THE RELATIONSHIP BETWEEN DIETARY ADVANCED GLYCATION END PRODUCTS AND DIABETES RELATED COMPLICATIONS

BY

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DISSERTATION

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ABSTRACT

One of the research areas that has gained interest in recent years in order to explain diabetes-related complications is the accumulation of a group of compounds called advanced glycation end products (AGEs). AGEs can be formed in the body and contribute to vascular damage in diabetes. In addition, AGEs can be found in some foods rich in protein and fat (high cooking temperatures increases its formation) and some research shows that they can also accumulate in the body and could have a role in diabetes complications. Some studies also have shown that higher intake of AGEs could increase the risk of diabetes-related complication by elevating inflammatory and oxidation markers even in states when glucose levels are normal. However, the association between AGEs consumption and diabetes-related complications has not been demonstrated.

In this study, we tried to identify if high intake of AGEs results in an increased risk for complications in patients with DM type 2 in two different ethnicities (Mexicans and non-Hispanic Whites). Because the association between AGEs consumption and diabetes-related complications has not been studied due to the long-term data needed for AGE intake. In addition, we were also interested in developing an assessment tool (a food frequency questionnaire) to categorize whether AGEs intake is high, moderate or low in order to assess AGEs intake related to DM complications in the different populations studied.

This study showed that for each unit increase in the transformed dietary AGEs (LogAGEs), participants were 3.7 times more likely to have moderate-high risk for cardiovascular disease. The present study also found that the food frequency questionnaire is comparable to 7 days of Food Records to measure dAGEs. In conclusion, dietary AGEs were associated with the risk level for diabetes-related cardiovascular complications, which should be explored in future research.

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LIST OF SYMBOLS

AGEs	Advanced glycation end products
AGER1	Advanced glycation end products receptor 1
CML	Carboxymethyl-lysine
CML-LDL	Carboxymethyl-lysine-low density lipoprotein
CRP	C reactive protein
dAGEs	Dietary advanced glycation end products
ELISA	Enzyme-linked immunosorbent assay
DM	Diabetes Mellitus
FFQ	Food Frequency Questionnaire
FMD	Flow-mediated vasodilation
FR	Food Record
HbA1c	Hemoglobin A1c
HDL	High-density lipoproteins
HOMA	Homeostatic model assessment
ICAM-1	Intracellular adhesion molecule 1
IL-6	Interleukin 6
LDL	Low-density lipoproteins
MAPK	Mitogen-activated protein kinases
MDA	Malondialdehyde
MG	Methylglyoxal
NF-κB	Nuclear factor kappa B
PI3-K	Phosphatidylinositol-3 kinase
RAGE	Receptor for advanced glycation end products
TBARS	Thiobarbituric acid-reactive substance
TNF-α	Tumor necrosis factor α
VCAM-1	Vascular adhesion molecule 1

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Chapter 1
Introduction

Recent data show an increase in the prevalence of diabetes mellitus (DM) in the United States (US) from 10.7 in 2007 to 11.3% in 2011 in adults 20 years of age and older. A closer analysis of the 2011 data shows important differences by race/ethnicity; the prevalence in non-Hispanic whites is 7.1% while in Mexican-Americans it is 13.3 %, just below the prevalence of DM in Mexico (14.4%) according to the 2006 national survey [1,2]. A recent survey in Mexico (ENSANUT 2012) reveals that 9.1% of the total population has been diagnosed with diabetes, and a rough estimation for the total prevalence (adding those without confirmed diagnosis) could be as high as 18% [3]. In addition, complications of diabetes are the 7th leading cause of death in the US, and it is estimated that the average medical expenditure for people with diabetes is 2.3 times higher than people without the disease [2]. Understanding the factors that could have a role in diabetes complications is important to improve the individual's quality of life and reduce the economic burden caused by diabetes complications. One of the research areas that has gained interest in recent years is the accumulation of a group of compounds called advanced glycation end products (AGEs). AGEs can be formed in the body and contribute to vascular damage in diabetes [4]. In addition, AGEs can be found in some foods rich in protein and fat (high cooking temperatures increases its formation) and some research shows that they can also accumulate in the body with subsequent impact on health [5]. Most of the studies with dietary AGEs (dAGEs) are clinical research studies, where a high or a low AGEs diet is assigned to a treatment or to a control group, and the effects of these diets are measured in terms of levels of blood AGEs, and levels of inflammation markers. In these studies, AGEs intake has been assessed with 3-day food records. However, there is still not a standardized assessment method for AGEs quantification in the diet, and the association between AGEs consumption and diabetic complications has not been studied due to the lack of longitudinal data on both AGEs intake and complications development.

It must be noted that my research to date has included Mexicans, non-Hispanic whites, and Mexicans in Illinois because this dissertation is a continuation of my research for my Master's degree at the University of Guanajuato, Mexico. That work included a randomized six week prospective study in two groups of patients: one with a standard diet (n=13), and another with low dAGEs (n=13), both with similar amounts of calories, carbohydrates, lipids, and proteins. At the beginning and the end of study, we collected anthropometric measures, and circulating glucose, hemoglobin A1c (HbA1c), lipids, insulin, serum fluorescent AGEs, protein C-reactive (CRP), tumor necrosis factor alpha (TNF-α) and malondialdehyde (MDA), insulin, and the homeostatic assessment model was calculated to measure insulin resistance (HOMA-IR). Adherence to diet was reviewed weekly. Changes in TNF-α levels were different for the standard diet (12.5±14.7) as compared with low dAGEs (–18.36±17.1, $p<0.00001$). Also, changes in MDA were different in the standard versus the low dAGEs group (2.0±2.61 and –0.83±2.0, $p<0.005$), and no changes were found for insulin levels or HOMA-IR. In conclusion, dAGEs restriction decrease significantly TNF-α and MDA levels [6].

For this dissertation, the differences and similarities among two ethnic groups in AGEs intake and differences in complications of diabetes and possible relationships with level of AGEs in diet were investigated.

1.1 Objectives

The long-term goal of my research is to delay the onset of diabetes complications that could be secondary to dAGEs. As a first step towards accomplishing this goal, the objective of this research is to investigate whether there is an association between different levels of AGEs intake and the extent of severity of complications in subjects with DM.

1.2 Hypothesis

My central hypothesis is that subjects with DM type 2 and with diabetes related complications would have a higher intake of dAGEs when compared with subjects without complications.

A review of the literature indicates that dAGEs increase circulating AGEs, accumulate in tissues, and act as a ligand for the advanced glycation end products receptor (RAGE). My previous work showed that by decreasing dAGEs the pro-inflammatory cytokine TNF-α and the oxidation marker MDA decreased as well [6].

The rationale for developing this research is that no previous study has shown a relationship between high intake of dAGEs and presence of complications in type 2 DM. In addition, there is evidence that Mexican-Americans have higher prevalence of diabetes and higher risk for diabetes-related complications than non-Hispanics whites. We are interested in knowing if differences in AGEs intake could explain this higher risk for complications independent of long-term healthcare.

1.3 Specific Aims

In order to test our central hypothesis the following 3 specific aims are proposed:

Specific Aim 1: Examine the dietary intake of AGEs in non-Hispanic white, Mexicans living in Mexico and Mexicans living in Illinois with DM that may vary due to different foods eaten and cooking methods. Also, explore the potential relation of AGEs intake and complications in diabetes.

The working hypothesis is that dAGEs intake is different in these 3 ethnic groups. This difference could explain the differences in diabetes related complications among these 3 groups. This would involve establishing differences between dietary intakes in the 3 groups, setting categories of AGEs intake and determining a score for categories for diabetes complications.

Specific Aim 2: Develop an assessment tool to categorize whether AGEs intake is high, moderate or low in order to assess AGEs intake related to DM complications in the different populations studied.

The working hypothesis is that a food frequency questionnaire is comparable to food records to establish categories of dAGEs intake. This would involve designing a food frequency questionnaire to assess and categorize AGEs intake, and measuring the

relative validity and reliability of this food frequency for future use in epidemiological studies.

Specific Aim 3: Identify if high intake of AGEs results in an increased risk for complications in patients with DM type 2.

The working hypothesis is that subjects with diabetes related complications have a higher AGEs intake when compared with subjects without complications. This would involve assessing actual and past intake of dAGEs in a control group (subjects without complications) and in a case group (subjects with complications).

1.4 Scope and statement of the problem

Diabetes-related complications are the 7th cause of death in the US, and, in Mexico, they are the leading cause of death [1,2]. Patients with diabetes-related complications have a diminished quality of life and the economic toll of the disease is becoming a real challenge. Longitudinal studies showed that glycemic control is the best strategy to delay diabetes-related complications. However, some studies also have shown that higher intake of AGEs could increase the risk of diabetes-related complications by elevating inflammatory and oxidation markers even in euglycemic states [7]. Because the association between AGEs consumption and diabetes-related complications has not been studied due to the long-term data needed for AGEs intake, we believe that this research could help to answer this research question.

1.5 Significance

At the completion of this research, we will have established if dietary AGEs represents a risk for complications in DM. These results will encourage the development of further research to establish a direct association between dietary intake of AGEs and complications. In addition, the results of this research could help in establishing the pertinent recommendations for AGEs intake in patients with DM with the purpose to decrease the health care burden that complications for diabetes represent in the actual health care system in developed as well developing countries.

For this thesis Chapter 2 and Chapter 3 served as literature review. The methodology and results for specific aim 1 are presented on Chapter 4, and additional results are presented on Appendix A. The methodology and results for specific aim 2 are presented on Chapter 5, and the methodology and preliminary results for specific aim 3 are presented on Chapter 6. Conclusions and future directions are presented on Chapter 7. The questionnaires used for the different studies are presented on Appendix B, C and D.

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Chapter 2
Dietary Advanced Glycation End Products and Aging[1]

Abstract: Advanced glycation end products (AGEs) are a heterogeneous, complex group of compounds that are formed when a reducing sugar reacts in a non-enzymatic way with amino acids in proteins and other macromolecules. This occurs both exogenously (in food) and endogenously (in humans) with greater concentrations found in older adults. While higher AGEs occur in both healthy older adults and those with chronic diseases, research is progressing to both quantify AGEs in food and in people, and to identify mechanisms that would explain why some human tissues are damaged, and others are not. In the last twenty years, there has been increased evidence that AGEs could be implicated in the development of chronic degenerative diseases of aging, such as cardiovascular disease, Alzheimer's disease and with complications of diabetes mellitus. Results of several studies in animal models and humans show that the restriction of dietary AGEs has positive effects on wound healing, insulin resistance and cardiovascular diseases. Recently, the effect of restriction in AGEs intake has been reported to increase the lifespan in animal models. This paper will summarize the work that has been published for both food AGEs and *in vivo* AGEs and their relation with aging, as well as provide suggestions for future research.

Keywords: advanced glycation end products; aging; Maillard reaction.

[1] This chapter appeared in its entirety in Nutrients and it is referred as to Luevano-Contreras C, Chapman-Novakofski. Dietary Advanced Glycation End Products and Aging. Nutrients, **2010,** *2*, 1247-1265.

2.1 Introduction

Advanced glycation end products (AGEs) are a heterogeneous, complex group of compounds that are formed mainly via the Maillard reaction. The Maillard reaction occurs when reducing sugar reacts in a non-enzymatic way with amino acids in proteins, lipids or DNA. This reaction has been studied for years in the food industry because its products add a desirable color and taste to foods. However, the study of the products of this reaction *in vivo* have received increasing attention in recent years due to association of AGEs with certain chronic diseases, such as diabetes mellitus, cardiovascular diseases, and Alzheimer's disease, as well as during the aging process.

2.2 Formation of AGEs

The formation of AGEs through the Maillard reaction occurs in three phases (Figure 2.1). First, glucose attaches to a free amino acid (mainly lysine and arginine) of a protein, lipid or DNA, in a non-enzymatic way to form a Schiff base. A Schiff base is a compound that has a carbon to nitrogen double bond where the nitrogen is not connected to hydrogen. The initiation of this first step depends on glucose concentration and takes place within hours. If the concentration of glucose decreases, this reaction is reversible. During the second phase, the Schiff base undergoes chemical rearrangement over a period of days and form Amadori products (also known as early glycation products). The Amadori products are more stable compounds (hemoglobin A1c is the most well known), but the reaction is still reversible. If there is accumulation of Amadori products, they will undergo complicated chemical rearrangements (oxidations, reductions, and hydrations) and form crosslinked proteins. This process takes place in weeks or months and it is irreversible. The final brownish products are called AGEs and some of them have fluorescent properties. They are very stable, and accumulate inside and outside the cells and interfere with protein function [1,2]. Besides the Maillard reaction, other pathways can also form AGEs. For instance, the autoxidation of glucose and the peroxidation of lipids into dicarbonyls derivatives by an increase in oxidative stress is another pathway described for the formation of AGEs [3]. These dicarbonyl derivatives known as α-oxaldehydes (glyoxal, methylglyoxal (MG),

and 3-deoxyglucosone) can interact with monoacids and form AGEs. The other well-studied mechanism for the formation of AGEs is the polyol pathway, where glucose is converted to sorbitol by the enzyme aldose reductase and then to fructose by the action of sorbitol dehydrogenase. Fructose metabolites (as fructose 3-phosphate) then are converted into α-oxaldehydes and interact with monoacids to form AGEs [4]. Thus, at least three pathways may form AGEs: The Maillard reaction; oxidation of glucose; and peroxidation of lipids and through the polyol pathway. Given these differing pathways, it is not surprising that AGEs are diverse in their chemical structure. Among the most widely studied AGEs are carboxymethyl-lysine (CML), pentosidine, and pyrraline, and, together with methylglyoxal (an α-oxaldehyde), they have been used as biomarkers for *in vivo* formation of AGEs [2,5,6]. CML (not fluorescent, not cross-linked AGEs) has been consistently used also as a biomarker for long-term protein damage and can be formed by the Maillard reaction and by α-oxaldehydes. As well as CML, pentosidine (a fluorescent protein crosslink) is formed by the Maillard reaction and by the α-dicarbonyl glyoxal, while pyrraline (not fluorescent, not cross-linked AGEs) is formed by the Maillard reaction [7].

The deleterious effects of AGEs in different tissues are attributed to their chemical, pro-oxidant, and inflammatory actions [1,2]. The biological effects of AGEs are exerted by two different mechanisms: One independent of the receptor (damage of protein structure and extracellular matrix metabolism); or one involving the receptor for advanced glycation end products (RAGE) [2,8] (Figure 2.2). The interaction of AGEs with the receptor RAGE triggers the activation of the mitogen-activated protein kinases (MAPKs) and the phosphatidylinositol-3 kinase (PI3-K) pathways that will lead to the activation of the transcription factor NF-κB (nuclear factor kappa B). After activation, NF-κB translocates to the nucleus where it will activate the transcription of genes for cytokines, growth factors and adhesive molecules, such as tumor necrosis factor α (TNF-α), interleukin 6 (Il-6), well known inflammation promoters, and vascular cell adhesion molecule 1 (VCAM1) [8-12]. NF-κB activation increases RAGE expression, creating a positive feedback cycle that enhances the production of inflammation

promoters. In addition, AGE-RAGE interaction activates NAD(P)H oxidase (a complex of enzymes which produces superoxide) and when this complex is upregulated, it increases intracellular oxidative stress. The sudden increase in oxidative stress by NAD(P)H oxidase in response to AGE-RAGE interaction will also activate NF-κB [13-15].

2.3 Implications for Health

Accumulation of AGEs has been found in healthy aging persons, and this accumulation is higher during high glucose concentrations. Microvascular and macrovascular damage, seen in diabetes, is attributed to the accumulation of AGEs in tissues, but it is also associated with atherosclerosis, Alzheimer's disease, end stage renal disease, rheumatoid arthritis, sarcopenia, cataracts, and other degenerative ophthalmic diseases, Parkinson's disease, vascular dementia and several other chronic diseases [16-19]. For instance, Bar *et al.* have demonstrated differential increases of AGEs products in Alzheimer's dementia and vascular dementia compared to controls [20]. It has also been suggested that AGEs are involved in the loss of bone density and muscular mass associated with aging [21]. We discuss briefly some of the health implications described in the older population.

2.3.1 Cardiovascular Diseases

The *in vivo* accumulation of AGEs over time contributes to changes in the structure and function of the cardiovascular system and presents as arterial stiffening, myocardial relaxation abnormalities, atherosclerotic plaque formation and endothelial dysfunction. Several authors have described some of the mechanisms for these changes. One of the proposed mechanisms involves additional cross-linking on collagen (whose normal structure already contains crosslinking) by glycation of its free amino acids. The collagen-AGEs cross-linking will produce stiffness of blood vessels. Sims *et al.* completed a histological study on 27 samples of post-mortem aortas from people with diabetes and controls and found a correlation between AGEs accumulation and aortic stiffness [22]. Another mechanism by which AGEs exert damage to the cardiovascular

system is reduction of low-density lipoproteins (LDL) uptake by cell receptors. This occurs through glycation of the LDL particle on the apolipoprotein B and in the phospholipid components of LDL. The glycated LDL is more susceptible to cross-linking with collagen on the arterial wall than non-glycated LDL, and it is not taken up into the cell and accumulates. Macrophages uptake of these modified LDL lead to foam cell formation, and the development of atheroma [23,24]. Furthermore, decreasing in nitric oxide (NO) activity is another mechanism described by AGEs damaging the cardiovascular system. NO (a vasodilator) biosynthesis in the endothelium counteracts some of the mechanisms for atherosclerosis. Some authors proposed that AGEs reduce NO synthase (eNOS) half-life in the endothelium. For instance, Xu *et al.* found a decreased in eNOS activity after exposure to CML. They also found that after 30 minutes of exposure with CML-albumin, there was a reversible inhibition of endothelium and vascular response dependent on NO *in vivo* and *in vitro* [25-28]. Additional work supports the role of increased androgens during and after menopause as a risk factor for cardiovascular events in women, with an associated increase in AGEs. A study of 106 postmenopausal women found significant correlations between testosterone and free androgen indices *versus* AGEs after adjustment for age, body mass index, insulin resistance indices, and fasting glucose and insulin levels [29].

Therefore, the accumulation of AGEs could be explained by some of the cardiovascular changes associated with aging, such as vascular stiffening, diastolic dysfunction and endothelial dysfunction [24]. A study with long-term (24–30 weeks) administration of aminoguanidine (an inhibitor of AGE formation) showed prevention of the age-related cardiac hypertrophy and arterial stiffness [30]. It has also been found that CML, a predominant AGE, can serve as a predictor of cardiovascular mortality. Semba *et al.* studied a group of 559 women aged 65 and older for 4.5 years from the Women's Health and Aging Study I (WHAS I). During this time 22% of the population died; 43.9% from cardiovascular disease. They measured CML as a marker for AGEs and found that the highest risk for dying of cardiovascular disease were for women in the highest quartiles of CML [31]. From these reports, it appears that high concentrations of AGEs

could be a risk factor for cardiovascular disease, but further evidence is needed to support this statement.

2.3.2 Sarcopenia

Loss of muscle mass and strength (sarcopenia) is a serious problem among older populations [32]. In accordance with recent studies, one third of women and half of men older than 60, have sarcopenia in the U.S. [33]. The pathogenesis of sarcopenia is multifactorial, and may include hormonal changes, increased oxidative stress and inflammation, changes in vasculature, and inactivity [34-36]. AGEs may also contribute to this condition by increasing oxidative stress and inflammation. Some studies have found a relation between AGEs and muscular function in older population. Haus *et al.* found that pentosidine concentrations were 200% higher in a group of older individuals with a mean age of 78 y (n=22) compared with their younger counterparts with a mean age of 25 y (n=20). The authors concluded that AGEs may contribute to the decline of muscular function observed in aging [37]. Dalal *et al.* conducted a study of older women to measure the AGEs concentration in blood and handgrip strength. Serum carboxymethyl-lysine (CML), the circulating soluble form of receptor for advanced glycation end products (sRAGE), endogenous secretory receptor for advanced glycation end product (esRAGE) and grip strength, were measured in 559 moderately-to-severely disabled women, age 65 and older, in the Women's Health and Aging Study I in Baltimore. The authors found that women with higher concentrations of CML had less grip strength than women with lower CML concentrations. The authors concluded that women with higher AGEs have more muscle weakness [38]. These studies presented interesting results, but they are not conclusive of a causal relationship between AGEs and sarcopenia, and more studies will need to further address this health problem among older population.

2.3.3 Renal Disease

The relationship between renal disease and AGEs has largely been studied in patients with type 2 diabetes mellitus, and to a lesser extent in older populations. Semba *et al.*

[39] demonstrated that in an older population (n=1008), elevated circulating AGEs were an independent predictor of renal function. The study was carried out in men and women, age 64 and older, participating in the InCHIANTI study in Tuscany, Italy. The results of the study demonstrated an elevated plasma concentration of CML independently associated with chronic kidney disease and the estimated glomerular filtration rate (an index of kidney function) at baseline, after three and six years of follow-up. These findings suggest that the potential adverse effects of AGEs on the kidney are applicable to the general population of older community-dwelling adults [39]. In another study of 548 women from the Women's Health and Aging Study I in Baltimore, 51.6% of women had decreased glomerular filtration rate, which was associated with increased serum levels of CML and sRAGE. However, more follow-up studies on the elderly population are needed to establish if high levels of CML could predict decreased in renal function [40].

