabetes was reduced by 36% and the risk of developing a CVD event was halved: there was 13 heart attacks in the participants, only one of which was in the acarbose-treated group.<sup>195</sup> Because the trial was in people who did not yet have diabetes it can be easily argued that CV pathology was not long-standing or yet irreversible, therefore making it more likely that beneficial effects could be seen. Nonetheless, it shows that CVD can be impacted by lowering post-prandial glucose, especially if done during the diabetes trajectory of increasing blood glucose levels. Use of acarbose in the US fell out of favor, despite the obvious beneficial effects on glucose homeostasis and CVD, because of the gastrointestinal effects of flatulence, bloating and stool changes.

Two new classes of glucose-lowering agents have become available: GLP-1 receptor agonists (since 2005) and sodium/glucose cotransporter (SGLT2) inhibitors. New RCTs were designed and conducted to test the hypothesis that these new compounds, which are more expensive than existing glucose-lowering drugs, were beneficial to CVD versus placebo or compared to existing glucose-lowering agents. The two large RCTs LEADER (9,340 patients, mean age 64 years) and SUSTAIN-6 (3,297 patients, mean age 65 years) found that two GLP-1 agonists, liraglutide and semaglutide, favourably affect the risk of developing CVD in type 2 diabetes.<sup>196, 197</sup> Liraglutide reduced CVD death and all-cause mortality compared to placebo, and even resulted in less hospitalizations because of heart failure, with differences becoming even more apparent after 24 months.<sup>196</sup> When subgroup analysis was conducted in patients older than 60 years of age in the LEADER study however, the effect was only marginally significant. Participants on semaglutide treatment had fewer heart attacks and nonfatal strokes compared to those on placebo.<sup>197</sup> However, another agent in this class, lixisenatide, when studied in the ELIXA trial (6,068 patients, mean age 60 years old) over two years, did not show CVD benefit.<sup>198</sup>

The EMPA-REG OUTCOME trial (7,020 patients, mean age 63 years), evaluated CV safety and efficacy of empagliflozin (SGLT-2 inhibitor) versus placebo, in a population with established CVD. Subjects with diabetes and known CVD were followed for three years. Empagliflozin use lead to a 38% relative risk reduction in CVD—an impressive result. When subgroup analysis was done in patients older than 65 years of age, the beneficial effect persisted. Additionally, similar to use of GLP-1 agonists, hospitalizations for heart failure were reduced by 35% using empagliflozin. Similar results are appearing for canagliflozin, especially in the older than 65 years subgroup (CANVAS trial with 10142 patients, mean age of 63 years), and therefore point to a class effect.<sup>199, 200</sup>

## **A MODEL OF AGING, BODY COMPOSITION CHANGES AND INSULIN RESISTANCE**

Based on this review of the literature and our own work, we propose a model (Figure 6) that links the possible dysregulation of physiological pathways leading to obesity and diabetes, both a form of accelerated aging and risk factors for CVD, as a consequence of chronic excessive food consumption. The changes in body composition that are intrinsically connected with the biology of aging together with over indulgence in food and the types of food modify gut microbiota. Changes in gut microbiota, together with nutrients alter intestinal permeability which lead to absorption/infiltration of different types of molecules from the gut lumen including lipopolysaccharides (LPS), large molecules from the outer membrane of gram-negative bacteria. The excess calories and LPS may then lead to upregulated ECS, mitochondrial dysfunction, and chronic inflammation and, over time, increased cellular senescence and adipose tissue dysfunction. Excessive adipose accumulation then causes insulin resistance and eventually, in some people, diabetes. Diabetes and obesity are both forms of accelerated aging and independent risk factors for CVD. Based on this model, diet and exercise should be essential component of treatment of insulin resistance in older persons in preventing development of diabetes. This concept has

already been eloquently studied in the Diabetes Prevention Program which showed that diet and exercise were more effective than metformin in preventing development of diabetes.<sup>201</sup>

## SUMMARY

Over the past 60 years, tremendous progress has been made in understanding the pathogenesis of age-related glucose dysregulation and cardiovascular disease. Despite knowing that excessive food consumption is the predominant cause of obesity, diabetes, and cardiovascular disease, no progress has been made in reversing the prevalence of these diseases. More research, basic, translational and clinical, is needed to better understand interaction of gut microbiota, inflammation, endocannabinoids and whole-body energy consumption in the genesis of diabetes and other metabolic diseases in humans. This knowledge can help us better reinforce selection in “quality” food consumption. Collaborative efforts across disciplines such as behavioral science, mental health, and nutrition are needed so we can get a better understanding of human behavior in overeating and finetune public policies in curbing the obesity and diabetes epidemics. We need to tackle the root cause of the obesity epidemic, which is excessive calorie intake, before we can reverse the tide of ever increasing prevalence of diabetes and cardiovascular disease. Based on our review of the topic, it also appears self-evident that we need to intervene earlier in the glucose trajectory, especially in the post-prandial period, to maintain glucose homeostasis as close to ‘normal’ as possible and before people are given a biochemical diagnosis of diabetes. Blood glucose is a continuum of risk for CVD and it is likely that changes in the vascular epithelium that cannot be reversed simply by lowering blood glucose have already occurred by the time of diabetes diagnosis.

## Acknowledgments

### SOURCES OF FUNDING

This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

## Non-standard abbreviations:

<b>2hG</b>	2hr plasma glucose
<b>BLSA</b>	Baltimore Longitudinal Study of Aging
<b>CDC</b>	Centers for Disease Control and Prevention
<b>CVD</b>	Cardiovascular disease
<b>FPG</b>	Fasting plasma glucose
<b>GIP</b>	glucose-dependent insulinotropic polypeptide
<b>GLP-1</b>	glucagon-like peptide-1
<b>IMGU</b>	Insulin-mediated glucose uptake
<b>NIA</b>	National Institute on Aging

<b>NIH</b>	National Institutes of Health
<b>NIMGU</b>	noninsulin-mediated glucose uptake
<b>OGTT</b>	oral glucose tolerance test
<b>RCT</b>	randomized control trial
<b>SGLT2</b>	sodium/glucose cotransporter 2
<b>USDA</b>	US Department of Agriculture

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### Box 1: Brief description of glucose clamp technique

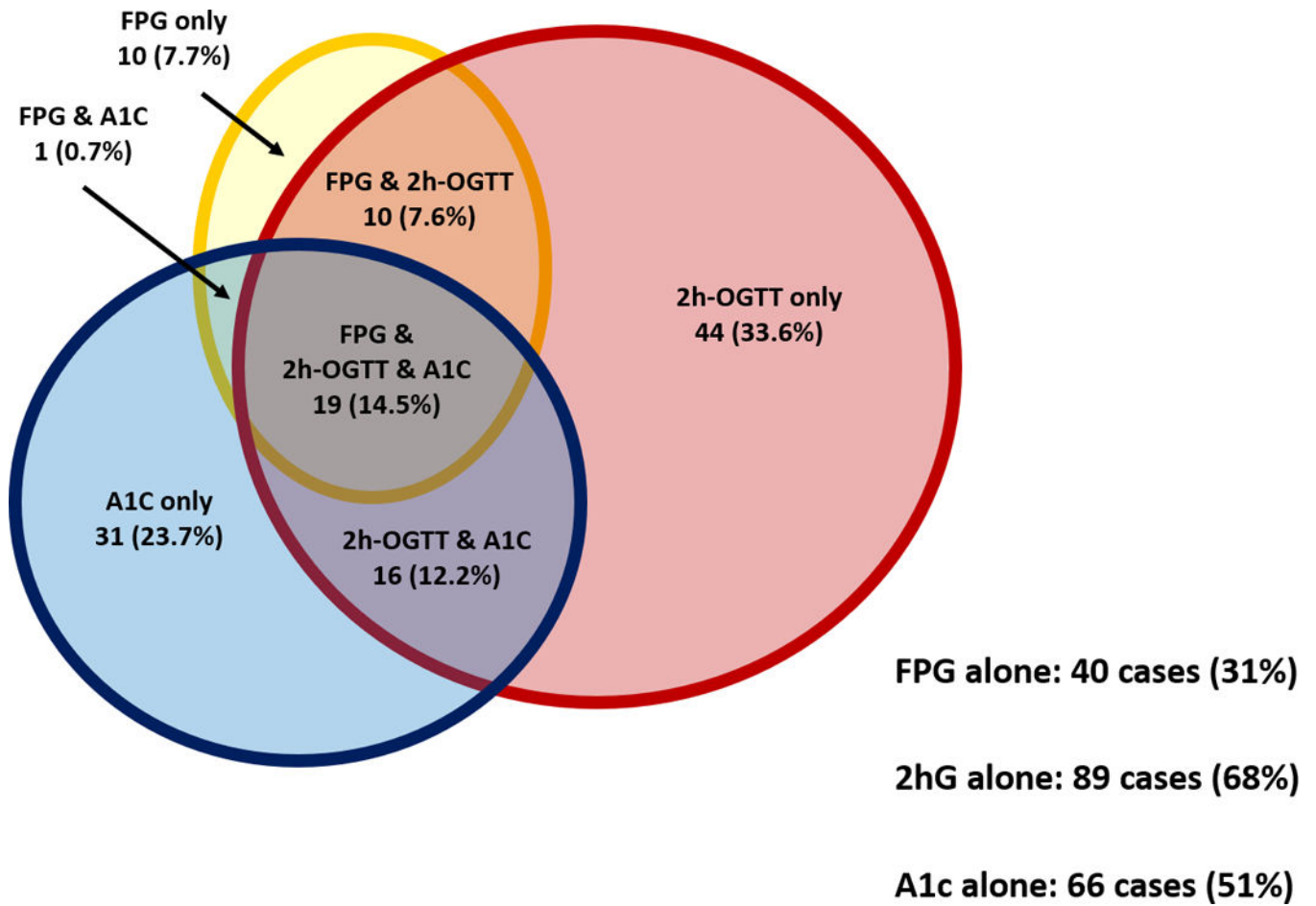
The glucose clamp technique was developed in the 1960s by Andres and colleagues at the National Institute on Aging to conduct *in vivo* assessment of glucose metabolism in humans.<sup>71</sup> Ground breaking studies were done to understand hormonal regulation and pathophysiology of carbohydrate metabolism.<sup>54, 202–205</sup> To this day, this clamp technique is still considered the reference standard for studying whole body glucose homeostasis as it is generally considered the most rigorous and reproducible test for studying glucose homeostasis of the whole body or even a specific organ.