2.3.4 Alzheimer's Disease

Although a definitive etiology for Alzheimer's disease is unknown, oxidative stress has been identified as a primary risk factor for the disease. Both aging itself and the presence of AGEs are thought to be risk factors through their role in chemical, pro-oxidant, and inflammatory actions as previously described. A comparison of normal control and Alzheimer's disease patients' brain tissue found higher AGEs and RAGE expressions in age-matched controls [41]. In addition, there is evidence that RAGE mediates the blood-brain barrier transport of amyloid peptides in certain situations [42]. A recent review has described possible links between Alzheimer's disease and diabetes, which include AGEs, advancing age, as well as oxidative stress and hypercholesterolemia, although exact mechanisms and relationships require additional research [43].

2.3.5 Diabetes

Hemoglobin A1c is the most widely recognized early glycation product, and is also used as an indicator of blood glucose management in those with diabetes. Hyperglycemia

increases the glycation process, and is especially apparent in insulin independent tissues such as red blood cells, peripheral nerve tissue cells, endothelial cells, eye lens cells, and kidney cells [44]. It is also hypothesized that glycation of proteolytic enzymes in diabetes reduces their efficiency, resulting in more build up of glycated end products [44]. Not surprisingly, AGEs have also been implicated in delayed wound healing associate with diabetes, presumably through vascular, neurological, or intermediary metabolic modifications [45].

2.4 Exogenous Sources of AGEs

In addition to *in vivo* production, AGEs can also be found in cigarettes and in foods. The curing of tobacco leaves has been proposed as the source for compounds that can readily increase *in vivo* AGEs. Cerami *et al.* found that glycotoxins from cigarettes are inhaled into the alveoli, and then they are transported to blood stream or to lung cells where they can interact with other glycation products and contribute with AGEs formation [46].

2.4.1 Dietary AGEs

Heat has been used for treatment of foods to improve their safety, bioavailability and taste. In addition to these positive effects, overheating of foods can also provoke protein degradation and other deteriorative reactions [47]. Heat treatment in some foods results in promotion of the Maillard reaction, which adds desirable flavor, color and aroma. In the food industry, the Maillard reaction has been used for caramel production, coffee roasting, and bread baking among others. Some products of the Maillard reaction can be added to industrialized products such as sodas and juices among others [48]. There is growing evidence that the average Western diet is a plentiful source of exogenous AGEs. The AGEs content of a diet depends on the nutrient composition (foods rich in protein and fat have the highest content) and on the way food is processed [49,50]. AGEs formation can be rapidly accelerated by increasing the time and degree of exposure to heat and can be introduced into the body in heat-processed foods [47,49,50]. These findings were demonstrated using an AGE-specific, enzyme-linked

immunosorbent assay (ELISA), and it was estimated that ≈10% of ingested immunoreactive AGEs are transported into circulation, two-thirds of which remain in the body, and are incorporated covalently in tissues. Only one third is excreted via the kidneys [51].

However, it has been controversial whether dietary AGEs are harmful to human health. One of the reasons for this controversy is that, as well as those found *in vivo*, Maillard reaction products formed in foods are heterogeneous and only a few have been characterized. Some of the products formed during this intricate reaction are furfurals, pyrralines and dicarbonyl compounds such as methylglyoxal. The products formed in the last reaction of this process are known as melanoidins in food science [52]. As mentioned before, regardless of the diversity of AGEs, CML has been reported as one of the most abundant *in vivo* and it was one of the first to be characterized in foods (milk and milk products). For this reason in most studies CML is chosen as a marker of AGEs in foods and *in vivo* [53].

Studies on the effects of AGEs from foods not only are limited to CML, but also to the melanoidins found in bread crust, bakery products and coffee. Some positive and negative effects of melanoidins have been studied. Ames *et al.* found that melanoidins increased the number of anaerobes, clostridia, and bifidobacteria in a culture of human fecal bacteria [54]. These results indicate that a mixture of melanoidins can stimulate growth of health-beneficial bacteria in the gut. Borrelli *et al*. found similar results showing that melanoidins from bread crust can promote growth of some bifidobacterias strains, indicating a possible potential prebiotic effect of bread crust melanoidins. Somoza *et al.* carried out a study in rats fed with malt and bread crust to measure the activity of chemopreventive enzymes such as glutathione-S-transferase (GST) and UDP glucuronyl-transferase (UDP-GT) [55]. The activity of GST in kidney increased by 18% on the group fed with bread crust, while UDP-GT in liver increased by 27%. The authors concluded that diet malt and dietary bread crust increased chemopreventive enzymes in rats.

On the other hand, several studies, mostly with CML and MG, have shown that the intake of dietary AGEs modifies circulating AGEs levels in human subjects and animals with or without diabetes or renal disease. In a study with 90 healthy subjects, Uribarri *et al.* estimated the amount of AGEs from three days food records using a database with the AGEs content of certain foods. They found a significant correlation between the ingested AGEs and the plasma levels. A subgroup was exposed to a dietary restriction of AGEs and their plasma levels decreased as well. These results are similar to previous reports on patients with diabetes and renal failure patients [56]. Those findings support the view that the intake of dietary AGEs is an important contributor to the body AGEs pool [56-60]. Besides the endogenously formed AGEs, dietary AGEs have also been shown to act as RAGE ligands and activate major signal transduction pathways *in vitro* [11,61,62]. Dietary AGEs, together with those made endogenously, could promote a systemic glycoxidant burden, oxidant stress and cell activation, which increases vulnerability of target tissues to injury [63,64].

2.4.2 Dietary AGEs Metabolism

Several studies have focused on understanding the absorption, metabolism and excretion of dietary AGEs. Forster *et al.* carried out a study to try and understand the bioavailability and the kinetics of elimination of some Maillard products found on custard, pretzels and brewed coffee. They found that pretzel sticks are a rich source of pentosidine and pyrraline. The study was carried out with 18 healthy subjects who received specific amounts of these foods on a single day. Urinary excretion of pyrraline and pentosidine was measured by chromatographic methods for the following three days. The urinary excretion of both Maillard products increased after ingestion and the rate of recovery in urine was around 50% for pyrraline and around 60% for pentosidine. However, the metabolic fate of the pyrraline and pentosidine is unknown [65].

The mechanisms of intestinal absorption of AGEs are not yet well understood. A recent study trying to answer this question found that pyrraline is absorbed by the peptide transporter hPEPT1. This study is the first one addressing this question and studies on the absorption mechanism for more AGEs are needed [66].

A few studies about intestinal absorption of different AGEs have been conducted. However, the complete extent of absorption of each individual AGE is not well known. As mentioned before, the most studied dietary AGEs are CML, pyrraline and pentosidine. Several studies have shown different rates of absorption of each of them. However, their metabolic pathways have not been elucidated. More studies on this area are needed to understand the impact of dietary AGEs on health and aging.

2.5 AGEs in the Elderly

The serum levels of AGEs are dependant of endogenous production, exogenous intake and renal and enzymatic clearance, which together produce transient increases and decreases in serum AGEs levels. Several enzymes (glyoxalase I, II and carbonyl reductase) and a receptor (AGER1) have been shown to be part of a detoxification and counterregulation system against the prooxidant effects of glycation [67,68]. In addition, renal excretion eliminates excess of AGEs production under physiological conditions. Some authors have proposed that with aging as well as in some pathological conditions there is imbalance in this steady-state. This imbalance can be due to an increased endogenous production, or an increased exogenous intake that, in combination with lower renal AGEs clearance, leads to the accumulation of AGEs observed in older population [68,69].

Uribarri *et al.* investigated whether AGEs intake correlated with glycotoxin levels, markers of inflammation and oxidative stress (OS) comparing older *versus* younger healthy adults. They studied 172 healthy volunteers in two groups (18–45 years) and (60–80 years). The CML and MG derivatives were higher in the older group. The concentration of AGEs in serum correlated with levels of inflammation markers and OS. Additionally, the level of dietary glycotoxins correlated independently with CML and MG derivatives, as well as hsCRP. The association found between sCML and the homeostasis model assessment (HOMA an instrument to measure insulin sensitivity) levels of normal persons could be linked to metabolic processes, which may precede insulin resistance, diabetes mellitus, or vascular dysfunction at any age [70]. Vlassara *et*

al. found similar results in a study with 325 healthy participants and 66 participants with kidney disease (CKD). Serum CML and MG were higher in the group of older participants, and serum CML correlated negatively with eGFR and positively with age [67].

The accumulation of AGEs in tissues may contribute to increased OS, and as a final result, impair organ function [16]. Indeed, within the complex the association between OS and aging ovarian follicles, AGEs may have an important role as they accumulate over the lifespan [71]. More recent work suggests a mechanism for this in that detoxification of an AGEs precursor is significantly diminished in older mice, allowing AGEs to increase [72]. Additionally, slowly diminishing renal function with age [73] could affect the ability to excrete AGEs. Uribarri *et al.* suggest this as a possible explanation as to why they found high AGE levels in the older group, even though the intake of dietary AGEs by the older age group was reduced [70]. More evidence is needed, but these results suggest the important role of dietary and circulating AGEs in chronic degenerative diseases, which are more prevalent in the elderly.

2.6. Strategies for AGEs Reduction

Some of the strategies studied to lower the extra load of AGEs are reducing dietary AGEs, pharmacological treatment and, more recently, exercise.

2.6.1 Dietary Restriction of AGEs

Findings in several intervention studies, both human subjects and animals, indicate that the high intake of dietary AGEs contributes to tissue damage that can be prevented by dietary AGEs restriction. These intervention studies reduced dietary AGEs by decreasing the heat during the preparation of foods [74-79]. Sebekova *et al.* found that long-term consumption of AGEs in rats leads to a dose-dependent increase in proteinuria that overtime could induce renal damage [80]. In addition, the high long term consumption of AGEs has also been associated to higher levels of fasting glucose, insulin and serum AGEs, as well as increased AGEs localization and RAGE staining in

ovarian tissue of rats [81]. In studies of mice, reduced dietary AGEs have been found to attenuate insulin resistance, increase the prevention of diabetes and, in diabetic mice, reduce diabetic vascular and renal complications, and improve impaired wound healing [74-76].

Whereas in human studies, Uribarri *et al.* demonstrated that intake of dietary AGEs by people with type 1 and 2 diabetes promotes the formation of pro-inflammatory mediators, leading to tissue injury [82]. Patients with uremia, with and without diabetes, in whom the intake of AGEs was reduced, showed reduced levels of inflammatory molecules such as TNF-α and high sensitivity C-reactive protein (hsCRP) [79]. In another study in patients with type 2 diabetes mellitus, decreasing the intake of AGEs for six weeks contributed to decreased levels of circulating AGEs and inflammatory markers [60]. The effects of reducing dietary AGEs have also been studied in nondiabetic peritoneal dialysis patients, a group that has very high AGE levels, and the results showed significant reduction in the levels of AGEs and C-reactive protein [79].

2.6.2 Role of Restriction of Dietary AGEs in Lifespan

It has been demonstrated that caloric restriction increases lifespan in *C. elegans* and mice. Several centenarian populations have been studied, and they have one thing in common: a lower caloric intake [83,84]. It has been postulated that positive outcomes of caloric restriction (CR) in mice could be explained in part by a decrease in the intake of AGEs and concomitant decrease of OS [85].

To investigate whether a reduction in CR would decrease the AGE intake, and whether this decrease could explain the benefits of CR, investigators studied three groups of mice assigned to one of three diets (n=22 per group): CR diet, regular diet; or CR diet high in AGEs [86]. A longer lifespan in CR mice *versus* Reg or CR-high mice (median and maximal survival 13.2% and 6%, respectively) was reported. Additionally, survival in CR-high mice was shorter than in Reg mice. There was a significant increase of OS in the CR-high group and accelerated aging-related cardiovascular and renal disease and

a shorter lifespan. In these studies, the high levels of AGEs in the CR-high diet compete with the benefits of CR, but the mechanism remained uncertain [86].

2.6.3 Exercise and AGEs

Published reports of research linking exercise and AGEs are sparse. One of the first studies in this subject suggested a decrease in levels of AGEs in exercise-trained diabetic rats as compared with sedentary diabetic rats. A possible explanation for the decrease is that adaptation to systemic physical exercise in diabetic animals affects not only enzyme-regulated metabolism but also non-enzymatic processes involving protein glycation. This study was published in Russian, so access was only to the abstract. Another study explored whether exercise prevented the age-associated changes in non-enzymatic cross-linking of myocardial collagen, and thus may improve cardiac performance in an animal model. In this study long-term exercise training appeared to attenuate age-related deteriorations in cardiac contractility and myocardial stiffness and was related to decreases in myocardial pathologic collagen cross-linking (AGEs cross-linked collagen) in old rats (n=7) when compared with controls (n=7) [87]. Boor *et al.* carried out a study in Zucker rats where one group (n = 8) had a training program for five weeks and the other group (n=8) did not perform any exercise. It was found that the group with the training protocol had lower levels of CML in plasma, renal cortex and in glomeruli, in comparison with the sedentary group [88].

Few studies have explored the effects of exercise on AGEs on the human model. One of them showed the effect of Tai Chi in a healthy Malaysian population older than 45 years. The subjects were randomized either to practice Tai Chi two times per week or to a control group. Measures were taken at 0, 6, and 12 months. The subjects in the Tai Chi group had a decrease in concentration of AGEs and malondialdehyde MDA (a lipoxidation marker) after 12 months of intervention [89]. In another study, researchers reported the acute effect of exercise in subjects with coronary artery disease, finding no difference between the levels of RAGE before and after the training session [90]. Recently, Yoshikawa recruited seventeen healthy women (30–60 y) who participated in

a lifestyle modification protocol for three months to measure changes in AGEs. The protocol aimed to increase physical activity among the intervention group, which was measured by a pedometer. An education session was given at the beginning of the study and participants attended supervised sessions once per week. Levels of CML decreased in the treatment group compared to the control group (n=12) and CML decrease was negatively correlated with the number of daily steps [91].

2.6.4 Pharmacological Interventions

Several pharmacological agents have been studied as blockers of AGEs crosslinking or as blockers of their actions using cellular, animal and human models: benfotiamine (a B1-like vitamin with higher bioavailability), metformin, aminoguanidine, aspirin, and inhibitors of the renin-angiotensin system for example. Saha *et al.* showed that Candersatan, an angiotensin receptor blocker, administered for 12 weeks reduced levels of CML in the urine of patients with diabetic kidney disease [92]. Aminoguanidine, a scavenger of α-dicarbonyl, showed in some studies promising results as an inhibitor of AGEs formation. However, a clinical trial carried out in diabetic patients was stopped due to safety concerns and lack of efficacy. Patients presented secondary effects that included gastrointestinal disturbance, abnormal tests for liver function, and flu-like symptoms [93]. Additionally, metformin has shown to decrease circulating AGEs in patients with diabetes, as well as decreasing the activity of NFκB [94]. In a study of 22 women with polycystic ovary disease (PCOS), metformin therapy for six months resulted in a reduction of serum AGEs [95]. In a similar intervention with 21 women with PCOS, Orlistat, a lipase inhibitor, reduced serum AGEs after a high AGEs meal as compared to 15 women without PCOS by decreasing AGEs absorption [96]. A six month study evaluating the effect of calorie restriction and Orlistat found serum AGEs reduction in both the PCOS and obese groups, independent of body mass index changes [97]. Some of these agents are in preclinical or clinical phase trials, and it could be a long time before any of these treatments emerge as effective and safe therapeutic agents to inhibit and counteract AGEs effects.

2.7 Limitations of Dietary AGEs Studies

Formation of AGEs in foods is a complex process involving several reactions and many end products. Only a few of them have been characterized and measured in foods. Some of these products have fluorescence properties. By taking advantage of this property, some studies have measured AGEs using chromatography methods while other studies have measured AGEs by immunohistochemical techniques [53,98]. There is as yet no agreement among the different groups studying dietary AGEs as to which is the best. Recently Uribarri *et al.* published a food database with approximately 500 food items with CML content using ELISA [49]. This database represents a great tool to asses CML content of the diet of larger populations. However, more extensive research with other methodology is needed to validate CML content of this database.

Intervention studies on the implications of reducing dietary AGEs have been performed mainly in patients with renal disease or diabetes. A few studies exploring the short term effects (2–4 weeks) of reducing dietary AGEs in healthy subjects have been performed mainly in young population [99,100]. Long-term clinical studies with older individuals are needed to determine the health effects of dietary AGEs on this population, but the methodological design of long-term studies represents a great challenge. One of the difficulties is achieving diets with different dietary AGEs content, but with similar content in other nutrients, such as heat-sensitive vitamins. Pouillart *et al*. demonstrated that diets with different dietary AGEs content but similar thiamine, vitamin E and other heat-sensitive vitamins content, are challenging but possible [101]. Future intervention studies addressing the impact of dietary AGEs will need to include diets with varying AGEs content, adequate content of all nutrients, as well as be attractive and tasteful to achieve high compliance during these long-term interventions.

2.8 Conclusions

Although the data are not conclusive, the convergence of data from diverse experimental studies suggests an important role of AGEs in healthy aging, as well as chronic disease morbidity. Certainly the data are supportive that endogenous AGEs are

associated with declining organ functioning. It appears that dietary AGEs may also be related. There are no conclusive results about the damaging effect of Maillard products from foods, but it appears from several studies that Maillard products coming from foods rich in protein and fat are more damaging than Maillard products from bread crust, and roasted coffee. Foods rich in protein and fat seem to have a higher content of CML and Methylglyoxal; in contrast, bread crust has lower content of these two AGEs. However, characterization of AGEs in food and biomarkers of these AGEs require additional research before reaching a conclusion.

Although many promising pharmacologic anti-AGE therapies exist, their efficacy and safety are still under study. Research in this area is in the earliest phase, and a long time could pass before the U.S. Food and Drug Administration could approve a drug targeting AGE formation or modification.

As of today, restriction of dietary intake of AGEs and exercise has been shown to safely reduce circulating AGEs, with further reduction in oxidative stress and inflammatory markers. More research is needed to support these findings and to incorporate these into recommendations for the elderly population.

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Figure 2.1 Formation of AGEs.

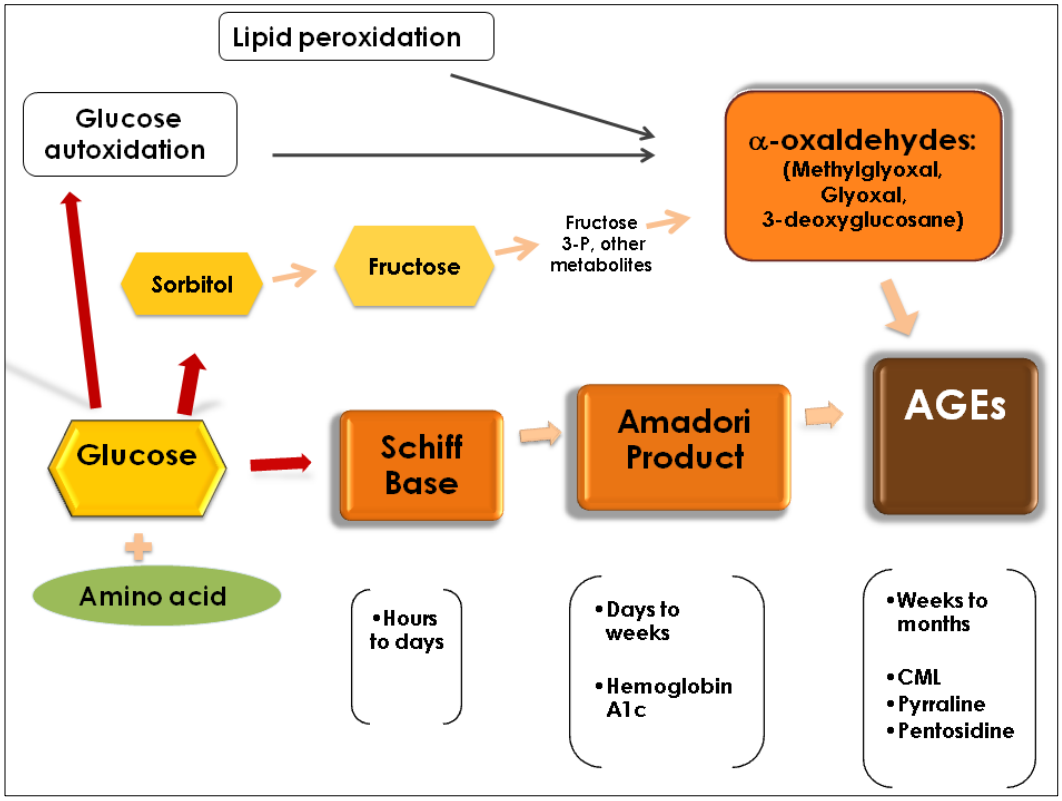
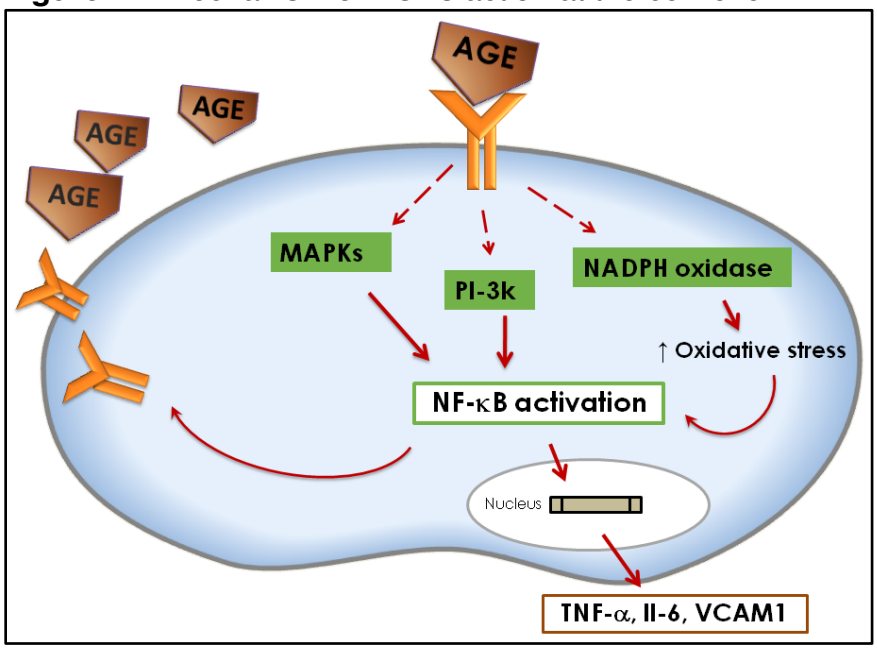


Figure 2.2 Mechanism of AGEs action at the cell level.