Although protocols for clamp come in many forms, most commonly used are the hyperinsulinemic-euglycemic and the hyperglycemic clamps. In all clamp protocols, overnight fast is required for at least 8 hours prior to the procedure. An antecubital intravenous catheter is inserted for infusion of 20% dextrose, insulin, peptides, or isotope. A second catheter is inserted in a retrograde fashion in a dorsal hand or wrist vein for blood sampling. This hand is then placed in a warming box with temperature set at 50°C for about 15 minutes to arterialize the blood prior to starting the clamp. Three to four blood samples are drawn at 10 min intervals to establish basal glucose level. All plasma glucose samples are assayed in real-time.

The hyperinsulinemic-euglycemic clamp procedure is designed to assess *in vivo* insulin sensitivity and best method for studying hepatic glucose production. This procedure begins with an infusion of priming dose of regular insulin administered as a step function with decreasing level every 2 min for the first 10 minutes then decrease to the desired insulin dose that is maintained for the rest of the clamp procedure. The most common dose use is 6nmol/m<sup>2</sup>/min (40 mU/m<sup>2</sup>/min) with insulin doses up to 200 mU/m<sup>2</sup>/min had been used.<sup>206</sup> Four minutes after the start of insulin infusion, glucose infusion is also begun and the glucose infusion rate is adjusted every 5 minutes based on the plasma glucose determined from the sample to maintain the plasma glucose levels at the basal level. Glucose utilization usually reaches a steady state around 80 minutes after the initiation of insulin infusion. At this juncture, the glucose infusion rate is approximately equal to glucose utilization, assuming that hepatic glucose production is completely suppressed by the exogenous hyperinsulinemia. The amount of glucose metabolized is designated as M. For more insulin resistant individuals, hepatic glucose production may not be completely suppressed, and amount of glucose infusion would be lower and hence a lower M value would be obtained.

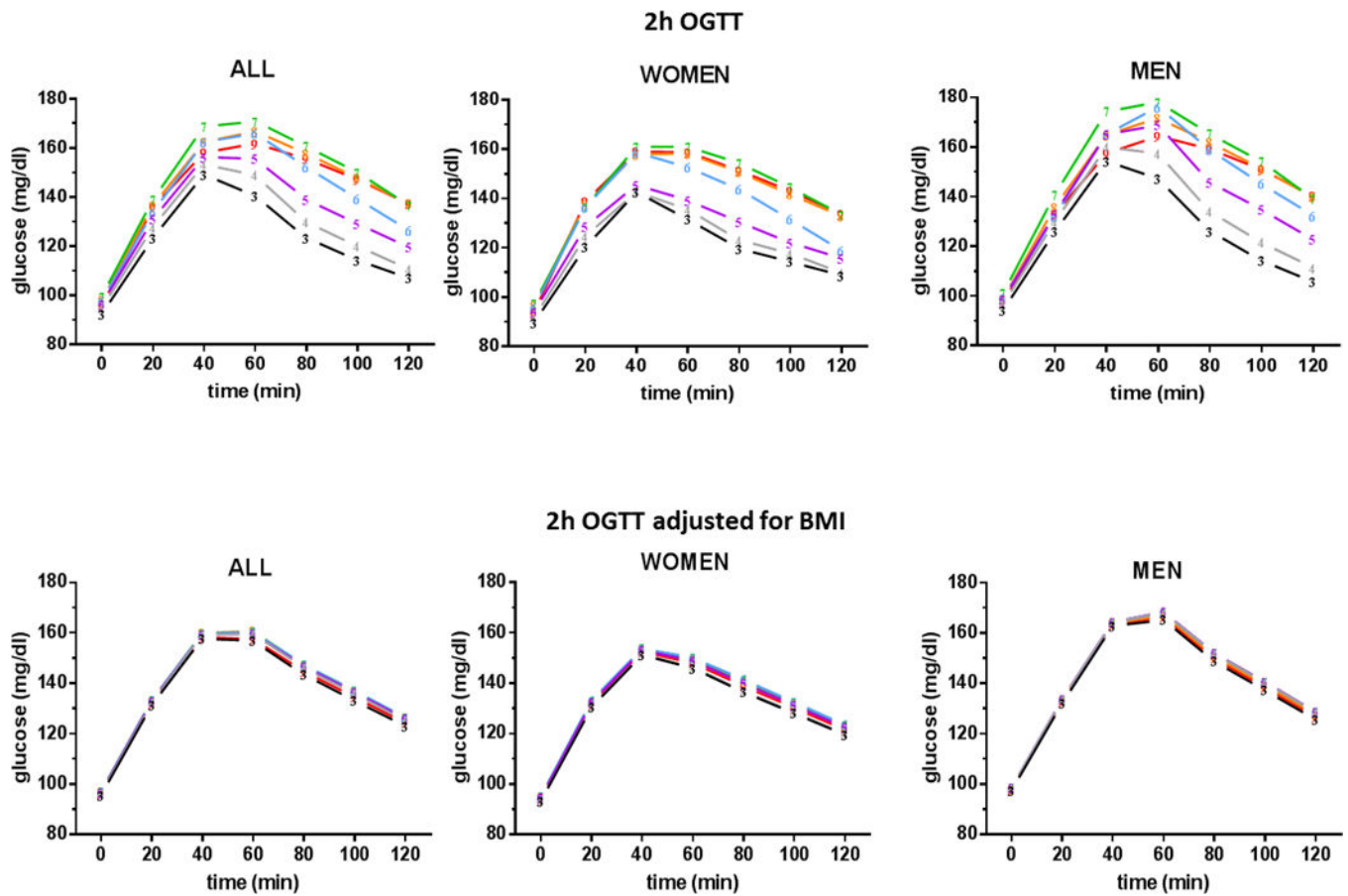
The hyperglycemic clamp technique is designed to assess  $\beta$  cell function in addition to insulin sensitivity. The procedure begins with an infusion of priming dose of 20% dextrose administered as a step function with decreasing level every 2 min for the first 10 minutes. For the first 10 minutes, blood samples are obtained for glucose measurement every 2 minutes. For the rest of the clamp, plasma glucose samples are obtained every 5 min so that dextrose infusion can be adjusted as to maintain the plasma glucose level at a predetermined level, usually 5.5 mmol/L (98 mg/dL) above basal glucose levels. A range of target glucose levels of 3.0 – 12.8 mmol/L (54–230 mg/dL) above basal glucose levels had been used in studies. Hyperglycemic clamp enables assessment of both  $\beta$ -cell function and peripheral insulin sensitivity. The crucial difference between insulin

sensitivity obtained from the hyperglycemic clamp and the hyperinsulinemic-euglycemic clamps is that the insulin sensitivity is in response to variable endogenous insulin release in the former and to stable and reproducible exogenous insulin administration in the later.