Chapter 3
Role of Dietary Advanced Glycation End Products in Diabetes Mellitus[2]

Abstract: Dietary AGEs can be formed via the Maillard reaction and several alternative pathways. AGEs exert their deleterious effects by damaging protein structure and function, as well as through activation of cellular mechanisms. At the cellular level, the damaging effects of AGEs have been attributed to several AGE-binding proteins. Increased levels of AGEs have been implicated in several chronic diseases, including diabetes-related complications such as renal diseases, retinopathy, neuropathy, and cardiovascular diseases, as well as delayed wound healing. To investigate the role of AGEs thoroughly, a reliable assessment of dietary AGEs is needed. Varying methodology, diverse food preparation, and quantification of a variety of dietary AGEs makes this a complex goal. In addition, some antiglycation food products may balance or offset the negative impact of dietary AGEs.

Keywords: AGEs (advanced glycation end products), dietary AGEs (dietary advanced glycation end products), carboxymethyl-lysine, diabetes mellitus.

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3.1 Introduction

According to the latest report from the International Diabetes Federation, 366 million people have diabetes mellitus worldwide with almost 80% of them living in low-to-middle income countries. The International Diabetes Federation also reports that 11% of the healthcare expenditures are due to diabetes mellitus [1]. In the United States the prevalence of diabetes is 8.3%, and it is the 7th leading cause of death according to the 2011 report by the Centers of Disease Control and Prevention (CDC) [2]. According to the 2011 National Diabetes Fact Sheet by the CDC, diabetes complications represent a health care burden with diabetes being the leading cause of new blindness and the leading cause of renal failure and heart diseases [2].

A causal relationship between chronic exposure to hyperglycemia and microvascular and macrovascular diseases found in diabetes mellitus has been well established [3]. There are at least three theories about how high levels of blood glucose could lead to the development of complications in diabetes mellitus: oxidative stress, protein kinase C activation, and the accumulation of a heterogeneous, complex group of compounds called advanced glycation end products (AGEs) that are formed mainly via the Maillard reaction [4]. The activation of the protein kinase C system in insulin independent cells has been identified as one of the mechanisms responsible for the metabolic consequences of hyperglycemia. Dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, glycolytic intermediates, promote the formation of diacylglycerol, which is a co-factor for protein kinase C activation. The activation of protein kinase C triggers the transcription of several growth factors including the transforming growth factor-β, which has been involved in the thickening of capillary basement membrane (an abnormality seen in tissues in diabetes) [5]. Hyperglycemia also could increase intracellular production of reactive oxygen species (ROS) by three mechanisms: the mitochondrial electron transport chain, the autoxidation of glucose, and the production of AGEs. Oxidant stress appears to be involved in the activation of the protein kinase C system as well as in AGEs formation [4,6].

The Maillard reaction occurs when a reducing sugar reacts in a non-enzymatic way with amino acids in proteins, some lipids or DNA to produce AGEs. This reaction has been studied for years in the food industry because its products add a desirable color and taste to foods. However, the study of the products of this reaction in vivo has received increasing attention in recent years due to the association of AGEs with certain chronic diseases, such as diabetes mellitus, cardiovascular diseases, and Alzheimer's disease, as well as during the aging process [7].

In addition to those AGEs formed in vivo, the role of dietary AGEs (dietary AGEs) on health and on diabetes-related complications has been studied. Clinical studies have measured the effects of high dietary AGEs in terms of levels of blood AGEs and levels of inflammation and oxidation markers. In these studies, dietary AGEs intake has been assessed with 3-day food records. However, there is not a standardized assessment method for AGEs quantification in the diet, and the association between dietary AGEs consumption and diabetic complications has not been studied due to the longitudinal data needed for both AGEs intake and complications development. In order to appreciate the complexity of AGEs and complications related to diabetes, this article will review AGEs formation, metabolism and excretion, as well as the current literature related dietary AGEs to diabetes complication. In addition, some challenges to researchers will be reviewed, including diverse analytical measurement of dietary AGEs and how to measure AGEs in the diet to generate data on usual intake and establish a safe intake. Finally, dietary strategies to lower AGEs damage will be discussed.

3.2 Formation of Advanced Glycation End Products

3.2.1 Formation in vivo

Several AGEs have been described and complex pathways for their formation have been elucidated. Their detailed description is outside the scope of this review, but a brief description on the Maillard reaction and alternative pathways is described.

Maillard reaction

The Maillard reaction consists of three well-described phases. First, the carbonyl group of a reducing sugar interacts in a non-enzymatic way with an amino acid to form an unstable compound called Schiff base. Lysine and arginine are the amino acids most often described as taking part in this reaction, but hydroxylysine and glycine have also been described. During the second phase, the Schiff base has two fates; it could undergo hydrolyzation and generate the original sugar and amino acid, or it could undergo cyclization and further Amadori rearrangements to form more stable compounds called Amadori products. Despite their stability and similarity to the first phase, under physiological and non-oxidative conditions 90% of Amadori products could sustain a reversible reaction to the initial sugar and amino acid [8]. In the last phase Amadori products can generate AGEs by two different ways, either oxidative or non-oxidative cleavage. The oxidative cleavage of Amadori compounds will yield two intermediates that after autoxidation and further rearrangements will produce AGEs. The principal AGEs produced in this form is carboxymethyl-lysine (CML), one of the first AGEs characterized in vivo and the major AGEs' biomarker in human tissues [9]. In contrast, the non-oxidative cleavage of Amadori products will produce the α-dicarbonyl derivative 3-deoxyglucosane. This derivative can react with an amino acid and also form carboxymethyl-lysine or other AGEs cross-links as pyrraline, pentosidine or imidazolone (Figure 3.1) [8-11]. A study of the kinetics of AGEs formation measured the fluorescence of final AGEs and it showed that ribose is the most reactive sugar, followed by fructose and lastly glucose. However, ribose and fructose concentration in tissues is significantly lower that the concentration of glucose. In addition, when glucose remains for a long time it will produce the same amount of fluorescence [12], which makes glucose the principal contributor for the Maillard reaction [11].

Alternative pathways, formation of α-dicarbonyl: methylglyoxal, glyoxal and 3-deoxyglucosane

Other pathways generate AGEs by producing short chain carbonyl compounds known as α-dicarbonyl or α-oxaldehydes as glyoxal, methylglyoxal and 3-deoxyglucosane. The

α-dicarbonyl compounds are very reactive, participating in the formation of intra- or inter-protein cross-links. In addition, α-dicarbonyls also have the ability to form AGEs either by directly reacting with an amino acid or by starting the Maillard reaction instead of a reducing sugar. For this reason, α-dicarbonyls have been suggested as the main precursors for AGEs formation, particularly methylglyoxal [13]. Among the pathways that produce α-dicarbonyls, the Namiki and the Wolff pathways are the more important. The Namiki pathway occurs when a Schiff base degrades and forms glyoxal, while the Wolff pathway involves the autoxidation of monosacharides. For instance, the autoxidation of glucose at physiological conditions produces α-dicarbonyls. In addition, other metabolic intermediates have been implicated in α-dicarbonyl production with subsequent generation of AGEs. For example, glycolytic intermediates (glucose-6-phosphate, glyceraldehyde 3 phosphate and dihydroxyacetone phosphate), a polyol pathway intermediate (fructose-6 phosphate), an intermediate from ketone body and threonine metabolism (acetol), and lipid peroxidation also generate methylglyoxal (Figure 3.1) [13-15].

3.2.2 Formation of dietary AGEs

Since its first description in 1912 by Louis Camille Maillard, the Maillard reaction has been extensively studied in food science to explain the nonenzymatic darkening of fruits, the modification of skim milk during storage, and the production of off-flavor and pleasant aromas of some foods. It has also been used for caramel production, coffee roasting and bread baking [16,17]. In the food industry, proteins are used for emulsifying, foaming, gelling and solubilizing foods, and protein glycation increases emulsifying activity, improves foaming properties, increases protein solubility, promotes the formation of compounds with antioxidant activity (extending shelf life on food via delaying lipid oxidation), and improves food texture [18].

As well as in in vivo formation, AGEs production in foods (dietary AGEs) involves the Maillard reaction. An amino acid from a protein, amine or phospholipids reacts in a non-enzymatic fashion with carbonyl groups from reducing sugars as well as with

degradation products of carbohydrates, lipids, and ascorbic acid, and the resulting products often are referred to in the food science literature as Maillard reaction products. The reaction occurs in three well-identified stages previously described. There are low molecular Maillard products such as aldehydes, ketones, acryl amides, and AGEs, as well as high molecular products as melanoidins [17,19]. Other products formed during this intricate reaction are furfurals, pyrralines and α-dicarbonyl compounds such as methylglyoxal. The last products formed in the Maillard reaction, very well studied in food science, are the melanoidins, which are brown pigments [10]. Some other dietary AGEs studied in foods are furosine, which is a degradation product of Amadori compounds, as well as carboxymethyl-lysine and pentosidine formed in the last stage of the Maillard reaction [20].

Another source of dietary AGEs could be autoxidation of fatty acids and amino acids [21]. Chao et al proposed that heat could cause oxidation of unsaturated fatty acids from soybean oils and some fishes such as salmon and cod. The oxidative products could participated in the rearrangement of Amadori adducts to form irreversible crosslinks like pentosidine or carboxymethyl-lysine [20].

Regardless of the diversity of AGEs, carboxymethyl-lysine has been reported as one of the most abundant in vivo and it was one of the first to be characterized in foods (milk and milk products). For this reason, in most studies carboxymethyl-lysine is chosen as a marker of AGEs in foods and in vivo [22].

3.3 Metabolism of AGEs

Increased levels of AGEs have been implicated in several chronic diseases, including diabetes-related cardiovascular complications and renal disease, as well as aging [23,24]. AGEs exert their deleterious effects by damaging protein structure and function, and also are implicated in activation of cellular mechanisms. Besides endogenous AGEs, dietary AGEs also have been shown to be a major source of the body's pool of AGEs, and it is believed that they are also involved in tissue damage. An important point

to review is how dietary AGEs are absorbed and if they can exert damage similar to that of endogenous AGEs.

From the quantitative point of view, the contribution of dietary AGEs to the total pool of AGEs in the body is considered more important than the contribution from endogenous AGEs by abnormal glucose metabolism or lipid peroxidation. Henle estimated that 10-50 times more dietary AGEs are supplied by a conventional diet that those found in plasma or tissues of subjects with uremia [25]. Evidence from human studies shows the contribution of dietary AGEs as carboxymethyl-lysine and methylglyoxal to the levels of circulating AGEs. Cross-sectional and randomized studies have demonstrated a correlation between dietary AGEs and circulating AGEs [26-30]. A cross-sectional study with 90 healthy subjects showed significant correlation between carboxymethyl-lysine estimated from 3 days food records and plasma levels of AGEs. When a subgroup decreased their intake of carboxymethyl-lysine their plasma levels also decreased [26]. A similar study of healthy volunteers compared older (60–80 years, n=56) versus younger (18–45 years, n=116) healthy adults. The plasma levels of carboxymethyl-lysine and methylglyoxal were higher in the older group and correlated with levels of inflammation markers and oxidative stress. Additionally, the level of dietary glycotoxins (measured by three days of dietary records) correlated independently with serum carboxymethyl-lysine ($r=0.46$, $p < .001$) and methylglyoxal ($r=0.37$, $p=0.001$), as well as with C reactive protein ($r=0.200$, $p=0.042$) [27]. Vlassara et al found similar results in a study with 325 healthy participants and 66 participants with kidney disease. Serum carboxymethyl-lysine and methylglyoxal were higher in the group of older participants, and serum carboxymethyl-lysine correlated positively with age and with dietary AGEs [28]. These findings support the view that the intake of dietary AGEs is an important contributor to the body's AGEs pool [26,27,29,30].

In contrast, a recent study by Semba et al found that urinary carboxymethyl-lysine was positively correlated with intake of starchy vegetables, whole grains, sweets, nuts/seeds and chicken, and negatively correlated with intake of fast foods. Intake of AGE-rich

foods such as fried chicken, French fries, bacon, sausage and crispy snacks were not significantly correlated with serum or urinary carboxymethyl-lysine. In contrast to other researchers, these researchers concluded that the high consumption of foods considered high in carboxymethyl-lysine was not a major determinant of either serum or urinary carboxymethyl-lysine [31].

Several authors have studied the metabolism and absorption of some dietary AGEs and their precursors. For instance, a study by Erbersdobler and Faist on Amadori products (dietary AGEs precursors) found that their intestinal absorption is by diffusion [32]. They used 14C-labelled fructose amino acids in rats and humans and found that urinary excretion of Amadori products after the ingestion of test meals showed a rapid elimination of the absorbed part, while the fecal output, although low, persisted for 3 days. Only 1-3 % of the ingested amounts of protein-bound Amadori products were recovered in the urine, leaving around 90% of Amadori products unaccounted [32]. A review regarding metabolism of premelanoidins and melanoidins showed data suggesting that on average 16 to 30% of absorbed premelanoidins were excreted in the urine and 1 to 5% for melanoidins. A possible explanation suggested for this low recovery of pre- and melanoidins was that digestive microbial enzymes degraded pre- and melanoidins during intestinal transit [33]. For instance, Wiame et al showed that bacteria that reside normally in the large intestine degrades around 80% of dietary Amadori products [34]. It also has been proposed that the rest of premelanoidins are metabolized and retained by different tissues [33].

Koschinsky et al conducted a study with 43 subjects with diabetes mellitus and 5 healthy subjects. The AGEs were measured using an AGE-specific enzyme-linked immunosorbent assay (ELISA) in urine and serum 24 hours before and 48 hours after a rich AGEs meal. It was demonstrated that ingested AGEs are absorbed, and it was estimated that ≈10% of ingested immunoreactive AGEs were transported into circulation, two-thirds of which remained in the body, and were incorporated covalently in tissues. Only one third was excreted via the kidneys [35]. Foerster and Henle have studied the bioavailability and kinetics of elimination of pyrraline and pentosidine. In a

first study, seven healthy subjects were asked to ingest a normal diet on days 1 and 5, and on days 2, 3 and 4 a diet virtually free of Maillard compounds. Urinary excretion of free pyrraline was directly affected by composition of the diet, decreasing from 4.8mg/day on day 1 to 0.3 mg/day on day 2, 3 and 4. The analysis of the 24-hour urine sample established that most of dietary pyrraline was absorbed and then rapidly excreted via the kidney within 48 hours [36]. Another study with 18 healthy volunteers who received specific amounts of custard, pretzels and brewed coffee on a single day found the rate of recovery in urine to be close to 50% for pyrraline and 60% for pentosidine. However, the metabolic fate of the pyrraline and pentosidine is unknown [37]. This study raises the question of how pyrraline or other dietary AGEs can cross the intestinal epithelial barrier. An in vitro study trying to answer this question concluded that, in general, free or protein-bound dietary AGEs could cross the intestinal epithelium by simple diffusion, by endocytotic processes or mediated by transport proteins. It was found that pyrraline is absorbed by the peptide transporter hPEPT1 in HeLa cells [38].

AGEs clearance

It has been proposed that levels of serum AGEs depend on the endogenous production, the intake of dietary AGEs, and on the clearance system. This clearance system consists of several enzymes (glyoxalase I, II and carbonyl reductase) and a receptor (AGER1), and they have been proposed to have a role in AGEs detoxification [28,39]. In addition, renal clearance is the predominant means of excretion of AGEs, mainly the low-molecular weight fraction (glycation-free adducts) [40]. The predominant plasma AGEs (imidazolones, carboxymethyl-lysine and carboxyethyl-lysine) have a high renal clearance [41] and their levels are inversely correlated with renal function [42]. The AGEs adducts and peptides are filtered through the glomeruli and a small proportion may be reabsorbed and degraded by proximal tubular cells [43]. It has been reported that the presence of near normal renal function is critical to maintain the body load of AGEs and possibly other oxidants at nontoxic levels [44]. However, the AGEs clearance system is altered in diabetes mellitus and aging, and although the kidney is key in the oxidative stress defense system, it is also a target for AGE-induced injury [45]. Serum

AGEs correlate directly with the levels of inflammatory markers and oxidative stress, and inversely with creatinine clearance and urinary AGE clearance also correlates directly with creatinine clearance [42]. Hence, persons with diabetes mellitus and renal disease display elevated serum AGEs levels and reduced urinary AGEs excretion [46]. Limited information concerning absorption, biodistribution and elimination of AGEs is available. Therefore, studies in this area would help us understand the extent of the role of dietary AGEs on disease.

3.4 Receptors of Advanced Glycation End Products

At the cellular level, the damaging effects of AGEs have been attributed to several AGE-binding proteins, such as RAGE (receptor for AGEs), AGEs receptor (AGER) 1, R2, R3, and scavenger receptors such as CD-36 [47] and SCR-II [48]. These receptors are present on vascular, renal, hemopoietic, and neuronal/glial cells, and they serve in the regulation of AGEs uptake and removal. The AGEs receptors also modulate cell activation, growth-related mediators, and cell proliferation, consequently influencing organ structure and function. Furthermore, these receptors have been shown to play distinct functional roles in AGEs toxicity or detoxification [49].

The most notable receptor is RAGE, and it triggers oxidative stress and inflammation in both acute and chronic diseases, including diabetes mellitus [50]. This receptor is a member of the immunoglobulin superfamily of cell surface molecules, [51] and increased serum and tissue levels of AGEs activate RAGE. The interaction of AGEs and RAGE promotes the activation of the mitogen-activated protein kinases (MAPKs), the phosphatidylinositol-3 kinase (PI3-K) and the nicotinamide adenine dinucleotide phosphate-oxidase (NADPH, a complex of enzymes which produces superoxide) and when this complex is upregulated, it increases intracellular oxidative stress (Figure 3.2). The activation of these pathways will lead to the activation of the transcription factor NF-κB (nuclear factor kappa B) which activates the transcription of genes for proinflammatory cytokines, growth factors and adhesive molecules, such as tumor necrosis factor α (TNF-α), interleukin 6 (Il-6), well known inflammation promoters, and

vascular cell adhesion molecule 1 (VCAM1) [52-55]. Induction of these proinflammatory molecules could contribute to cellular dysfunction and damage target to organs, and ultimately lead to complications as atherosclerosis, cardiovascular disease, and nephropathy [56-58].

Besides the endogenously formed AGEs, dietary AGEs have also been shown to act as RAGE ligands and activate major signal transduction pathways in vitro [59,60]. Dietary AGEs, together with those made endogenously, could promote a systemic glycoxidant burden, oxidant stress and cell activation, which increases vulnerability of target tissues to injury [61,62].

In contrast, AGER1 suppresses AGEs and their related oxidative stress and inflammation (Figure 3.2). This receptor is encoded by the gene DDOST, and it is a type 1 transmembrane protein of ~50kDa [63]. Overexpression of AGER1 inhibits the epithelial growth factor receptor, suppresses RAGE proinflammatory signaling pathways, and contributes to maintain AGEs homeostasis [63-65]. Likewise, AGE-R3 (a 32-kDa protein and member of the β-galactoside-binding lectin family) exhibits high binding affinity for AGEs, thus protecting against AGEs-induced proinflammatory response [66]. Hence, AGER1 and AGER3 are largely responsible for AGEs-recognition and high-affinity binding [67,68]. Lastly, AGER2 is implicated in several biological functions, including cell growth, proliferation, differentiation, and apoptosis [69]. It may undergo AGEs-induced phosphorylation and could play a role in signal transduction and cell activation associated with-receptor binding [70]. The AGE-receptor systems may be regulated by factors related to diabetes such as glucose, insulin, AGEs, and reactive oxygen species (ROS) [71].