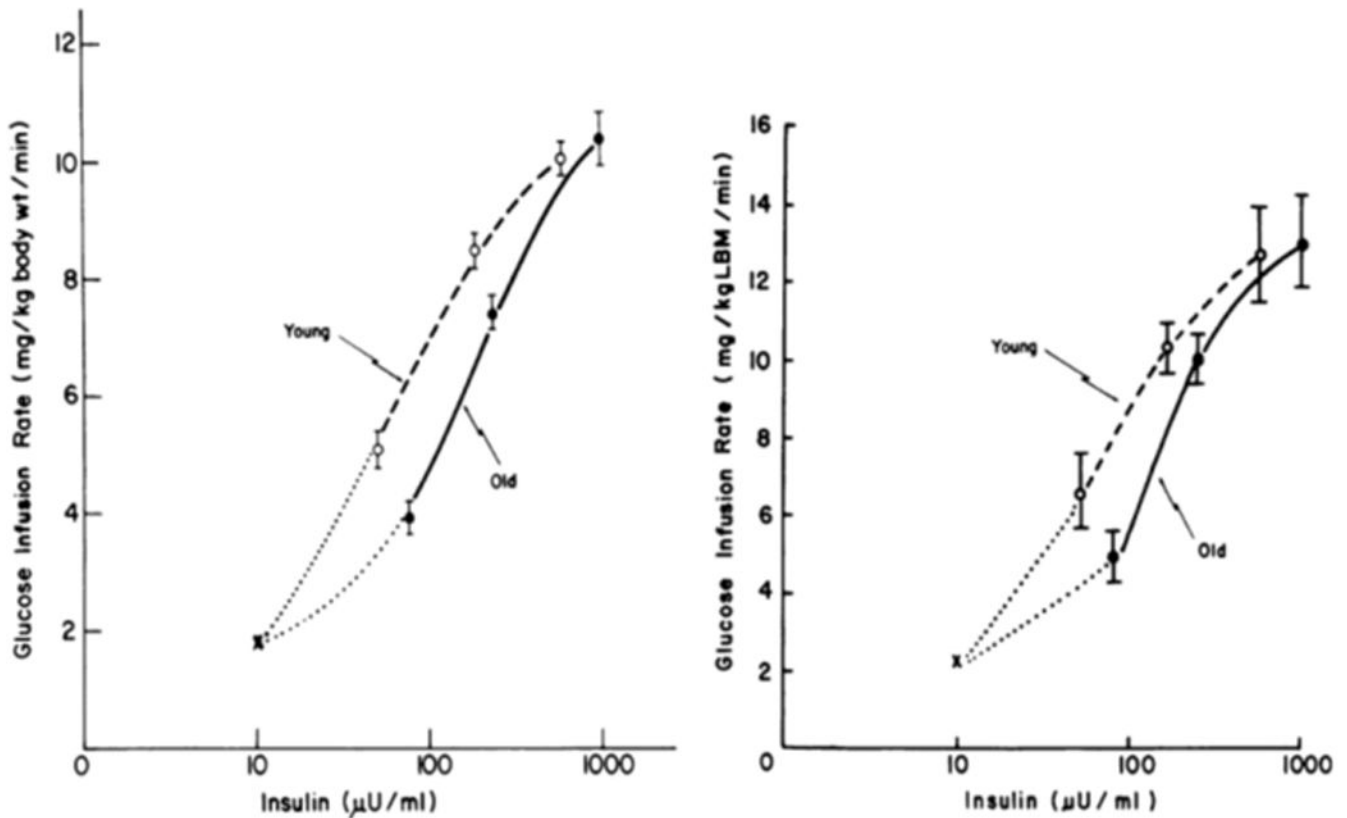
**Figure 1:**

Selected participants from the Baltimore Longitudinal Study of Aging with data on A1c and OGTT that had no history of diabetes and were not on antidiabetic medication. Out of 1152 participants, 131 individuals were newly diagnosed with type 2 diabetes using either A1c, FPG or 2hG. Using FPG (yellow oval) alone identified 40 (31%) cases, 2hrG alone (red circle) identified 89 (68%) cases, and A1c alone (blue circle) identified 66 (51%) cases. If only FPG and A1c are used for diagnosis, more than one-third of those with diabetes would be missed.