Some data suggests that AGER1 may be suppressed or saturated in the presence of high-AGEs induced oxidant stress. For instance, low expression of AGER1 in the kidney of non-obese diabetic mice was associated with high tissue AGEs levels and kidney disease [72]. Furthermore, human circulating mononuclear cells from diabetic

subjects with severe diabetes complications showed low expression of AGER1 and high serum AGEs [73]. Although, the cause of AGER1 down regulation in diabetes mellitus is not yet known, this effect is reversible by consumption of AGE-restricted diet in both humans and mice [28,74-76]. Indeed, a study with young and old mice fed high or low AGEs found that old mice maintained on high AGEs diets developed insulin resistance, decreased AGER1, increased RAGE, fibrosis in the heart and kidney, a depleted glutathione/oxidized glutathione ratio, and increased serum 8-isoprostane. In contrast, mice kept with low dietary AGEs had an enhanced antioxidant reserve, had no insulin resistance, had higher tissue AGER1 levels and had a reduction on systemic AGEs accumulation [75].

In addition, increased AGER1 expression also has been associated with extended lifespan in mice [75,76]. Similarly, a study with type 2 diabetic patients showed that the suppressed expression and function of AGER1 in diabetic peripheral blood mononuclear cells were nearly normalized by dietary AGEs restriction [74]. Another study showed that a moderate (30-50%) reduction of dietary AGEs by healthy participants substantially reduced the normal baseline levels of serum AGEs, oxidative stress and inflammation as well as AGER1 [28]. The defense mechanism exerted by AGER1 was lost in patients with diabetes mellitus and its efficiency decreased with aging [63]. It has been shown in animal studies that a way to restore this balance is by reducing dietary AGEs [75]. Therefore, efforts should be made to corroborate these results with human studies in those with and without diabetes.

3.5 Diabetes and Related Complications

Formation of AGEs in vivo depends of specific intra and extracellular conditions. Some of the studied factors involved in AGEs production are the rate of turnover of the proteins, oxidant stress in the intra or extracellular environment, and the degree of hyperglycemia [77]. It has been reported that intracellular AGEs formation significantly increases in endothelial cells after 1 week of hyperglycemia. Additionally, the type of reducing sugar also affects the rate of AGEs formation with intracellular proteins, with

the slowest reaction in the presence of glucose when compared with fructose, glyceraldehyde-3-phosphate, and glucose-6-phosphate [77]. Accumulation of AGEs in blood and in tissues has been found in healthy aging persons, and this accumulation is higher during high blood glucose concentrations. In addition, AGEs have been reported to be elevated in human tissues, plasma and urine in cases of metabolic and vascular disorders like diabetes mellitus, atherosclerosis, and renal disease [46,78-80]. Therefore, increased levels of AGEs in physiological matrices often serve as biomarkers for those diseases.

One of the main biomarkers of diabetes mellitus is hemoglobin A1c, an Amadori product that results from the reaction of glucose with the valine terminal of hemoglobin A [81], and it is used as an indicator of the average glucose levels in diabetes mellitus. As mentioned before, high levels of glucose increases glycation of the extracellular matrix, but this also occurs with intracellular proteins, especially in insulin-independent cells such as red blood cells, peripheral nerve tissue cells, endothelial cells, eye lens cells, and kidney cells [9]. It is also hypothesized that glycation of proteolytic enzymes in diabetes reduces their efficiency, resulting in accumulation of additional glycated end products [9]. One of the first studies showing that glycemic status correlates with AGEs levels was carried out by Portero-Otin et al, and their results on the quantification of pyrraline (a non-oxidative glucose-derived Maillard reaction product) in urine showed higher levels for individuals with diabetes mellitus when compared with healthy individual 3.4 and 1.1 mg/day respectively [82]. In addition to higher renal excretion of AGEs, Kalousova et al also found a slight elevation of serum AGEs in patients with diabetes mellitus type 2 (n=24) when compared with healthy controls (n=34) [79]. Furthermore, subjects with type 2 diabetes mellitus (n=50) had levels of AGEs in serum, skin and saliva that increased with the progression of complications in diabetes mellitus [46]. How these diabetes mellitus complications relate to elevated AGEs levels is not completely known. However, microvascular damage characteristic of diabetes in the kidney, retina, and microvasculature of peripheral nerves could occur when endothelial

cells from microvascular beds are damaged with subsequent capillary occlusion, ischemia and organ damage [83].

Retinopathy

Changes seen in retinopathy such as capillary occlusion and retinal ischemia could be due to high levels of glucose that could provoke dysfunction of intraretinal blood vessels, increased permeability of capillaries and progressive loss of retinal pericytes and endothelial cells [83]. The level of AGEs from retinal blood vessels has been found to correlate to the degree of retinopathy in subjects with type 2 diabetes mellitus [72], and it is hypothesized that they could be involved in the damage seen in retinopathy. In a review study, Ahmed described studies in cell cultures that have shown the toxicity of AGEs for the pericytes, and also that AGEs increase the levels of RAGE mRNA in pericytes and endothelial cells. The interaction of AGE-RAGE in retinal cells could promote upregulation of vascular growth factors as the vascular endothelial growth factor (VEGF). The vascular endothelial growth factor is capable of increasing angiogenesis and neovascularization (characteristic of proliferative retinopathy). In addition, subjects with retinopathy were found to have increased levels of AGEs and interleukin-6 (IL-6) in the eye vitreous. Interleukin-6 could also promote angiogenesis by increasing expression of the vascular endothelial growth factor [23].

Renal disease

The relationship between renal disease and AGEs has been studied in animal models. A recent study by Coughlan et al [84] described a study in which urinary carboxymethyl-lysine excretion was increased four weeks after diabetes induction, which preceded the excretion of urinary albumin and continued to rise progressively after 32 weeks. They concluded that the most informative marker of progressive renal damage linked to the AGEs pathway in experimental diabetic nephropathy was urinary excretion of carboxymethyl-lysine.

Researchers have also investigated these outcomes in patients with type 2 diabetes mellitus, and to a lesser extent in older populations. Semba et al [85] demonstrated that in an older population (n=1008), elevated circulating AGEs were an independent predictor of renal function. The study was carried out with men and women, age 64 and older, participating in the InCHIANTI study in Tuscany, Italy. The results of the study included an elevated plasma concentration of carboxymethyl-lysine independently associated with chronic kidney disease and the estimated glomerular filtration rate (an index of kidney function) at baseline, after three and six years of follow-up. These findings suggest that the potential adverse effects of AGEs on the kidney are applicable to the general population of older community-dwelling adults [85]. In another study of 548 women from the Women's Health and Aging Study I in Baltimore, 51.6% of women had decreased glomerular filtration rates, which were associated with increased serum levels of carboxymethyl-lysine and sRAGE (the soluble form of RAGE) [86]. In normal renal function, circulating AGEs are cleared by the kidneys, but high levels of AGEs have been found in patients with uremia as well as in patients with diabetic nephropathy, probably because of inadequate renal clearance [87]. Levels of AGE were measured in a study with patients with diabetes mellitus and no renal damage (n=22), with end stage renal disease but no diabetes mellitus (n=8), with end stage renal damage and diabetes mellitus (n=12) and healthy controls (n=17). It was found that all groups had higher levels when compared with the healthy controls [88]. AGEs are associated with damage seen in renal disease such as glomerular basement membrane thickening, mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis [89]. Some of these changes could be mediated by an increase in the transforming growth factor β (TGF-β) that increases synthesis of collagen matrix components responsible for the basement membrane thickening [23].

Neuropathy

High levels of AGEs have also been found in the peripheral nerves of subjects with diabetes mellitus [89]. In a recent review, Ahmed found that in vitro studies have shown that there is increased glycation of myelin in diabetes. Nerve demyelination seen in

diabetic neuropathy could be explained by phagocytosis of the glycated myelin by macrophages. In animal studies, when AGEs are injected to peripheral nerves there is a reduction on sensory motor conduction velocities, nerve action potentials and blood flow [23]. However, the mechanism by which AGEs could be involved in diabetic neuropathy is not clear.

Cardiovascular diseases

As previously described, [90] the in vivo accumulation of AGEs over time contributes to changes in the structure and function of the cardiovascular system and presents as arterial stiffening, myocardial relaxation abnormalities, atherosclerotic plaque formation and endothelial dysfunction. Several authors have described some of the potential mechanisms for these changes. One of the proposed mechanisms involves additional cross-linking on collagen (whose normal structure already contains crosslinking) by glycation of its free amino acids. The collagen-AGEs cross-linking will produce stiffness of blood vessels. Sims et al completed a histological study on 27 samples of post-mortem aortas from people with diabetes and controls and found a correlation between AGEs accumulation and aortic stiffness [91]. Another mechanism by which AGEs exert damage to the cardiovascular system is reduction of low-density lipoproteins (LDL) uptake by cell receptors. This occurs through glycation of the LDL particle on the apolipoprotein B and in the phospholipid components of LDL. The glycated LDL is more susceptible to cross-linking with collagen on the arterial wall than non-glycated LDL. Because of this, it is not taken up into the cell and accumulates in circulation. Macrophage uptake of these modified LDL lead to foam cell formation, and the development of atheroma [88,92,93]. Furthermore, decreasing nitric oxide activity is another mechanism by which AGEs can damage the cardiovascular system. Nitric oxide (a vasodilator) biosynthesis in the endothelium counteracts some of the mechanisms for atherosclerosis. Some authors proposed that AGEs reduce nitric oxide synthase half-life in the endothelium. For instance, Xu et al found a decrease in nitric oxide synthase activity after exposure to carboxymethyl-lysine-albumin both in vivo (rabbit femoral artery) and in vitro (rabbit aortic ring). They also found that after 30 minutes of exposure

to carboxymethyl-lysine-albumin, there was a reversible inhibition of endothelium and vascular response dependent on nitric oxide in vivo and in vitro [4,94]. Therefore, the accumulation of AGEs could explain some of the cardiovascular changes associated with the cardiovascular diseases seen in diabetes, such as vascular stiffening, diastolic dysfunction and endothelial dysfunction [95].

Wound healing

Not surprisingly, AGEs have also been implicated in the delayed wound healing associated with diabetes, presumably through vascular, neurological, or intermediary metabolic modifications [96]. Interaction of AGE-RAGE increases the production of inflammatory molecules such as tumor necrosis factor α (TNF-α) that could create a state of chronic inflammation in diabetes patients as well as production of destructive matrix metalloproteinases. These two events could prevent deposition of matrix components that are necessary for wound healing [23].

Even when some of the mechanisms responsible for the complications of diabetes are not well described, an association between the accumulation of endogenous AGEs and those complications is clear. In addition, several research groups [74,97,98] have addressed the role that exogenous AGEs could have on diabetes and its complications.

3.6 Managing Dietary AGEs for Health

3.6.1 Analytical measurement of AGEs in foods

One of the challenges in studying dietary AGEs is knowing the amount of AGEs in foods. Formation of dietary AGEs is a complex process involving several reactions and many end products, and only a few of them have been characterized and measured. Some of the analytical techniques used for this purpose are capillary electrophoresis, autoimmune assays, mass spectrometry, high performance liquid chromatography or gas chromatography. Some AGEs have fluorescence properties. By taking advantage of this property, some studies have measured AGEs using fluorescence

spectrophotometry. However, according to Zhang et al, high performance liquid chromatography and gas chromatography coupled with fluorescence, flame ionization or with mass spectrometry are more specific and sensitive methods for AGEs quantification [10]. However, other authors have measured AGEs by immunohistochemical techniques [99]. There is as yet no agreement among the different groups studying dietary AGEs as to which is the best.

Analytical measurement of AGEs in foods was for a long time focused on monitoring the quality of food products and for detecting indications of thermal damage in foods, mainly in milk and milk products. Furosine, pyridosine, pyrraline and carboxymethyl-lysine have been used for this purpose [100]. Since dietary AGEs have been associated with chronic diseases, some authors have measured dietary AGEs in foods more extensively.

Delgado-Andrade et al studied in a clinical setting how two different diets (one rich in AGEs and one low in AGEs) affected protein digestibility in adolescents (n=18) in Spain. They prepared two 7-day diets and measured the total furosine, hydroxymethylfurfural and fluorescence by high performance liquid chromatography in both diets [101]. In another study in Spain, the levels of glyoxal and methylglyoxal were measured in a mixture of 26 commercial cookies. The mean level of glyoxal was 15 mg/kg and 29.9 mg/kg for methylglyoxal [102]. Methylglyoxal was measured in 13 commercial carbonated beverages, 11 of which contained high fructose corn syrup as the sweetener and 2 of them contained an artificial sweetener. Levels of methylglyoxal were in the range of 23.5–139.5 μg/100 mL for the 11 beverages with high fructose corn syrup and less than 10 μg/100 mL for the beverages with artificial sweetener [103]. In another clinical study measuring risk factors for diabetes mellitus and cardiovascular disease in healthy individuals, the carboxymethyl-lysine content of a standard and a steam diet (foods were cooked with steam techniques) was measured by gas chromatography–mass spectrometry. Total carboxymethyl-lysine intake was 5.4±2.3 and 2.2±0.9 mg/d for subjects in the standard and steam diet, respectively. Individual analysis of foods

showed that bread, dough, cookies, meat and fish were the main contributors of carboxymethyl-lysine to the standard diet [97].

Chao et al measured pentosidine, carboxymethyl-lysine and furosine in some sauces used in Asian cuisine: soybean sauce, sour-sweet sauce, and barbecue sauce, as well as in sauce-treated chicken, pork, beef, salmon. They used three cooking methods: boiling, deep-frying and baking. Carboxymethyl-lysine, and pentosidine were measured by high performance liquid chromatography followed by fluorescence detection. The content was reported as μg per 100 mg of food. Cooking the food increased the amount of furosine, pentosidine and carboxymethyl-lysine in all the foods tested in comparison with the raw sample. They found that salmon and cod fried in soybean oil (temperature used 356ºF, 180ºC) had higher pentosidine and carboxymethyl-lysine than baked samples (temperature used 446ºF, 230ºC). The authors concluded that the amount of glycation products produced was affected by the interaction of the hot frying oil and food. The authors also noted that cooked salmon had higher levels of pentosidine, carboxymethyl-lysine and furosine than cooked cod, suggesting a role of polyunsaturated fatty acids, which are higher in salmon than cod, in the formation of dietary AGEs. The authors also concluded that when food was treated with the sauces tested, the sauce and heat could have a synergistic effect on dietary AGEs formation. Their hypothesis was that heat releases amino acids from the tested foods and led to the interaction with reducing sugars found in the sauces, thereby forming dietary AGEs [20].

The first attempt to measure a considerable number of foods to create an AGEs food content database was made by Goldberg et al [99]. They selected 250 typical foods and measured carboxymethyl-lysine by enzyme-linked immunosorbent assay (ELISA) with an anti-carboxymethyl-lysine monoclonal antibody. The results were expressed as AGEs units per milligram of protein or lipid, and then the AGE value was multiplied by the amount of protein or lipid per gram of food. The cooking methods used were boiled in water (212ºF, 100°C), broiled (437ºF, 225°C), deep fried (356ºF, 180°C), oven fried

(446ºF, 230°C), and roasted (350ºF, 177°C). The food group with the higher mean values of carboxymethyl-lysine was the fat group. Butter, cream cheese, cream and mayonnaise had the highest values followed by oils and nuts with values ranging from 1,300 to 450 kU of AGEs. Foods rich in protein and fat also had a high content of carboxymethyl-lysine that increased depending on the cooking method. Foods that were oven fried had higher amounts of carboxymethyl-lysine followed by deep frying, broiling, roasting and lastly boiling. The authors concluded that the presence of free radicals from lipoxidation reactions could increase formation of AGEs, and that glycoxidation and lipoxidation increased by heat could be responsible for the carboxymethyl-lysine content in foods rich in fat with lower protein content. The groups with lower levels of carboxymethyl-lysine were milk, fruits and vegetables; however infant formula had a 100-fold higher content of carboxymethyl-lysine than cow's milk, which was consistent with similar finding from Sebekova et al [104]. Even when cereals had a lower content of carboxymethyl-lysine, some products in this group had higher amounts depending on the processing. For instance, breakfast cereals with processing temperatures over 446ºF (230º C) had higher amounts of carboxymethyl-lysine [99].

Recently, Uribarri et al published a larger food database with approximately 500 food items, including those published by Goldberg et al, with the carboxymethyl-lysine content using the same enzyme-linked immunosorbent assay [105]. Additionally, they also measured methylglyoxal in some food items to validate their findings. They found the same pattern of carboxymethyl-lysine content in foods depending on their food composition and method of cooking. They attributed the higher amount of carboxymethyl-lysine in meats to the amount of intracellular components in muscle, such as amino-lipids and reducing sugars as fructose or glucose-6-phosphate, which in the presence of heat accelerates new AGEs formation. They also highlighted that higher amounts of carboxymethyl-lysine in some cheeses could be due to the curing or aging processes, and that the high content in fats could be due to the extraction and purification procedures involving heat and dry conditions. In addition to exposure to higher temperatures, they also noted that lower moisture increased the amount of

carboxymethyl-lysine for the same food. Therefore, frying, broiling, grilling, and roasting would produce higher carboxymethyl-lysine than boiling, poaching, stewing and steaming. A new observation in this study was that marinating meat in acidic solutions such as lemon juice or vinegar for at least 1 hour could reduce production of new carboxymethyl-lysine by half in comparison with foods not marinated [105]. This database represents a great tool to assess the carboxymethyl-lysine content of the diets. However, corroboration of the carboxymethyl-lysine values using different technology would help validate this AGEs database.

3.6.2 Restriction of dietary AGEs

Findings in several intervention studies, both human subjects and animals, indicate that the high intake of dietary AGEs contributes to tissue damage and increased levels of inflammatory markers that can be prevented by dietary AGEs restriction. Clinical trials on restriction of dietary AGEs in human subjects are presented on Table 3.1. The effects of reducing dietary AGEs have been studied in non-diabetic peritoneal dialysis patients, a group that has very high AGE levels, and the results showed significant reduction in the levels of AGEs. For instance, Uribarri et al studied 18 non-diabetic patients with peritoneal dialysis, in whom the intake of AGEs was reduced for 4 weeks by exposing meat to different cooking methods by participants. Subjects with the low AGEs diet showed 34% reduction in serum carboxymethyl-lysine and 35% reduction in methylglyoxal when compared to baseline. Subjects with high AGE diet had elevation of serum carboxymethyl-lysine and methylglyoxal by 29% and 26% respectively [106]. In a similar study, a decreased in vascular adhesion molecule 1 (VCAM1) and tumor necrosis factor α (TNF-α) were also found in the low AGE diet compared with the high AGE diet [107]. Another study with overweight and obese volunteers randomized to a low- and high-AGE- diet during 2 weeks found that renal function and the inflammatory profile improved following the low-AGE diet [108].

Intervention studies with patients with diabetes mellitus have had similar results. In a study of 13 patients with type 2 diabetes mellitus, decreasing the intake of dietary AGEs

(meals were provided to participants) for six weeks contributed to decreased levels of circulating AGEs and inflammatory markers (vascular adhesion molecule 1 and tumor necrosis factor α) [30]. In another study, 24 patients with diabetes mellitus were randomized to one of two groups with different diets for 6 weeks (meals were provided), one with a high level of dietary AGEs and the other with low AGEs. It was found that the low-density lipoprotein (LDL) in the group with high dietary AGEs intake was more glycated than in the group with low AGEs intake [62]. In addition, the acute effect of a meal rich in dietary AGEs has also been measured in type 1 and type 2 diabetes mellitus. It was found that flow-mediated dilation of the brachial artery (used as a measure of endothelial function) decreased after a single challenge with dietary AGEs and inflammatory markers as vascular adhesion molecule 1 increased [109,110]. Finally, a recent study with type 2 diabetes mellitus patients (n=18) and healthy controls (n=18) found a lower homeostatic model assessment (HOMA) and lipoxidation markers in subjects with lower dietary AGEs intake. The participants were randomly assigned to a low dietary AGEs (instructions were given for lowering AGEs in foods) or left with their usual dietary AGEs intake (around 20 equivalent of AGEs, measured by 3 days of dietary records) during 4 months. Patients assigned to the low dietary AGEs diet had lower levels of serum carboxymethyl-lysine, methylglyoxal, 8-isoprostane (a lipoxidation marker) and insulin in serum, as well as a lower homeostatic model assessment when compared with the subjects with the regular dietary AGEs intake [74].

These results suggest that dietary AGEs could exert similar effects as those studied from endogenous AGEs. However, it is important to note that the same research group has performed most of the intervention studies presented here, and more studies from different groups are needed to corroborate these results.

3.6.3 Anti-glycative dietary factors

There are a number of different pathways by which glycation of end products may be interrupted. These include cleaving AGEs cross-links; blocking of RAGE; blocking carbonyl groups on reducing sugars, Amadori products, and 3-deoxyglucosones;

deglycating Amadori products (Amadoriases); protecting against glycation-derived free radicals (antioxidants); and preventing autoxidation of glucose and Amadori products through chelators that remove transition metals. These last four processes are thought to occur through food or food components. The first two processes occur through pharmacological means [111].

The most profuse process for antiglycation is through antioxidants, which scavenge free radicals. Antioxidants are abundant in foods containing large amounts of vitamins C and E, carotenoids, and selenium, as well as phenolic compounds such as anthocyanins, flavonoids, catechins, and lipoic acid. These nutrients and food components are present in fruits and vegetables (vitamin C, anthocyanins, flavonoids), green tea (catechins), nuts seeds and oils (vitamin E), wine and chocolate (flavonoids), and liver, spinach, broccoli, and potatoes (lipoic acid). Additional food components with antioxidative properties include curcumin, and aged garlic extract [111].