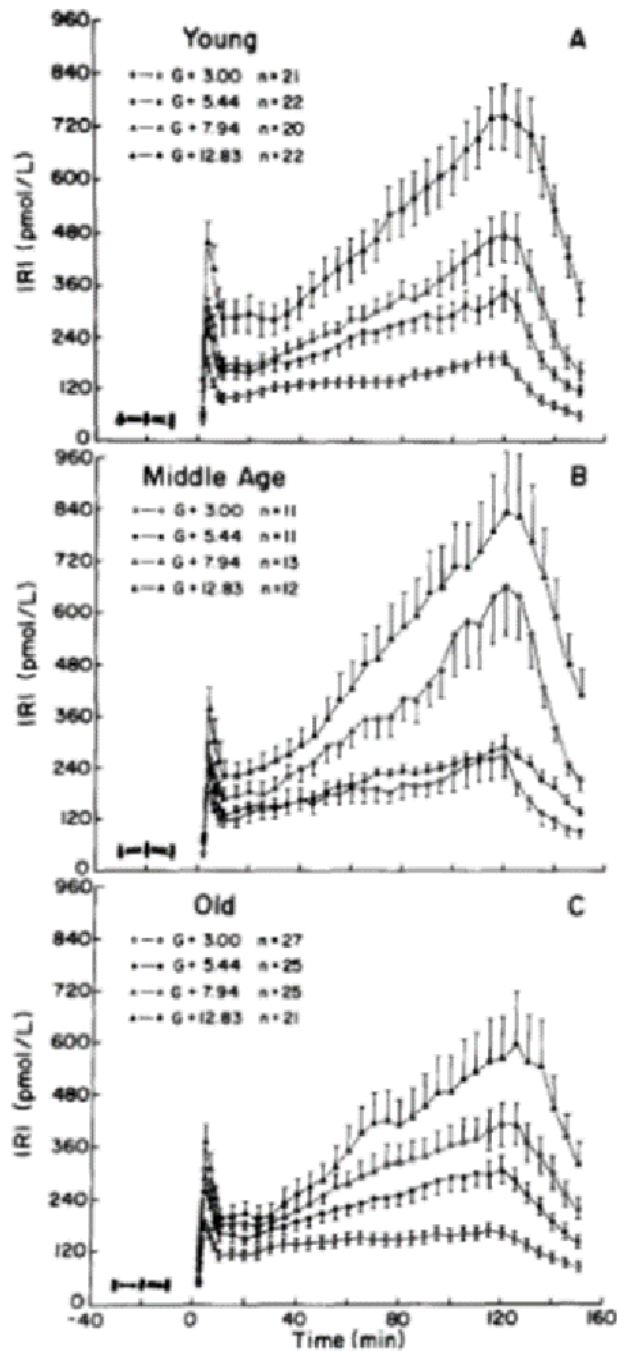


**Figure 2: Oral glucose tolerance test**

Top panel shows OGTT results in 2,777 nondiabetic participants from the Baltimore Longitudinal Study of Aging. Each line corresponds to one decade. Plasma glucose levels during OGTT rise consecutively for every decade of age until they peaked at the 7<sup>th</sup> decade. Men and women have similar patterns with men having more severe glucose intolerance. Bottom panel shows the OGTT results adjusted for body mass index (BMI).



**Figure 3:** Dose-response curve for insulin-mediated whole-body glucose infusion rate during hyperglycemic clamps in young (---) young and old (—) subjects. Left panel presents glucose infusion rate in mg/kg body weight/min. Right panel presents glucose infusion adjusted for lean body mass. “x” represents basal hepatic production rate (reprint with permission).<sup>55</sup> An age-associated decrease in glucose utilization was demonstrated with no change in maximal tissue responsiveness or maximal glucose uptake. The insulin-glucose dose-response curve shifts to the right with age (left panel). The half-maximal glucose uptake occurred when plasma insulin levels were 54 uU/mL in the young and 113 uU/mL in the old. This association held significance after accounting for lean body mass (right panel).



**Figure 4:** Bimodal time course of the endogenous plasma insulin response during 2h hyperglycemic clamps at four different hyperglycemic plateaus: 54, 98, 142, 230 mg/dL above basal glucose levels. Top panel: young participants; middle panel: middle age participants; bottom panel: old participants (reprint with permission).<sup>70</sup> There is a first-phase insulin secretion that occurs during the first 10 min with a peak response at ~4 min; then, plasma insulin level falls reaching a nadir at ~ 10 min; next, the second insulin secretion phase begins and insulin levels increase linearly until a plateau is reached. Within each age group, higher glucose