Aged garlic extract contains a higher concentration of antioxidants than whole garlic. During the aging and extraction over 10 months, the garlic can become odorless as the organosulfur compounds become water soluble [112]. The aged garlic extract contains many compounds that have been investigated for health benefits, including lectins, fructooligosaccharides, apigenin which is a flavonoid, fructosyl arginine which is a Maillard reaction product, and tetrahydrocarbolines [113]. Although aged garlic extract has primarily been investigated for cardiovascular [114,115], cancer [116-118] and immune system effects [119], it has also been used in in vitro experiments to determine its ability to inhibit AGEs products. Aged garlic extract inhibited the cross-linked glycation end products and an ingredient of garlic extract, S-allyl cysteine, also inhibited non-crosslinked AGEs [120]. An in vitro study also found that aged garlic extract scavenged superoxide radicals in at dose dependent manner over three dosages [112].
Curcumin is a major component of turmeric, a popular ingredient in Asian foods. Curcumin has been reported to interact with a number of immunoregulatory enzymes and transcription factors, and therefore has been evaluated for its role in cancer

treatment and prevention [121,122], as well as inflammatory conditions [123], dementias [124], and obesity-related disorders [121]. However, intestinal metabolism of curcumin results in metabolites with short half-lives and limited cell permeability [125]. Nevertheless, numerous studies have reported anti-diabetic properties of curcumin [126]. In one animal study, diabetic rats fed curcumin with their diet for 16 weeks had lower fasting blood glucose and urinary glucose as compared to control animals. A variable effect of curcumin on lysosomal enzymes was found depending on which lysosomal enzyme and where in the body it was measured [127]. However, one animal study reported increased oxidative stress and AGEs formation with hyperglycemic animals fed curcumin, as evidenced by faster cataract formation. Non-diabetes rats showed beneficial effects at very low levels of curcumin [128]. Recently, in vitro work concluded that curcumin induced gene expression of AGE-receptor 1(AGER1), which facilitates the clearance of AGEs [129].

Other antioxidants have also shown promising results in animal and in vitro studies. A number of fruits, vegetables and herbs were evaluated using in vitro techniques to assess their antiglycation ability. Ginger, cinnamon, and cumin have been reported to reduce AGEs formation in vitro by more than 50% at 1.0 mg/ml [130]. In one animal study, the phenolic acids caffeic and ellagic acid were added to mouse chow at 2.5 or 5 g per 100 g diet and fed to diabetic mice for 12 weeks. Hemoglobin A1c, glycated albumin, and renal carboxymethyl-lysine levels were lower for animals fed the phenolic acids, with more significant effects seen with the higher intakes [131]. A study to investigate the effects of Pu-erh tea, which is a fermented tea, on AGEs accumulation associated with diabetic nephropathy found that Pu-erh tea prevented diabetes-induced accumulation of AGEs and led to decreased level of RAGE expression in glomeruli [132]. In another animal study, green tea extract was administered to rats for 4 weeks (300 mg per kg body weight per day). Fluorescence of AGEs, blood glucose and hemoglobin A1c was significantly reduced when compared to diabetic rats not receiving the extract ($P < .05$) [133]. However, in a study where green tea or vitamins C and E were added to the rats' drinking water, there was no difference in glycemia when

compared to control animals, but did decrease lens fluorescence. Surprisingly, the group fed the additional vitamins C and E exhibited worsened collagen glycoxidation in certain tissues. Additionally, tendon breaking time in urea, which can be used as a marker for total cross-linking, increased with both vitamin C and E administration and with green tea, leading the authors to speculate that treatment of oxidative stress alone may not ameliorate complications of diabetes, but that other stresses as well as attention to intracellular and extracellular activities, could be important [134]. Indeed, in vitro experiments suggest some caution concerning flavonoids and AGEs. In general, lower concentrations were found to be inhibitory while higher concentrations were more often enhancing of carboxymethyl-lysine production, presumably through increased hydrogen peroxide production [135].

Few studies have evaluated these foods or ingredients in human studies. However, Klein et al evaluated mate tea's impact on blood glucose and lipid values in diabetes and pre-diabetes. Each group was divided into receiving the mate tea, an educational intervention, or both the tea and education. Those instructed to drink the tea were to make it from 6.6 g of yerba mate leaves and 330 mL of boiling water, drinking the 330 mL three times per day for 60 days. No differences were found in the pre-diabetes group across treatments. In the diabetes group, those drinking mate tea demonstrated significant decrease in blood glucose and hemoglobin A1c [136].

Of course, drugs have been developed for their antiglycation properties, particularly alagebrium, which has been shown to have positive effects in animal models [108,137].

3.7 Summary, Implications for Research

The health impact of dietary AGEs has increasingly been studied since recognition of their potential deleterious effects. Some of these effects can be attributed to changes in the nutritional composition of the foods from which the dietary AGEs are derived. The nutritional consequences of the Maillard reaction in foods include the loss of some amino acids, such as lysine, arginine, tryptophan and histidine. Good indicators of loss

of lysine in foods are furosine, pyrraline and carboxymethyl-lysine [100]. There is also some evidence that AGEs could affect mineral metabolism. Studies in rats have shown increased urinary excretion of calcium, magnesium, sodium zinc, and copper compared with animals fed a control diet [17]. Similar studies in adolescents have shown impaired iron bioavailability [138].

Secondly, dietary AGEs can have an impact on health through a variety of systematic mechanisms. Studies focusing on the effects of dietary AGEs have been shown to increase circulating AGEs, to accumulate in tissues, to affect endothelial function, to increase pro-inflammatory cytokine and oxidation markers and to act as a ligand for the advanced glycation end products receptor (RAGE). Furthermore, data from our group shows that AGEs intake was higher in participants with presence of cardiovascular disease complications when compared with those without complications [139].

Although a role for AGEs in diabetes-related complications seems well supported by the literature, in other areas the role of AGEs in general and dietary AGEs in particular is less clear. For instance, there is no consensus among the researchers investigating dietary AGEs about their health impact, probably because of the diversity in AGEs, their effects, and dose-response issues. Indeed, some studies have found some beneficial or no effects from dietary melanoidins (late products of Maillard reaction in foods) or other AGEs such as pentosidine. For instance, Ames et al. found that melanoidins from bread crust increased the number of anaerobes, Clostridia, and Bifidobacteria in a culture of human fecal bacteria [140], indicating a possible potential prebiotic effect of bread crust melanoidins [141]. In addition, other study showed an increase in the activity of chemopreventive enzymes as glutathione-S-transferase [142].

Moreover, AGEs heterogeneity has made measuring them in foods and comparing data from different research groups difficult. Also, only short term effects have been measured and information about long term effects have not been studied yet. The methodological design of long-term studies represents a great challenge. One of the

difficulties is achieving diets with different dietary AGEs content, but with similar content in other nutrients, such as heat-sensitive vitamins. Pouillart et al. demonstrated that diets with different dietary AGEs content but similar thiamine, vitamin E and other heat-sensitive vitamins content, are challenging but possible [143].

Future studies on the role of dietary AGEs on diabetes mellitus and their related complications also should address the methodological problem for their measurement in the diet. Most studies of dietary AGEs have used 3 days food records for their quantification in the diet. However, 7 days food records are better for measuring proteins and fat [144]. While the database for dietary AGEs has grown, consensus about how dietary AGEs should be measured has not been reached. Finally, human studies including antiglycation food products need to be conducted to elucidate any valid role these foods and food components may have in health.

Authors' Contributions

Claudia Luevano-Contreras provided the first draft of the manuscript with Ma. Eugenia Garay-Sevilla submitting the section on Metabolism and Receptors of AGEs and Karen Chapman-Novakofski submitting the section on Antiglicative dietary factors. All authors reviewed the final draft with minor modifications.

Declaration of Conflicting Interests

The authors declared no conflicts of interests with respect to the authorship of this article.

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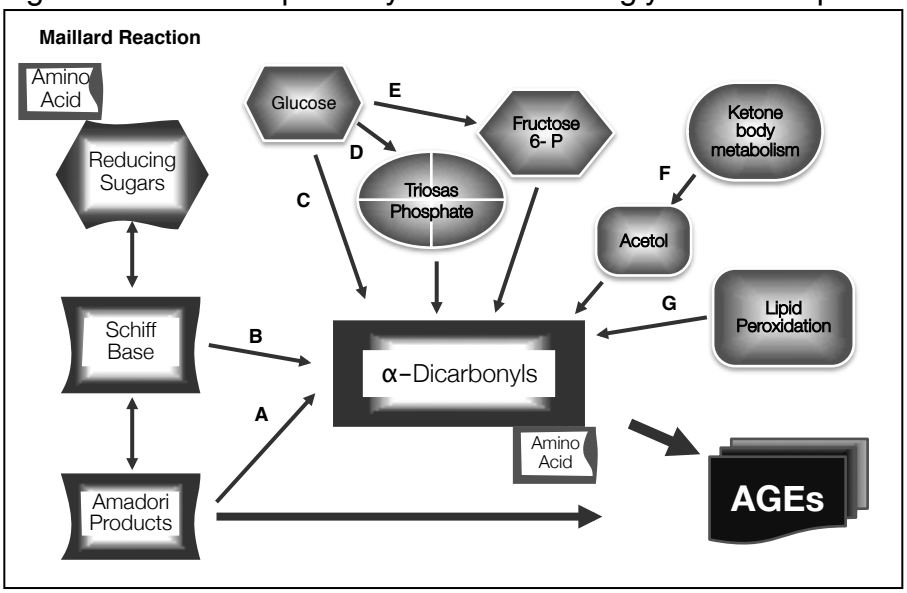
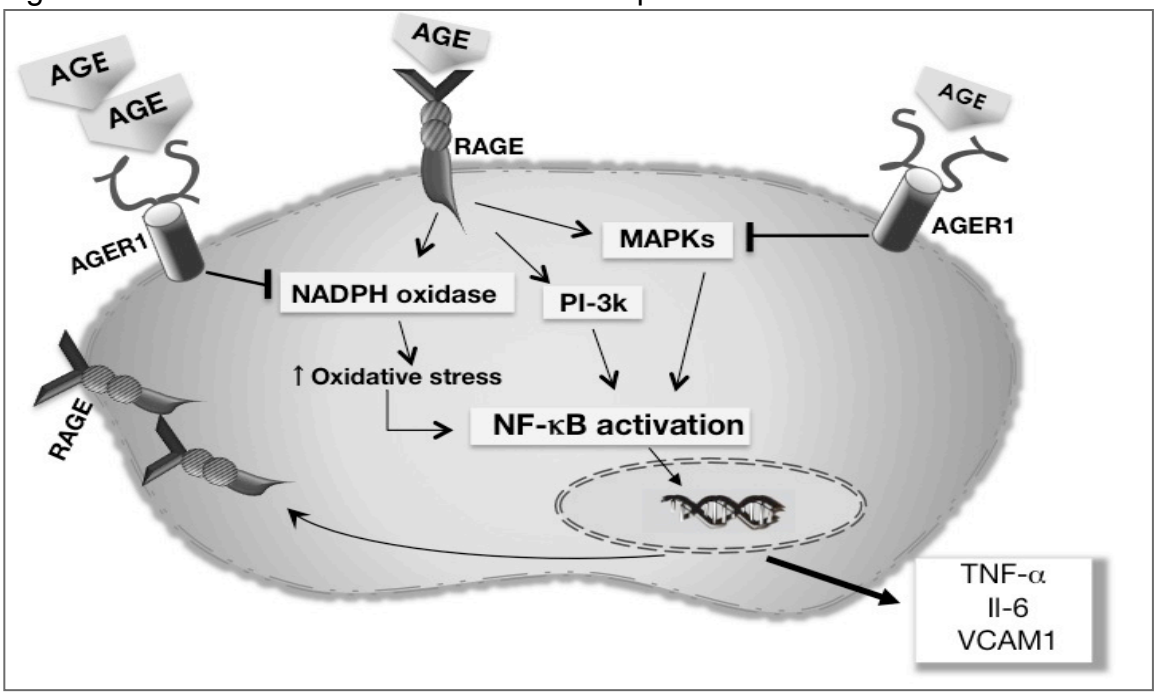
Figure 3.1 Different pathways for advanced glycation end products formation in vivo



A) Non-oxidative Amadori product cleavage B) Namiki pathway C) Wolff pathway, glucose autoxidation D) Glycolytic pathway E) Polyol pathway F) Ketone body and threonine metabolism G) Lipid peroxidation

Figure 3.2 Interaction of AGEs with their receptors



AGE = Advanced glycation end product; RAGE = receptor for AGE; MAPK = mitogen-activated protein kinases; AGER1 = AGEs Receptor 1; PI-3k = phosphatidylinositol-3 kinase; NAD(P)H oxidase = enzymes complex which produces superoxide; TNF-α = tumor necrosis factor α; IL-6 = Interleukin 6; VCAM-1 = vascular adhesion molecule 1.

Table 3.1 Clinical studies with dietary AGEs restriction in human subjects

	Study population	Design	Diets	Results
Vlassara 2002[30]	11 subjects with diabetes (DM)	2 weeks crossover; meals were provided to participants with different AGEs content	Low AGEs: 3.67±1.2 x 106 AGEs units	Serum AGEs ⇓ 30% VCAM-1 ⇓ 50%
			High AGEs: 16.3±3.7 x 106 AGEs units	Serum AGES ⇑ 64%
	13 subjects with DM	6 weeks study; meals were provided with different AGEs content	Low AGEs: 3.67±1.2 x 106 AGEs units	CML ⇓ 40%, CRP ⇓ 20%, VCAM-1 ⇓ 20%
			High AGEs: 16.3±3.7 x 106 AGEs units	CML ⇑ 28%, CRP ⇑ 35%, TNF- α ⇑ 86%
Uribarri 2003[106]	26 subjects with peritoneal dialysis (non-DM)	4 weeks study; participants ate meat cooked with different methods.	Low AGEs, Basal: 13.8±3 Final: 17±3 x106 AGEs units	Serum CML ⇓ 34% MG ⇓ 35% and CML-LDL ⇓ 28%
			High AGES Basal: 12.4±1.5 Final: 5.5±0.9 x106 AGEs units	Serum CML ⇑29%, MG ⇑ 26% and CML-LDL ⇑ 50%
Cai 2004[62]	24 subjects with DM	6 weeks study; meals with different AGEs content	Low AGEs: 3.67±1.2 x 106 AGEs units	LDL was 50% less glycated and 80% less oxidized
			High AGEs: 16.3±3.7 x 106 AGEs units	⇑ MAPK phosphorylation, NF-κB activity, and VCAM-1 production on endothelial cells
Negrean 2007[109]	20 subjects with DM	6 days study; acute effect of 2 different AGEs diets on days 4 and 6; single meal was provided	Low AGEs meal: 2750 KU	CML and MG ⇓
			High AGEs meal: 15100 kU	CML and MG ⇑ after 4 h. Impaired macrovascular function: Flow mediated dilation ⇓ 36.2%. Markers of endothelial dysfunction: E-selectin ⇑ 51%, ⇑I-CAM and ⇑VCAM. Marker of oxidative stress, TBARS ⇑ 21%
Stirban 2008[145]	20 subjects with DM	6 days study; acute effect of 2 different AGEs diets on days 4 and 6; single meal was provided	Low AGEs meal: 2750 KU	
			High AGEs meal: 15100 kU	Leptin and adiponectin ⇓
Vlassara 2009[28]	40 healthy subjects	4 months study; instructions to change cooking methods	> 13 AGE 106 AGEs units	Low AGEs Reduction on CML, MG and AGER1, RAGE, and p66Shc, 8-isoprostane, VCAM-1 and TNFα
	9 CKD-3 subjects	4 weeks study; instructions to change cooking methods		
Birlouez-Aragon 2010[97]	62 healthy subjects	4 weeks study; meals were provided with different cooking preparations	Low AGEs 2.2±0.9 mg	⇓ Total cholesterol, HDL and triglycerides
			High AGEs: 5.4±2.3 mg	⇑ CML, fasting insulinemia, HOMA
Uribarri 2011[74]	18 healthy and 18 subjects with DM	4 months study; instructions to change cooking methods	High AGEs: 20000 KU AGEs	Low AGEs Lower levels of CML, MG, 8-isoprostane (a lipoxidation marker) and insulin in serum, as well as a lower HOMA

AGER1=AGEs Receptor 1; CML=carboxymethyl-lysine; CML-LDL=carboxymethyl-lysine-low density lipoprotein; CRP=C reactive protein; HDL=high-density lipoproteins; HOMA=homeostatic model assessment; ICAM-1= intracellular adhesion molecule 1; MAPK=mitogen-activated protein kinases; MG=methylglyoxal; p66 Shc=Shc adaptor protein; RAGE =receptor for AGE; TBARS=thiobarbituric acid-reactive substance; TNFα =tumor necrosis factor α; VCAM-1= vascular adhesion molecule 1.

Chapter 4
The Relationship Between Dietary Advanced Glycation End Products And Indicators Of Diabetes Severity In Mexicans And Non-Hispanic Whites: A Pilot Study[3,4]

Abstract: Diet is an important source of exogenous advanced glycation end products (AGEs). Dietary AGEs content depends on nutrient composition and on the way food is processed/cooked. The objective of our study was to compare AGEs intake of 2 different ethnic groups (Mexicans and non-Hispanic whites) with type 2 diabetes mellitus (DM) and to study the relationship between dietary AGEs and diabetes-related complications. Complications were self-reported by subjects (n=65) and categorized according to a published DM disease severity index as low risk or moderate-high risk. Dietary records for 10 days were used to estimate dietary AGEs from a published food table. Non-Hispanic whites had higher intake of dietary AGEs (natural logarithm was used, LogAGEs) when compared with Mexicans, which was consistent with their higher intake of saturated fat. Additionally, for each unit increase in the LogAGEs, a participant was 3.7 more likely to have moderate-high risk for cardiovascular disease.

Key words: Dietary AGEs, cardiovascular complications, carboxymethyl-lysine (CML).

[3]This chapter appeared in its entirety in the International Journal of Food Science and Nutrition and it is referred as to Luevano-Contreras C, Preciado-Puga M, Garay-Sevilla ME, Chapman-Novakofski K. The relationship between dietary advanced glycation end products and indicators of diabetes severity in Mexicans and non-Hispanic whites: a pilot study. Int Journal of Food Sci and Nutrition, **2013**, *64*,16-20. It is available at http://www.informahealthcare.com using DOI: 10.3109/09637486.2012.704905

[4]Additional analysis and tables not included on the published manuscript are included on Appendix A, and questionnaires used for this study are included on Appendix B.

4.1 Introduction

The prevalence of diabetes mellitus (DM) is dramatically increasing worldwide, especially for some ethnic and racial groups. For instance, Mexican-Americans have higher prevalence, as well as higher risk and prevalence of diabetes-related complications when compared to non-Hispanic whites [1]. Several mechanisms have been proposed for the development of diabetes-related complications. A research area gaining interest in recent years is the accumulation of a group of compounds called advanced glycation end products (AGEs). AGEs can be formed in the body (endogenous) and contribute to vascular damage in diabetes. In addition, exogenous sources of AGEs can be found in some foods rich in protein and fat, with high cooking temperatures increasing AGEs formation. Several AGEs have been characterized, and one of the most widely used as a marker of AGEs in foods and *in vivo* is carboxymethyl-lysine (CML) [2].

The effects of dietary AGEs have been measured in clinical research studies in terms of levels of blood AGES and inflammatory and oxidation markers. Patients in the groups with higher AGEs intake had higher levels of inflammatory and oxidation markers such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and C-reactive protein (CRP) than patients in the lower AGEs intake group. In these studies, AGEs intake has been assessed with 3-day food records. However, while some authors have found that 3 days of food records are good for providing a reasonable estimate of the quality of the diet, they are not very accurate in estimating individual nutrients, and at least 7 to 10 nonconsecutive days are necessary to represent usual intake of energy and macronutrients [3,4]. The length of time needed to adequately represent AGEs intake has not been studied, but AGEs intake is highly dependent on the macronutrients fat and protein [5].

Because Mexican-Americans are one of the minorities with higher prevalence of DM, one of the objectives of this study was to compare the diets of Mexicans (living in Mexico or in USA) and non-Hispanic whites with diabetes. In addition, we wanted to

study the relationship between dietary AGEs and diabetes parameters, as well as to recognize tertiles of AGEs intake in these groups using 10 days of food records. Our hypothesis was that subjects with higher intake of AGEs could have higher risk for self-report diabetes complications.

4.2 Methods

A convenience sample of Mexican-Americans (n=15) and non-Hispanic white (n=15) adults in USA and Mexican adults (n=35) in Mexico with type 2 DM were recruited directly by investigators, through key informants, or email bulletins. All participants were over 18 years of age and had type 2 DM by self-report. Pregnant or breastfeeding women were not included, nor vegan (strict vegetarian), vegetarians, nor participants with a protein restricted diet. Participants must have had a recent (past 3 months) glycosylated hemoglobin A1c (HbA1c) test (self-report). The Institutional Review Boards in both countries approved the protocol.