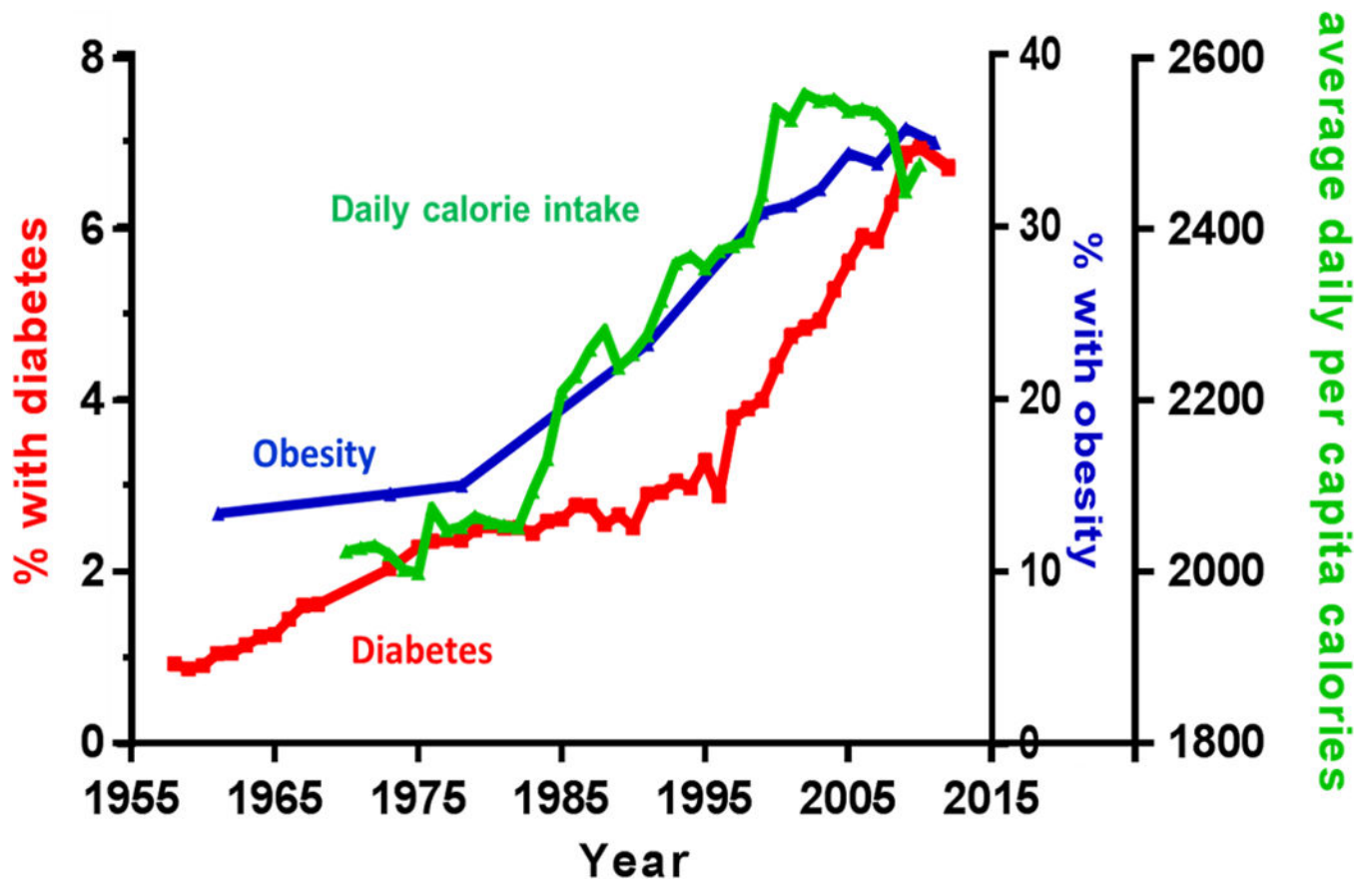
doses corresponded with statistically higher insulin responses at all levels but differences in insulin responses among the age groups were not significantly different.