In a first meeting, participants had a brief training (10-15 minutes) about how to complete the 10-day food records after they had read and signed the informed consent (Spanish or English, by preference). For the training, food models and measuring cups were used to increase the accuracy of the reported food amounts. Reporting cooking methods and food brands was emphasized. After patients reported the first day, they were interviewed again to increase the likelihood that all foods were included, cooking methods reported, portions were as accurate as possible, and to answer any questions the participants may have had. A final meeting clarified any inconsistencies or omissions.

In addition, they completed background questionnaires: a history of diabetes mellitus questionnaire which included type of medication, history of complications, health-related visits, history of smoking and alcohol intake, self-reported HbA1c, blood glucose levels, weight, and height; and a vitamins and supplements intake questionnaire [6,7]. The

body mass index (BMI) was calculated with self-reported weight and height according to the formula weight (kg)/[height (m)]^2.

4.2.1 Dietary assessment

For the quantification of micro- and macronutrients, Food Processor software (Salem, Oregon, 2008) was used for the analysis of US dietary records, with foods added according to label nutrient content when needed. For the Mexican dietary records, the software Nutrikal (2007) was used. A registered dietitian reviewed all the analyses.

To develop a classification of AGEs intake, we reviewed six previous clinical studies that assessed low and high AGEs intake in humans [8-13]. The average intake for the low AGEs groups was 3900 KU (2700-5500 KU), and the average intake for the high AGEs was 15000 KU (12200-16300 KU). Therefore, for the present study, consumption higher than 15000 KU was considered high, between 4000 and 15000 KU was considered moderate and less than 4000 KU was considered low. The AGEs content of food was taken from a published database with around 500 foods with the amount of CML expressed as AGE Kilounits (KU) [14]. In addition, because higher caloric intake may influence the intake of AGEs, dietary AGEs were divided by caloric intake to derive AGEs density.

4.2.2 Complications

The Michigan Diabetes history form was used for background medical history data [6]. Participants answered the questionnaire at home and were advised to contact their primary physician with questions regarding their medical history. Three categories of complications were used: cardiovascular disease, eye disease, and peripheral disease. For cardiovascular disease, questions included those pertaining to the presence of hypertension, high levels of cholesterol, angina, heart attack, heart failure, artery bypass surgery, angioplasty, and heart catheterization. For eye disease complications, questions ranged from presence of cataracts, detached retina, blurred vision, retinopathy, blindness, macular degeneration, macular edema, and laser treatment or

cataract surgery. For peripheral disease questions, presence of peripheral vascular disease, intermittent claudication, peripheral neuropathy, foot ulcers, gangrene, amputation of the toe, foot or leg were asked. To categorize the self-reported information about complications, we used a published disease severity index for DM, but we used only two risk levels (low or moderate-high) instead of the original four [15]. Participants were classified as low risk if they answered ”no” to all the questions regarding a specific complication category or as moderate-high risk if they answer “yes” to any of the questions regarding that complication. Another section of the diabetes history questionnaire was regarding health care. The questions included those related to the last visit to the ophthalmologist, diabetes educator and dietitian, as well as how many visits they had with a health care provider in the last 12 months.

4.2.3 Statistical Analysis

Normality was assessed by the Shapiro-Wilk test. Skewed data is presented as median and interquartile range, and dietary AGEs were transformed using the natural logarithm (LogAGEs). One-way analysis of variance (ANOVA) was used for testing differences among groups with a Bonferroni correction test for post-hoc analysis, and Tamhane test when homogeneity of variance was not assumed. For non-normally distributed data and non-continuous variables the test Kruskal-Wallis was used. Stepwise linear regression was used to evaluate the relationship between dietary variables, and dietary AGEs (LogAGEs) as dependent variable. An influential outlier was eliminated in our final model. In addition, logistic regression was used to evaluate the relationship between each complication category and other independent variables as health care variables, smoking, metformin use, BMI, LogAGEs, and AGEs density. All statistical analyses were performed in SPSS (version 17.0, 2008 Chicago IL).

4.3 Results

We analyzed data for 65 participants, 35 in Mexico, 15 non-Hispanic white Americans and 15 Mexican-Americans in USA. Sixty-nine participants started the study, but 4 of them did not complete it (2 Mexicans, 1 non-Hispanic White and 1 Mexican-American).

For the Mexican group, 74% of the participants were females and 26% males. For the non-Hispanic white and Mexican-American groups, both included 47% females and 53% males. The HbA1c was similar for Mexicans and Mexican-Americans (8.2±1.8% and 8.1±2%) and lower for non-Hispanic whites (7.1±1.2%), but not statistically different. Mexicans had the lowest BMI (29.0±4.9 kg/m^2) followed by Mexican-Americans (32.3±7 kg/m^2) and non-Hispanic white (36.2±8.8 kg/m^2), but only Mexicans and non-Hispanic whites were statistically different ($p<0.01$).

Results from the dietary analysis are shown in Table 4.1. There was a trend of lower intake of energy, fat and saturated fat for Mexicans followed by Mexican-Americans. Regarding dietary AGEs (LogAGEs), there was a significant difference among groups; Mexicans had the lower intake followed by Mexican-Americans, and the highest consumption was for non-Hispanic whites. These results remained consisted after accounting for differences in weight (AGEs/kg) and differences in energy intake (AGEs density). After using stepwise linear regression, dietary AGEs (LogAGEs) were associated with protein and saturated fat. The association remained significant after adjusting for group ($F=55.12$, $p<0.0001$, $R^2=0.78$)(Table 4.2).

4.3.1 Severity Index for Diabetes-related Complications

Participants were classified into two risk levels (low or moderate-high) for each complication category. For cardiovascular complications, all of non-Hispanic whites were classified as moderate-high risk level (100%) followed by Mexican-Americans (80%) and Mexicans (69%); non-Hispanic whites and Mexicans were statistically different. Regarding peripheral disease complications, Mexicans had the highest frequency of moderate-high risk level (57%), followed by Mexican-Americans (40%), and lastly non-Hispanic whites (20%); Mexicans and non-Hispanic-whites were statistically different. For eye disease complications, the frequencies were very similar among groups.

Regarding health care variables, the only significant difference was for ophthalmologic visits 80% of non-Hispanics whites participants had visited the ophthalmologist in the last 12 months, but only 37% of Mexicans participants had visited the ophthalmologist in the last 12 months.

4.3.2 Relationship between Complications and dietary AGEs

Logistic regression was used to find a relation between each category of complication and dietary AGEs. Only cardiovascular complications were associated with dietary AGEs (LogAGEs) when adjusting for HbA1c, metformin intake and smoking habits. It was found that the odds ratio for LogAGEs was 3.7 (Table 4.3). Therefore, we find that one unit increase in the LogAGEs is associated with a 270% increase in the predicted odds for being at the moderate-high risk level for cardiovascular disease. The association was still significant after adjusting for energy intake (AGEs density), but the odds ratio for AGEs density was 1.5 (Table 4.3). It is important to note that no association was found for saturated fat (results not shown). Participants in the moderate-high risk level for cardiovascular disease had a significant higher intake of AGEs (11,921±8,671 KU) when compared with those in the low risk level (7,414±4,168 KU)($p<0.01$).

4.4 Discussion

One of the most interesting findings of our study was the association between dietary AGEs (LogAGEs) and the risk level for cardiovascular disease. There are no similar studies comparing intake of AGEs between subjects with diabetes-related complications and without complications. However, clinical studies have found that DM subjects assigned to a high intake of dietary AGEs had more glycated low-density lipoproteins (LDL) than subjects with the low AGEs intake [12]. In addition, the acute effect of a meal rich in AGEs has also been measured in type 1 and type 2 DM. It was found that after a single challenge with dietary AGEs flow-mediated dilation of the brachial artery (used as a measure of endothelial function) decreased, and inflammatory markers as VCAM-1 increased [10,11]. Furthermore, Chao et al. studied subjects with type 2 DM and found a positive correlation between high dietary AGEs intake and levels of inflammatory

cytokines (IL-1α, and TNF-α), levels of the oxidation marker 8-isoprostane and levels of glycated LDL [16].

Dietary intake for energy, fat and saturated fat was significantly higher in non-Hispanic whites when compared to Mexicans, but there were no difference between non-Hispanic whites and Mexican-Americans. For Mexican-Americans and Mexicans, only saturated fat intake was significantly different; it was higher for Mexican-Americans. A study among 4 adult female groups: Mexicans, Mexicans-Americans (MA) born in Mexico, MA born in USA and non-Hispanic whites found statistical differences in energy, fat and saturated fat intakes between Mexicans and MA born in Mexico, as well between MA born in Mexico and non-Hispanic whites. The authors concluded that an increase exposure to US environment among Mexicans shifted the diet toward an increase in energy, fat, saturated fat and sugar intake. [17]. Although, we did not find a significant difference between Mexicans-Americans and non-Hispanic whites there was a trend of lower intakes for Mexican-Americans.

The intake of AGEs was different among groups; Mexicans had the lower intake followed by Mexican-Americans and non-Hispanic whites which is consistent with their intake of saturated fat. However, it is important to have in mind that the database used was created considering usual intake of an urban population in USA, and is lacking usual Mexican foods. In a study in subjects with end-stage renal disease where 43% of them had diabetes, the mean AGE intake in those with diabetes was 14±7 KU X 10^6 [18] which is below the intake of AGEs in non-Hispanic whites. Similar to our results, a study in healthy subjects found that the intake of AGEs ranged from 20 KU X 10^6 to 14 KU X 10^6 [19]. Regarding the association of AGEs with other dietary variables, findings by Uribarri et al. showed a significant correlation of AGEs with the intake of protein, fat and saturated fat, and the multiple regression model showed that dietary AGEs was associated with dietary fat but not with protein or saturated fat ($r=0.69$; $p=0.01$)[18]. Likewise, we found a relation between protein and saturated fat intake and dietary AGEs.

Similar to our results, a study comparing diabetes-related complications among Hispanics and non-Hispanic whites from NHANES 2006 found that there was an increased risk for peripheral vascular disease among Hispanics [20].

This study had some limitations. First, years with DM were not measured and it should be considered for future studies. There were no non-Hispanic whites without cardiovascular disease and so we could not test for any effect of ethnicity. Body weight, height and complications were self-reported. In addition, we did not measure AGEs levels in blood, however findings from several studies support the view that the intake of dietary AGEs is an important contributor to the body's AGEs pool [16,21,22]. One of the strengths of this study was the comprehensive analysis of AGEs content in the diet; we used 10 days of food records and a recent published database with 500 foods with AGEs contents.

4.5 Conclusions

Non-Hispanic whites had higher intake of dietary AGEs when compared with Mexicans, which is consistent with their higher intake of saturated fat. For each unit increase in the LogAGEs, participants were 3.7 more likely to have moderate-high risk for cardiovascular disease. Despite its limitations this study shows a significant association between dietary AGEs intake and the risk level for diabetes-related cardiovascular complications, which should be explore in future research [23]

Acknowledgment

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Declaration of interests

The authors declare no conflict of interests.

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Table 4.1. Dietary Characteristics by Group.

	Mexicans (n=35)	***Mexicans-Americans (n=15)***	***Non-Hispanic Whites (n=15)***
Dietary AGEs (KU)	6303 ± 2842 *	11600 (7508, 15996) †	19798 ± 8707 †
Log Dietary AGEs¶	8.6 ± 0.5 *	9.3 ± 0.5 †	9.8 ± 0.4 ‡
AGEs density	3.9 ± 1.5*	6.9 ± 2.1 †	9.9 ± 2.6 ‡
Dietary AGEs by body weight (Kg)	92.7 ± 38.9 *	154.8 ± 84.9 †	198.7 ± 83.9 †
Energy intake (Kcal)	1575 ± 330 *	1603 (1307, 1900) *	1984 ± 582 †
(Kj)	6594 ± 1381*	6711 (5472, 7954)*	8306 ± 2436 †
Carbohydrates (g)	217.5 ± 53.1*	196.1 (159.8, 216.5)*	226.2 ± 67.4*
Protein (g)	72.4 ± 22.2*	69.7 (60.2, 90.1)*	85.9 ± 21.1*
Fat (g)	54.1 ± 15.1 *	60.2 (50.6, 75.1) *	83.2 ± 31.2 †
Saturated fat (g)	13.5 ± 4.2 *	18.7 (14.1, 22.7) †	27.7 ± 11.2 †
AGEs Intake	(n)	(n)	(n)
Low <4000 KU	9	0	0
Moderate 4-15000 KU	24	11	4
High >15000 KU	2	4	11

Results are presented as mean±SD or median and interquartile range. ¶Natural logarithm transformation used because skewed data. One-way Anova used for differences among groups. Different symbols show differences between groups ($p<0.05$).

Table 4.2. Multiple Linear Regression for Dietary AGEs with Independent Dietary Variables Adjusted by Group.

Independent variables	***Mexicans (n=35) β (SE)***	***Mexicans-Americans (n=15) β (SE)***	***Non-Hispanic Whites (n=15) β (SE)***
Constant	7.5 (0.1)*	7.9 (0.1)*	8.2 (0.1)*
Protein	0.010 (0.002)*	0.010 (0.002)*	0.010 (0.002)*
Saturated Fat	0.025 (0.006)*	0.025 (0.006)*	0.025 (0.006)*

Coefficients (β) and their standard error (SE) are presented. $F=55.1$, $p<0.0001$, $R^2=0.79$.
*p-value<0.001 for individual coefficients.

Table 4.3. Logistic Regressions for Cardiovascular Disease Complications

Model 1	***β (SE)***	***Odds Ratio***	***95% Confidence intervals***
Constant	-10.7 (5.7)		
LogAGEs	1.3 (0.6)*	3.7	(1.2, 11.9)
Metformin intake	-1.5 (0.9)	0.2	(0.04, 1.52)
Number cigarettes	-0.2 (0.1)	0.8	(0.58, 1.04)
HbA1c	0.2 (0.2)	1.2	(0.81, 1.87)
Model 2 AGEs density	0.4 (0.2)*	1.5	(1.1, 2.1)

Model 1: Chi-square=11.75, p=0.019, R^2=0.26. Model 2: Chi-square=12.8, p=0.012, R^2=0.3. The coefficient for the constant was -1.5, for Metformin intake, Number cigarettes and HbA1c were similar to the first model (±0.1). *p-value<0.05.

Chapter 5
Development, Relative Validity, and Reliability of a Food Frequency Questionnaire for a Case-Control Study on Dietary Advanced Glycation End Products and Diabetes Complications[5, 6]

Abstract: Dietary advanced glycation end products (dAGEs) could be involved on diabetes complications, yet their quantification is not standardized. The objective of this study was to design a food frequency questionnaire (FFQ) for dAGEs, and to assess its reliability and validity. For the design, data from 30 subjects was used. The final instrument had 90 food items. To measure reliability and validity, 20 participants with type 2 diabetes filled out twice the FFQ (FFQ-T1, FFQ-T2) and 7-day food records (7-dFR). The Shrout-Fleiss coefficient was 0.98 showing good reliability. For validation, the results for the weighted kappa were 0.55 (moderate agreement) for FFQ -T1 and 0.64 (good agreement) for FFQ-T2, and 75% and 80% of subjects respectively were correctly classified into tertiles; Bland-Altman graphics showed no systematic bias. This FFQ is comparable to 7-dFR for measuring dAGEs. To our knowledge this is the first questionnaire designed to measure specifically dAGEs.

Keywords: diabetes mellitus type 2, dietary intake, survey validation, dietary advanced glycation end products.

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[6]Note: Questionnaires used for this study are included on Appendix C.

5.1 Introduction

Diabetes complications are the 7th leading cause of death in the US, and it is estimated that 20% of health care dollars are spent on people diagnosed with diabetes [1]. The accumulation of endogenous compounds called advanced glycation end products (AGEs) could be involved in the development of these complications [2]. In addition, results from studies in animals and humans suggest a role of dietary AGEs (dAGEs) on diabetes. Dietary AGEs could accumulate in tissues and exert the same damage attributed to endogenous AGEs. Clinical studies have shown that high dAGEs intake increased circulating AGEs levels, pro-inflammatory cytokines, oxidation markers, and affected endothelial function [3-5]. However, the long-term effects of dAGEs have not been studied, mainly because of the methodological challenges for their measurement in the diet.

Among the different instruments for measuring dietary intake, the 7-day food record with weighing, measuring or estimation of portion size provides a reasonably accurate method of actual intake, but it is time consuming and it demands a high degree of cooperation from participants [6]. A good alternative to food records (FR) is the food frequency questionnaire (FFQ), because it decreases the participant's burden of filling out several days of food records [7]. Among the advantages of this questionnaire is that it is inexpensive to administer and less time consuming for analysis. However, the foods selected for the FFQ are very important, as well as the correct choice of portion size [8]. The FFQ should have defined food categories [9]. Food frequency questionnaires and diet history methods are the best methods for assessing past diet and are therefore the only good choices when designing case-control studies [10].

Food frequency questionnaires are also useful when the focus is on measuring a specific nutrient intake [11,12]. The list of foods used in this case should include foods with higher amounts of the nutrient of interest or with moderate levels, but consumed in larger amounts by the population of interest. For this reason, using previous dietary survey information collected from the population is highly recommended [7]. In

semiquantitative FFQs, the frequency of the food eaten is multiplied by the nutrient content of the food. In this fashion, nutrient intake can be classified in tertiles for low, medium and high intakes [13]. When a new FFQ is designed, a validation study is necessary in a sub-sample of the main study population, and weighed records or several days of FR are among the best reference methods [7].

At this time, there is no standardized methodology for the quantification of AGEs in the diet. Having a reliable and valid FFQ specific for AGEs will increase the accuracy of associations between diabetes and dAGEs; decrease participant burden during studies; and decrease analysis time of food eaten. Thus, a FFQ could help to assess dAGEs in a time and cost saving manner. For this reason, the aim of this study was to design a FFQ using information on usually consumed foods from participants from a previous study [14]. In addition, we assessed the reliability and validity of a food frequency questionnaire for assessing dAGEs when compared with the results of 7 days of FR.

5.2 Methods

5.2.1 Development of the FFQ Specifically for dAGEs

For the development of the FFQ, the dietary data from 30 subjects from a previously published research study were used [14]. These subjects had type 2 diabetes mellitus (DM) and they completed 10 days of FR. We calculated the dAGEs from the 10 days of FR using a database with the AGEs content in food [15]. The mean intake of dAGEs for these subjects was 16372±8898 KU AGEs. In order to identify which foods or beverages were the main contributors of dAGEs and to decide which foods would be included in the FFQ, steps suggested by Block were followed (Figure 5.1) [16].

First, all foods and beverages reported on the 10-day FR along with their amount (g or mL) were entered into Excel (Microsoft, 2011). The objective was to create a comprehensive list of foods consumed by this population as suggested by Block [16]. If a subject reported eating the same food several days or several times per day that food was entered only once, but the total amount (g or mL) for the 10 days were recorded.

After entering all foods in Excel as reported by all subjects, a list of 335 foods along with their amount was compiled.

Second, dAGEs (measured as AGE KU) were quantified using Uribarri's database for content of AGEs in foods [15]. Only 271 food items (out of 335) were quantified for dAGEs, and the remaining 64 foods items were not quantified because no similar food was found in the database or no disaggregation was considered pertinent. The food items that could not be quantified were assigned a value of zero AGE KU. Of these 64 food items, 32 were fruits or vegetables, 8 were different types of sauces (sweet and sour sauce, green sauce), 4 of them were complete dishes and 20 were miscellaneous foods.

Third, similar foods were grouped into food item categories [17]. The objective was to reduce the food list to include in the FFQ. After grouping into categories, the list was reduced to 130 food items. For example, Italian salad dressing and ranch salad dressing were grouped into the food item category "salad dressing", and for cookies all different types were grouped into the food item category "cookies". In addition, if a food had different preparation methods it was also grouped into the same food category, for example "grilled chicken" and "steamed chicken" were grouped into the food category "chicken".

Fourth, the relative contribution for each food item was calculated [16]. For this purpose, the total amount of dAGEs consumed by all subjects was calculated by summing the amount of dAGEs in all servings of all foods reported. The relative contribution for a specific food item was calculated by the following formula, [(total AGEs KU by a specific food/total AGEs KU in all foods)*100] [16].

Finally, food items were ranked by their relative contribution and the cumulative frequency calculated. According to Block, a FFQ should contain 80 or 90 percent of the original food list [16]. For this FFQ it was decided to include foods representing 95% of

the cumulative frequency because we were interested in capturing most of the dAGEs intake of our population. Figure 5.2 shows the 8 foods with higher relative contribution to the amount of dAGEs.

The final FFQ for dAGEs had 90 food items (out of 335), some of them asking for specific cooking methods (meats group). These were divided into 7 groups: cereals, pastries/cookies, snacks, fats, meats, cheese, and combination foods. The frequencies used were similar to those used in the National Health and Nutrition Examination Survey (NHANES) FFQ [18]: never, 1 to 6 times per year, 7 to 11 times per year, once a month, 2 to 3 times per month, once a week, 5 to 6 times per week, 2 to 4 times per week, once per day, 2 or more per day. The questionnaire measured the consumption of food over the last year, and was interviewer-administered.

Regarding portion size, participants were allowed to choose their portion size for each food. For this purpose, Nasco three dimensional food models (Nasco Lifeform®), measuring cups and a portion size booklet were used [19]. The food frequency questionnaire was evaluated for completeness and for ease of use and understandability by an expert panel.