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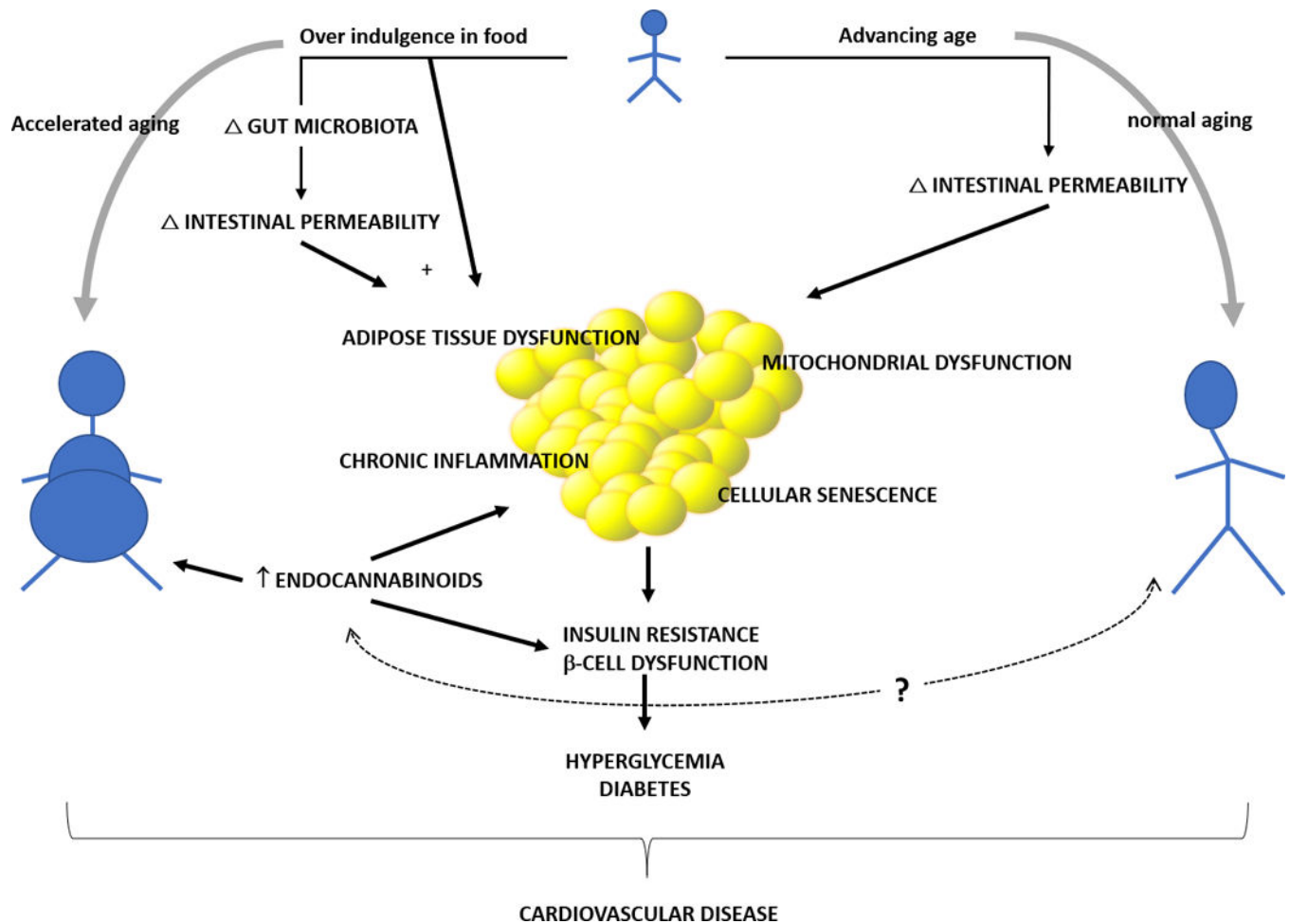
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**Figure 5:** Increase average daily per capita food consumption, prevalence of obesity and diabetes over the past 60 years in the United States (graphs drawn using data from the CDC and USDA).  
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**Figure 6:**

A model of aging, body composition changes and insulin resistance that links the possible dysregulation of physiological pathways leading to obesity and diabetes, both a form of accelerated aging and risk factors for CVD, to chronic excessive food consumption. The changes in body composition that are intrinsically connected with the biology of aging together with over indulgence in food and the types of food modify gut microbiota. Changes in gut microbiota, together with excess food intake alter intestinal permeability which lead to absorption/infiltration of different types of molecules from the gut lumen including lipopolysaccharides (LPS), large molecules from the outer membrane of gram-negative bacteria. The excess calories and LPS may then lead to chronic inflammation and an upregulated endocannabinoid system, and, over time, increasing the amount of senescent cells and adipose tissue dysfunction. Excessive adipose accumulation then leads to insulin resistance and, in some people, eventually to diabetes. Diabetes and obesity are both forms of accelerated aging and independent risk factors for CVD. (Illustration Credit: Ben Smith).