5.2.2 Food Record Questionnaire

The food record questionnaire asked for place, time and amount of food and beverages consumed. In addition, it included prompts to record cooking method and brand of food, when possible. Although our past study of the FR [14] asked participants to record 10 days, analysis of variance supported a shorter time frame of 7 days for the current study. Briefly, the average intake of energy and dAGEs for 10 days was compared with the average intake of the first 7 days and the first 3 days. There was no significant difference when comparing 10 days versus 7 days or when comparing 10 days versus 3 days. However, for the 3 days of FR, 21.5% of subjects were classified in a different tertile category. In contrast, for the 7 days of FR only 3.1% of subjects were

misclassified (SPSS, version 17.0, 2008, Chicago, IL, USA). Therefore, we decided to use 7 days of FR (7-dFR) for the validity of the FFQ.

5.2.3 Reliability and Relative Validity

Reliability was evaluated by repeated completion of the FFQ in a test-retest manner, to determine if the instrument was stable over time. Validity of the FFQ was evaluated with comparison to the 7-dFR, both for total AGEs content and types and amounts of food eaten.

5.2.4 Subjects

This was a convenience sample (n=20), and both men and women with type 2 DM were recruited. They were not included if they were following a protein-restricted, vegan or vegetarian diet. Participants from a previous study [14] were contacted by mail and 7 of them consented to participate in the present study. Additionally, 15 participants were recruited through email bulletins and flyers. Two participants that reported major changes in their diet during the study were excluded. The final sample size was 20 participants. In addition to dietary questionnaires, participants were asked to report their age, years with diabetes, weight and height. Body mass index (BMI) was calculated with the formula weight (kg)/ [height (m)]^2 [20]. All procedures were approved by the Institutional Review Board at the University of Illinois at Urbana-Champaign.

5.2.5 Study Procedures

During a first meeting (T1) and after consent, brief instructions and examples were given on how to complete the FFQ. The FFQ was interviewer-administered and a trained researcher carried out all the interviews. The FFQ did not include portion size, hence the subjects were asked to estimate their usual portion size with aid of food models, measuring cups and a portion size booklet [19]. These data are referred to as FFQ-T1.

During a second appointment (arranged two weeks after the first one), participants answered the interviewer-administered FFQ for a second time (T2) for reliability testing, following the same procedures used during the first interview. At the end of the second appointment, a closed package containing 7 days of FR (7-dFR) was given to the participants. A brief training was given about how to fill out the 7-dFR, such as how to estimate food serving sizes, and to include brand names, if possible, as well as cooking method and cooking time/temperature. The participants were asked to begin completing the 7-dFR one week after their second appointment, to minimize any influence from the food frequency completion. They received an email or phone call (their preferred contact) one day before they were to begin. After participants completed the first day's FR, it was reviewed to assess completeness, by e-mail, or by telephone. During a last appointment, completeness was reviewed for all FR, and subjects were asked to complete any missing items, amount, or preparation method.

5.2.5 Analysis of Questionnaires

Data from the 7-dFR were analyzed using Food Processor software (ESHA Research, 2011) for calories, carbohydrate, fat and protein content. Disaggregation of dishes into single ingredients was done before entering the data on Food Processor. Recipes websites (allrecipes.com, foodnetwork.com) were used to determine ingredients and amount based on what was reported. Nine new foods or recipes were added to Food Processor because initially they were not in the software database. The food composition was obtained either from food package labels on actual packages or on the internet, or adding the recipes by its component ingredients. In addition, in some instances the reported amount was not found in Food Processor and the website *What's In The Foods You Eat Search Tool* [21] was used to decide the amount to be entered in the software.

The compilation of the food composition database was completed using an indirect method [22]. We used the data obtained by Uribarri et al. for AGEs content in foods [15]. Data from the 7-dFR and the FFQ for AGEs (completed twice) were entered in the

AGEs food content Excel database (Microsoft, 2011) for quantification of total AGEs intake from the two sources. The following frequency codes were used for the FFQ [18]; 0 for never, 0.01 for 1 to 6 times per year, 0.028 for 7 to 11 times per year, 0.033 for once a month, 0.08 for 2 to 3 times per month, 0.14 for once a week, 0.29 for twice per week, 0.5 for 3 to 4 times per week, 0.79 for 5 to 6 times per week, 1 for once per day, and 2 for 2 or more per day. The amount of dAGEs was calculated by multiplying the frequency code and the gram or milliliters of food consumed by the AGEs (KU) amount in 100 g of food. Total dAGEs was determined by summing the intakes from each food. The actual database contains around 500 foods, therefore some foods were not found in the database. These foods were calculated with averages from similar foods available in the database. For example, there was no AGEs KU amount for pecans, therefore we used data for walnuts to quantify AGES KU; there was only deli ham, therefore turkey/chicken lunch-meat was entered there; when a meat did not have similar cooking method, a similar meat with the same cooking method was used. All decisions were made by at least 2 researchers and all final analyses were reviewed for any possible mistakes by a registered dietitian.

5.2.6 Statistical Analysis

Normality was assessed by the Shapiro–Wilk test. Skewed data are presented as median and interquartile range. Data on dAGEs from the FFQ-T1 and FFQ-T2 were analyzed and compared for reliability and Shrout-Fleiss intraclass correlation coefficient was used. A high value of this coefficient indicates low within person variation [23]. Data from 7-dFR were analyzed and compared to FFQ-T1 and FFQ-T2 for relative validity by parametric or nonparametric analysis, as appropriate. Spearman correlation coefficient was used because dAGEs data were non-normally distributed. For measuring agreement between questionnaires, the Bland-Altman method was used. Data of dAGEs were log transformed because data were skewed and limit of agreement was calculated as suggested by Bland et al. [24]. Because log transformed data were used, to determine the limit of agreement data were back transformed and reported as ratios. In addition, to measure how well the FFQ classified subjects in tertiles of AGEs

consumption compared to the 7-dFR, the percentage of subjects classified in the same tertile and the percentage of subjects classified in opposite tertiles by the different methods were calculated. The cut-off points for the tertiles were 4000 KU and 15000 KU, thus consumption higher than 15,000 KU was considered high, between 4000 and 15,000 KU was considered moderate and less than 4000 KU was considered low [14]. The weighted kappa statistic was calculated, and Masson et al. criteria were used as a measure of agreement [25]. Subjects classified into the same tertile were assigned a weight of 1, 0.5 for adjacent tertiles and 0 for opposite tertiles. Values of kappa > 0.80 indicate very good agreement, between 0.61 and 0.80 good agreement, 0.41 to 0.60 moderate agreement, 0.21 to 0.40 fair agreement and < 0.20 poor agreement [25]. All statistical analyses were carried out in SPSS (version 17.0, Chicago, IL, USA, 2008). The performance of the FFQ was considered adequate when a correlation coefficient was above 0.5, and 50% of subjects would be classified correctly into the same tertile and less than 10% grossly misclassified into opposites tertile, or weighted kappa values of at least 0.4 were found [25].

5.3 Results

The mean age of the 20 subjects (13 women; 7 men) was 56.6±10.2, years with diabetes were 6.9±4.8, and body mass index (BMI) was 37.4±7.7m^2/kg. Energy and macronutrients intake from 7-dFRs are described in Table 5.1. Data on dAGEs assessed by each questionnaire and the categories of dAGEs by each questionnaire are shown in Table 5.2.

5.3.1 Reliability and Relative Validity

For reliability, the Spearman's correlation coefficient between FFQ-T1 and FFQ-T2 was 0.89 ($p<0.01$), and the intraclass correlation coefficient was 0.98 with 95% CI (0.95, 0.99). These values showed good reliability of the FFQ. For validity, data from 7-dFR were compared to both FFQ-T1 and FFQ-T2 (Table 5.3). The Spearman's correlation coefficient showed good correlation for both FFQs. The weighted kappa showed moderate agreement for FFQ-T1 (0.55) and good agreement for FFQ-T2 (0.64). Both

FFQs had adequate percentages of subjects correctly classified in the same tertile, 75% for FFQ-T1 and 80% for FFQ-T2, and there was no misclassification into opposite tertiles for either of the FFQs. The FFQ-T1 overestimated dAGES by 93.6 KU in comparison to 7-dFR, but there was not a systematic bias as the average and the difference between both methods showed no correlation (R=-0.41, p=0.07). Figure 5.1 shows the Bland-Altman data for FFQ-T1 with 1.96±SD limits of agreement. Values after back transformation were 2 for the upper limit and 0.55 for the lower limit. In contrast, the FFQ-T2 underestimated dAGEs by 464.6 KU in comparison to 7-dFR, but there was not a systematic bias as the average and the difference between both methods showed no correlation (R=-0.16, p=0.5). Figure 5.2 shows the Bland-Altman data for FFQ-T2 with 1.96±SD limits of agreement which values after back transformation were 1.67 for the upper limit and 0.55 for the lower limit. Visual inspection of Bland-Altman data showed that for lower intakes, FFQs at each time measurement overestimated dAGEs. The data can also be interpreted, conversely, that at lower intakes, 7-dFR underestimated intake.

5.4 Discussion

The main aim of the present study was the development of a FFQ to measure the amount of AGEs in the diet of subjects with diabetes. In addition, we were interested in testing the reliability and the relative validity of this instrument. To our knowledge this is the first specific questionnaire design to measure dAGEs. Although researchers may use slightly different methodology in developing and evaluating a new FFQ, identifying the foods to be included and the amounts that will differentiate intake is useful for determining usual intake of a nutrient [13].

The present study found that the FFQ is comparable to 7 days of FR to measure dAGEs. Other studies have used only 3 days of FR. However, according to Block [6], 7 days of FR are better to measure macronutrients, and several studies showed that dAGEs are predicted better by amounts of protein and fat.

This FFQ has 90 food items. It was decided not to design a comprehensive FFQ to diminish burden in study participants, and only food items representing 95% of cumulative frequency were included. Our FFQ was shorter than other questionnaires measuring specific nutrients from the diet [12,22,26], although longer than others [11]. Some authors established that lengthy questionnaires impaired the accuracy of the answers and that when the purpose of the FFQ is very specific a short list of food is recommended [7,27].

Ten frequency categories were used, similar to the NHANES FFQ. This FFQ was developed to be applied in a case-control study of diabetes-related complications and for this reason, measuring past intake was important. An arguable issue on the development of a new FFQ is if to include portion size. For this FFQ, participants described their portion size with visual aids (food models, portion size booklet and measuring cups). A trained interviewer did both interviews for the FFQs, assuring completeness of the questionnaires. A challenge of including a portion size is when participants do not consume the amount of the food as indicated by choices for servings. This represents a problem because subjects have to calculate how often they consume the specified portion size. A review by Cade, et al. [28] showed that correlation coefficients between FFQ and references methods when subjects are allowed to describe their portion size is higher (0.5-0.6) than when a portion size is specified (0.4-0.5). Similarly, correlation coefficients for this study between FR and FFQ-T1 was 0.68 and with FFQ-T2 was 0.8. Our analysis showed that the questionnaire is reliable over time. The correlation coefficient 0.89 and the intraclass correlation coefficient 0.98 showed good reliability and it was higher when compared with other studies [29,30].

Regarding the validation of the FFQ, the dAGEs FFQ showed moderate to good agreement when compared with 7-dFR. The performance of the FFQ was considered adequate according to the criteria of Mason et al. [25], with a correlation coefficient above 0.5, 50% of subjects classified correctly into the same tertile, and weighted kappa

values of at least 0.4. All of these requirements were met on both applications of the FFQ, with the FFQ-T2 having sligtly better scores than FFQ-T1.

There was no agreement concerning which statistical test should be used to measure validation, but using several methods increases the probability that a conclusion is true [25]. The analysis by the Bland-Altman method did not show systematic bias, but the limits of agreement were wide. However, Mason et al. [25] argued that for a FFQ expected to be used in epidemiologcal studies, it is more important that the instrument can classify subjects according to a category of intakes. Our results of cross-classification indicated that our FFQ correctly classified more than 70% of subjects correctly.

The database for this FFQ was compiled from published data, and as research in the field advances, additional food items with AGEs content will be added. At such time, this FFQ may need to be evaluated again as well. However, both the FFQ and the FR were analyzed with the same database which make any error similar on both methods. Other authors have faced similar challenges when attempting to validate a FFQ for compounds in foods not widely studied [22]. Currently, there is only one database with the amount of AGEs in foods, and the foods tested in the FFQ were the most commonly consumed within our population.

A limitation of this study was the sample size. We only included 20 participants. However, it is important to point out that this is the first attempt to develop a FFQ measuring dAGEs, and further validation and review should follow. When biomarkers have been developed to reasonably reflect dAGEs, this also will add to determination of dAGEs role in health and disease. Additionally, this FFQ was designed and validated in a group of subjects with diabetes and mean age of 56.6 years. Before it can be used in another population it will need further validation.

The study of dAGEs in relation to health impacts faces several challenges. One of these is the large number and heterogeneity of AGEs [31], which has made measuring AGEs in foods and comparing data from different research groups difficult. Also, only short-term effects have been measured and long-term effects have not been studied yet. The methodological design of long-term studies represents a great challenge. However, we hope that this FFQ could be used to generate the epidemiological data necessary to anwers the question about dAGEs and diabetes-related complications.

Acknowledgment

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Declaration of interests

The authors declare no conflict of interests.

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Figure 5.1. Development of the Food Frequency for dAGEs.

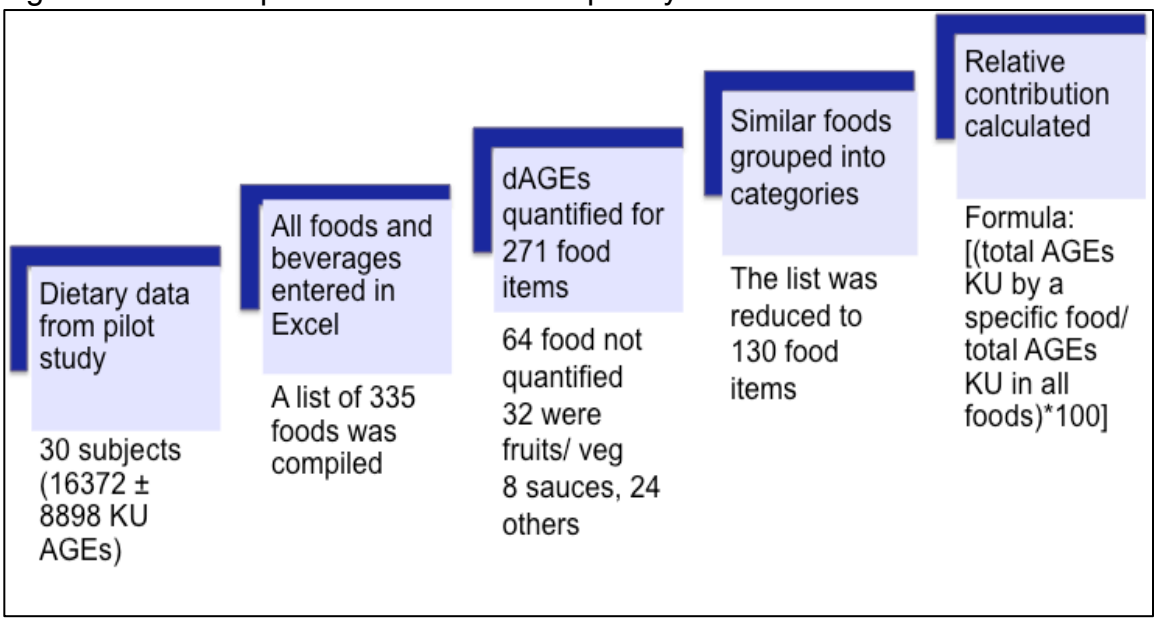


Figure 5.2. Relative Contribution.

Foods	dAGEs	Relative Contribution(%)
1.Chicken	467362	13.6
2.Pizza	401644	11.7
3.Beef	340683	9.9
4.Bacon	317614	9.2
5.Butter	249423	7.3
6.American cheese	129822	3.8
7.Double Hamburger	101725	2.9
8.Sausage	94868	2.8
:	:	:
:	:	:
:	:	:
Total: 130 food items	3427454	100%

Foods representing 95% of cumulative frequency were included on the FFQ.

Table 5.1. Energy and Macronutrients from 7-d FR (n=20)

	Mean±SD
Energy (Kcal) (KJ)	1948±504 8156±2114
Carbohydrates (g)	215±71
Protein (g)	90±36
Fat (g)	81±29
Saturated Fat (g)	28 ± 13

Results are presented as mean±SD.

Table 5.2. dAGEs from 7-d FR and FFQ

	Mean±SD	Median, IR	
dAGEs 7-d FR (KU)	16884±9364	14766 (10671, 18668)	
dAGEs FFQ-T1 (KU)	16978±9085	13629 (11848, 13629)	
dAGEs FFQ-T2 (KU)	16419±9942	12766 (10456, 20673)	
Categories of dAGEs intake	**Low**	**Moderate**	**High**
7-d FR (n)	1	9	10
FFQ-T1 (n)	0	12	8
FFQ-T2 (n)	0	13	7

Results are presented as mean±SD and median and interquartile range.

Table 5.3. Reliability for the FFQ.

	FFQ-T1 vs FFQ-T2
Spearman's correlation	0.89*
intraclass correlation coefficient	0.98**

*($p<0.01$), **CI (0.95, 0.99)

Table 5.3. Agreement between FFQs and 7-dFR

	FFQ-T1 vs 7-d FR	FFQ-T2 vs 7-d FR
Spearman's correlation	0.68*	0.80*
Weight Kappa ± SE (95% CI)	0.55±0.17 (0.23, 0.860)	0.64±0.15 (0.35, 0.92)
Cross-classification		
% classified into same tertile	75	80
% classified into next tertile	25	20
% misclassified	0	0

*P<0.01

Figure 5.3. Agreement between 7-d FR and FFQ-T1 for dAGEs

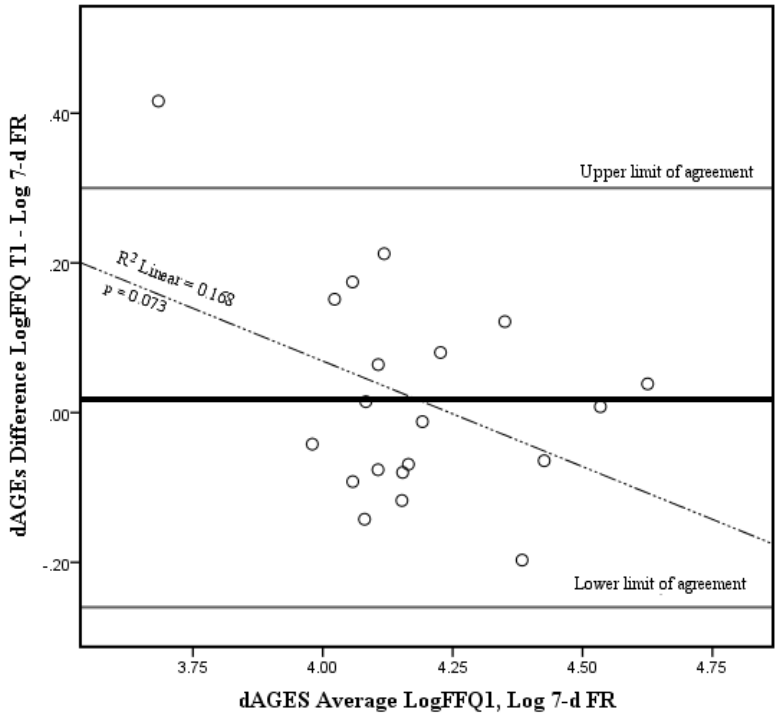
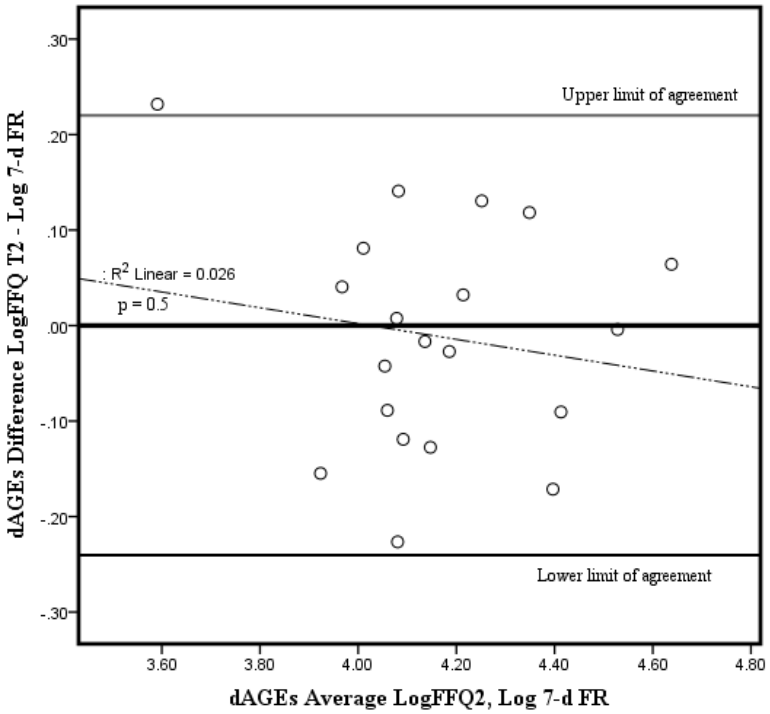


Figure 5.4. Agreement between 7-d FR and FFQ-T2 for dAGEs



Chapter 6
A Case-Control Study of Complications from Diabetes and Intake of Advanced Glycated End Products

6.1 Introduction

Diabetes-related complications classified as microvascular and macrovascular complications represent a health care burden. According to the 2011 National Diabetes Fact Sheet by the Center for Disease Control and Prevention (CDC), in 2004 heart diseases were noted on 68% of deaths and stroke among 16% of diabetes-related deaths. In 2005-2008, 67% of adults with self-reported diabetes had hypertension, and 28.5% had retinopathy. In addition, diabetes is the leading cause of new blindness and the leading cause of renal failure. Regarding nervous system diseases, 60-70% had mild to severe forms of nervous system damage, almost 30% had impair sensation in the feet, and more than 60% of lower limb amputation are due to diabetes [1].

Neuropathy (nervous system damage), nephropathy (renal system damage) and retinopathy (eye damage) can be classified as microvascular complications. On the other hand, cardiovascular disease (heart attack, chest pain, coronary heart disease, congestive heart failure), stroke, and peripheral vascular disease (leading to injuries, gangrene and amputation) can be classified as macrovascular complications [2]. Risk factors for microvascular and macrovascular are chronic exposure to hyperglycemia, age, years with diabetes, smoking, elevated triglycerides, and high BMI [2-4].

Hyperglycemia in diabetes leads to damage of vascular cells by non-enzymatic glycation (AGEs formation) and oxidative stress. High levels of glucose and subsequent autooxidation increase production of the AGEs precursors α-dicarbonyls (methylglyoxal, 3-deoxyglucosone, and glyoxal). These α-dicarbonyls are very reactive and they form AGEs independently of HbA1c formation. In people prone to diabetes-related complications, dicarbonyl and oxidative stress are selectively activated, increasing glycated proteins and lipids byproducts, but not hemoglobin A1c (HbA1c) changes [5].

Some studies have shown elevation of serum AGEs in patients with diabetes-related complications [6]. For instance, serum AGEs were found to be significantly elevated in diabetic patients with coronary heart disease (CHD), (8.1 U/mL) vs diabetic patients without CHD (7.1 U/mL) [7]. Furthermore, AGEs levels increased with the progression of complications in subjects with diabetes mellitus (DM) [8].

In addition to endogenous AGEs, several studies have pointed also to dietary AGEs as a modulator of vascular damage [9-11]. In addition, data from several intervention studies indicate that the high intake of dietary AGEs contributes to tissue damage and increased levels of inflammatory markers that can be prevented by dietary AGEs restriction [12-15]. These results suggest that dietary AGEs could exert similar effects as those studied from endogenous AGEs at least through accumulation in the body in chronic degenerative diseases, particularly in diabetes and renal diseases. Furthermore, results from our research group pointed out that subjects with a moderate-high risk for cardiovascular disease (presence of cardiovascular disease, hypertension and hyperlipidemia) had a higher intake of dAGEs than those without risk for cardiovascular disease [16]. For this reason, the objective of this research was to investigate whether there was an association between different categories of AGEs intake and cardiovascular complications in patients with DM.

6.2 Methods

This was an observational study; in particular a case-control study to compare patients with DM that have diabetes-related complications (cases) to those with no complications (control). Our primary outcome was cardiovascular disease complications, but as secondary outcomes other complications were recorded.

6.2.1 Sample Size

A power analysis was conducted with data from a previous work on mean AGEs intake between participants with presence of cardiovascular disease complications and without it, and it was found that in order to achieve 80% power, alpha 0.05, a sample size of 42

participants was needed, 21 with diabetes complications and 21 without complications. This allowed for 2 participants to drop out, which is below the drop-out rate in the previous study (2 out of 30).

6.2.2 Subject Characteristics

Subjects with DM were recruited (n=42) through a primary care clinic, an endocrinology clinic, through key informants, and through a general email flyer to University faculty and staff. Non-Hispanic whites subjects older than 18 years were included. They had to have had type 2 DM within 2 to 20 years since diagnosis. For the cases group, subjects needed to have a diagnosis of any of the following cardiovascular complications: myocardial infarction, angina, coronary angioplasty, heart failure, cardiovascular bypass, stroke, transient ischemic attack, claudication, peripheral vascular disease. For the control group, they needed to have no cardiovascular complications. They were excluded if they were smokers (another source of exogenous AGEs), if they were pregnant or breastfeeding women (dietary changes due to condition), if they were vegan (strict vegetarian) or participants with a protein-restricted diet (would affect dietary AGEs), or if they were blind (self-report), cannot read (self-report), or not oriented to time and place as assessed by three questions. Also, they were excluded if they did not complete the seven days food record or if they failed to complete the second interview.

6.2.3 Study Variables

Dependent Variables

Our main outcome variable was cardiovascular complications: myocardial infarction, angina, coronary angioplasty, heart failure, cardiovascular bypass, stroke, transient ischemic attack, claudication, and peripheral vascular disease. The subject's primary care physician evaluated the subject for the presence of these complications. After the subject's consent, the subject's clinical history was obtained from the health care provider as well as results of a complete lipid profile (total cholesterol, low density lipoproteins [LDL-cholesterol], and triglycerides).

As a secondary outcome, other complications such as eye disease and renal disease were recorded. The subject's primary physician also evaluated the subject for the presence of these complications. In addition, urine albumin and proteins lab results were requested, if available, to assess for renal disease complications.

Independent Variable

The exposure variable was the intake of dAGEs. We measured actual AGEs intake by seven days of dietary record and past intake by a food frequency questionnaire (FFQ).

The AGEs intake was categorized in tertiles of consumption: low, moderate or high intake. For the AGE intake categories, we reviewed six previous clinical studies as described previously [16] evaluating the effects of high and low dAGEs with the purpose of developing a classification of AGEs intake. Consumption higher than 15000 KU was considered high, between 4000 and 15000 KU was considered moderate and less than 4000 KU was considered low.

For the analysis of dAGEs, a published database was used with approximately 500 foods with their AGEs content [33]. Foods from food records were grouped into food categories, then, matched with specific foods from database with AGEs content. Foods with no specific match were estimated from similar foods from mean values. For the quantification of micro and macronutrients we used a commercial software *Food Processor* (ESHA Research, 2011).

In our previous pilot study [16], 10 days of food records were used. However, after statistical analysis, we found that recording 7 days is sufficient for classifying the different AGEs categories. A FFQ validated for AGEs intake was used to measure past intake of AGEs. Additionally, subjects were asked if they had had significant changes in their diets in the last six months, last year or since diagnosis of diabetes.

6.2.4 Research Procedures

Health care providers at Carle Foundation Hospital (Urbana, IL) gave an invitation letter (Appendix D, Figure D.1) to appropriate potential candidates. If potential participants were interested, they contacted the investigators through email or phone. In addition, participants were recruited through flyers at diabetes support groups and online bulletin (eweek). The inclusion/exclusion criteria were reviewed through email or phone before scheduling an appointment with potential participants (Appendix D, Figure D.2).

If they met the criteria and were interested, a first appointment was scheduled for the person to meet with the researcher to review and sign the consent. This first appointment took around one hour. Potential participants read the consent, and it was explained to them that if they agreed to participate they needed to confirm a release for some information from their medical records. This information was related to cardiovascular complications: myocardial infarction, angina, coronary angioplasty, heart failure, cardiovascular bypass, stroke, transient ischemic attack, claudication, and peripheral vascular disease. Participants recruited through eweek or diabetes support groups were asked to obtain this information from their online medical records when available.

After signing the consent, participants received instructions about how to answer a food frequency questionnaire (Appendix C, Figure C.4). Participants filled out the FFQ (45 minutes). Then, participants received training about how to fill out the food records. For the training, food models, a food portion booklet and measuring cups were used to increase the accuracy of the amounts of food that they reported. The importance of reporting cooking methods and brand of foods when possible was emphasized. They were given written instructions, a food portion booklet and food records forms for 7 days (Appendix C, Figure C.5 and C.6). They also received two additional surveys to fill out at home (Appendix B, Figure B.8, and Figure B.10).

Staff at Carle was notified when the referral was interested and was requested to fill out the Diabetes Complications Data. They reviewed the medical record for information about the cardiovascular complications related to DM, as well as a list of diabetes-related medications, including those related to cardiovascular, renal, eye, or peripheral conditions and as well as results of a complete lipid profile (total cholesterol, LDL-cholesterol, VLDL- cholesterol and triglycerides), and urine albumin and proteins lab results were requested, if available, to assess for renal disease complications. (Appendix D, Figure D.4). When participants did not receive their health care at Carle, they were asked to access their online medical records to provide their information regarding clinical data or to confirm with their physicians for the requested information. During recruitment, controls were matched to cases based on similar years since DM diagnosis.

After subjects wrote down the first day's food record, the investigator reviewed it for completeness, and to increase the likelihood that all foods were included, cooking methods, and portions, and to answer any questions the participants may have. This was done by phone or by e-mail.

A last appointment was scheduled after subjects finished recording their 7 days of food records and the other surveys. In this last meeting, the investigator reviewed every food record and asked participants for specific details when needed, and for any clarification of cooking method or portion size or brand name. Participants received remuneration ($50) upon completion of all questionnaires.

Statistical Analysis

Descriptive and categorical analysis of data, as well as logistic regression were used to find the odds of cardiovascular complications presence with high intake of dietary AGEs as the independent variable (SPSS). In addition, the weighted odds ratio will be calculated with the Mantel Haenszel method.

Smoking, years since diagnosis, HbA1c levels, some medications (metformin, lipid lowering medications) and antioxidants could be confounders for this study. Smoking was controlled at the design level by restricting participants that smoke. Cases and controls were matched by years since diagnosis and age. Levels of HbA1c, medications and antioxidants will be adjusted at the analysis phase.

In order to address selection bias both groups had similar access to health care services. The interviews to obtain the dietary information and the quantification of AGEs on the diet were carried out by the researcher following established methodologies to minimize bias.

Strengths

Some of the strengths of this study were that was efficient and less expensive in comparison to a cohort study. In addition, we can assess several risk factors along with dietary intake of AGEs.

Limitations

This is a retrospective study and we should have caution with inference of a temporal relationship. This is a small population sample and we could risk having no statistical significance for our results. The results of this study are not generalizable, because we are studying non-Hispanic whites.

Preliminary Results

To date, we have data for 27 subjects, 15 controls and 12 cases. For the control group, there are 10 female and 5 males and for the case group there are 8 females and 4 males. The mean and standard deviation for demographic characteristics and biochemical parameters are shown in Table 6.1. Preliminary data on dietary AGEs and dietary variables are shown in Tables 6.2 and 6.3. A t-test of these variables showed no significant differences between the group of controls and the group of cases. However, dietary AGEs was slightly higher in the cases, and this could indicate the trend for the

total sample size. For this study, cases were classified according to their presence of any of the following cardiovascular diseases: myocardial infarction, angina, coronary angioplasty, heart failure, cardiovascular bypass, stroke, transient ischemic attack, claudication, and peripheral vascular disease. It was decided not to include hypertension or dyslipidemias for classification purposes, but rather registered their presence and if pertinent adjust at the final analysis. For the group of controls, 67% of them had hypertension; in contrast, 91% of the cases had hypertension.

It was important to ask for past and recent changes in the diet and it was found that 20% reported changes in the last year and 73% since diagnosis for the control group and 17% for last year and 67% since diagnosis for cases. Subjects were classified by AGEs intake as previously described [16] as having a high intake, moderate or low intake, and their distribution by group is shown in Table 6.4. None of the subjects were classified as having a low intake. Therefore preliminary calculation of the odds ratio was done for high and moderate intake of AGEs. It was found that the odds ratio was 4, and after adjusting for years since diagnosis was 7.3 with a p-value of 0.057.

For our final analysis, in addition to the dietary variables shown on Table 6.2, simple sugars and antioxidants from food records will be reported and analyzed to explore potential detrimental or protective role on diabetes-related complications. Other information to include will be information related to lipid lowering medications.

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Table 6.1 Characteristics by Group.

	Control n=15		Case n=12	
	Mean	Standard Deviation	Mean	Standard Deviation
Year with DM	8.7	4.4	10.9	6.9
Age	57.8	11.6	58.5	10.5
BMI (kg/m^2)	38.1	10.9	38.9	7.1
HbA1c (%)	7.7	1.4	7.8	1.5
Fasting Glucose (mg/dL)	154.5	48.7	156.5	63.7
Triglycerides (mg/dL)	137.1	68.3	221.1	130.8
Cholesterol (mg/dL)	176.0	47.3	162.9	57.8
Creatinine (mg/dL)	0.93	0.44	1.0	0.5

t-test showed no significant differences between groups.
DM=diabetes mellitus; BMI=body mass index; HbA1c=hemoglobin a1c

Table 6.2 Dietary Variables by Group.

	Control n=15		Case n=12	
	Mean	Standard Deviation	Mean	Standard Deviation
Dietary AGEs (AGE KU)	15299	5183	21208	11346
AGEs density (AGE KU/Kcal)	8.6	3.5	10.7	5.5
Kilocalories (Kcal)	1947	633	2038	689
Carbohydrates (g)	207	81	204	53
Protein (g)	86	28	97	49
Fat (g)	88	40	93	39
Saturate fat (g)	30	17	33	17

t-test showed no significant differences between groups.

Table 6.3 Intake of dAGEs in Cases and Controls

Dietary AGEs	Case	Control	Total
High Intake	6	3	9
Moderate Intake	6	12	18
Total	12	15	27

Odds ratio was 7.3 after adjusting for years since diagnosis (p=.057).

Chapter 7
Conclusions and Future Directions

Longitudinal studies have shown that glycemic control is the best strategy to delay complications in diabetes [1]. However, recent large clinical trials [The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, the Action in Diabetes and Vascular Disease (ADVANCE) study, and the Veterans Affairs Diabetes Trial (VADT)] with aggressive treatment to normalize hyperglycemia (HbA1c lower 6.5%) have shown no significant decrease in incidence of cardiovascular events or in the rate of death from any cause [2]. The overall results from these clinical trials show that glycemia control decreases retinopathy risk better than cardiovascular disease risk. Targeting glycemia only lowers risk for cardiovascular disease in a modest way [3].

This evidence has prompted researchers to discuss the need for new biomarkers that could predict diabetes-related complications more accurately, for instance, the endogenous AGEs (CML). A review by Beisswenger discussed evidence from studies in subjects with diabetes-related complications showing that AGEs can be formed independently from HbA1c formation. The argument about how AGEs could be better markers for diabetes-related complications over time is also discussed [4].

The strongest data supporting this argument comes from an epidemiological study, the Joslin 50-year medalist study on subjects with Type 1 diabetes with more than 50 years with diabetes. They assessed retinopathy, neuropathy, nephropathy and cardiovascular complications (clinical data only). A lack of correlation between current HbA1c or long-term HbA1c (average of 20 measurements over a period of time) and complications was found. In contrast, they found that subjects with high levels (above the media) of plasma CEL (carboxyl-ethylysine) and pentosidine were 7.2 fold more likely to have any complication [5].

Similar to these results, our first study did not show a relation between HbA1c and risk

of complications. Our results showed that HbA1c was slightly high but not statistically different for Mexicans (8.2±1.8%) than for non-Hispanic whites (7.1±1.2%). However, non-Hispanics whites had a higher rate of cardiovascular risk than Mexicans. In addition, risk for diabetes-related complication could not be explained by HbA1c in the regression analysis [6]. However, a limitation of the study was that we did not measure endogenous AGEs to see if they explained better the differences in the rate of complications than HbA1c. Future studies should explore if there is a correlation between the amount of dAGEs estimated from dietary questionnaires with AGEs measured in serum or other tissues. In addition, preliminary results from our case-control study suggest no difference in values of HbA1c between cases and controls.

How AGEs damage tissues is still not completely understood. However, it is proposed that microvascular damage in the kidney, retina, and microvasculature of peripheral nerves could occur when endothelial cells are damaged with subsequent capillary occlusion, ischemia and organ damage [7]. Regarding cardiovascular complication, the proposed mechanisms are additional cross-linking on collagen by glycation of its free amino acids (collagen-AGEs cross-linking will produce stiffness of blood vessels), reduction of low-density lipoproteins (LDL) uptake by cell receptors, and reduction of nitric oxide synthase half-life in the endothelium [8]. This could explain some of the cardiovascular changes associated with the cardiovascular diseases seen in diabetes, such as vascular stiffening, diastolic dysfunction and endothelial dysfunction.

In addition to endogenous AGEs, the role of dietary components on diabetes-related complications should also be discussed. For instance, it is well established that high fat leads to a reduction in flow-mediated vasodilatation (FMD) of the brachial artery, a standard test for *in vivo* endothelial function in humans [9]. More recently Urribarri et al. and Negrean et al. demonstrated that dietary AGEs could also alter FMD. In these studies it was found that FMD of the brachial artery decreased after a single challenge with dietary AGEs and inflammatory markers, such as VCAM-1, increased [10,11]. Long-term effects of dietary AGEs on FMD have not been studied, but it is possible that

a prolonged exposure could provoke permanent damage of vascular tissue. Indeed, a clinical study carried out during 6 weeks found that LDL in the group with high AGEs intake was more glycated than the low AGEs intake [12]. Glycated LDL has been proposed as one of the mechanisms for developing atherosclerosis [13]. The longest intervention on the effects of dietary AGEs (4 months) showed that patients assigned to the low AGEs diet had lower levels of serum CML, methylglyoxal, 8-isoprostanes (a lipoxidation marker) and insulin in serum, as well as a lower homeostatic model assessment (HOMA) when compared with the subjects with the regular AGEs intake (around 20 equivalent of AGEs, measured by 3 days of dietary records) [14]. Our results from a pilot study showed that subjects with higher intake of AGEs have a higher risk level for cardiovascular disease. Dietary AGEs better explained a high level of risk for cardiovascular disease than other variables from the diet (saturated fat) or any other variable studied [6]. In addition, our preliminary results for the case-control study measuring dietary AGEs for the last year point to a higher intake of AGEs in the group of cases and an odds ratio that could be indicative of negative association between cardiovascular diseases and dietary AGEs.

Although a role for dietary AGEs in diabetes-related complications seems supported by the literature, there is yet no agreement among researchers working with dietary AGEs about important aspects of this area of research. For instance, *in vitro* cellular studies could help elucidate if intracellular signaling of AGEs interferes with the insulin pathways as MAPK and PI-3K. This could explain why some studies have found improvement in insulin resistance after a diet low in dAGEs [14]. In addition, at least three different areas also demand further research: 1) absorption, metabolism and accumulation of dietary AGEs; 2) additional analytical quantification of AGEs in foods; and, 3) measurement of dietary AGEs from food questionnaires.

The first area of research, absorption, excretion and metabolism studies have been broadly described before [15]. However, it is important to discuss a recent study by Roncero-Ramos et al. addressing accumulation of dietary AGEs in an animal model.

They fed rats with different fractions and amounts of CML for 88 days [16]. It was found that the heart and tail tendons of rats had higher concentration of CML in the group with the higher intake of CML. The correlation between CML intake and the concentration in the heart was 0.41 ($p=0.02$). Despite the use of an animal model, these results support that high intake of dietary AGEs could result in their accumulation in target tissues affected by diabetes-related complications in healthy individuals. Further research supporting this evidence needs to be done in a diabetes mellitus animal model and also the length of this accumulation should be addressed.

The second area of research, the analytical quantification of AGEs in foods is one of the most highly discussed among research groups. The heterogeneity of AGEs has made measuring them in foods and comparing data from different research groups difficult. Uribarri et al. used an immunohistochemical technique to quantify AGEs using arbitrary units (AGEs KU) for CML amount in foods. However, according to Zhang et al, high performance liquid chromatography and gas chromatography coupled with fluorescence, flame ionization or with mass spectrometry are more specific and sensitive methods for AGEs quantification [17]. For our study, we used the database published by Uribarri et al. [18]. Despite the use of arbitrary units and that comparison could not be made with other foods, this database have the larger amount of foods with content of AGEs. Food scientists need to standardize the methodologies for analytical quantification of dietary AGEs. Until then, Uribarri et al. database [18] represents a useful tool for estimating AGEs.

The last area of research needing further attention is the estimation of dietary AGEs from food questionnaires. Measuring dietary variables has always been an approximate task since bias from the subject reporting the diet and bias from the observer exists. Measuring dietary AGEs adds difficulty to the task since dietary AGEs highly depend on cooking methods. The work from this dissertation contributed most to this issue. Previous studies failed to describe how the evaluation of AGEs from food records was

analyzed. Our study of the food frequency questionnaire explains in detail how this was done, and our proposed food frequency questionnaire could help to facilitate this task.

It is true that long-term clinical studies are needed to corroborate the role of dietary AGEs on diabetes-related complications, however long-term dietary interventions always face compliance issues. For this reason, epidemiological observational studies could offer a good alternative for assessing the health impact of dietary AGEs especially in subjects with diabetes. Case-control studies addressing dietary exposures are always difficult to carry out because of a high possibility of recall bias. However, with outcomes having long latent periods, cohort studies are difficult to carry out and case-control are a good alternative to explore diet and disease interactions. For this reason, a case-control study was suggested for this work. One of the limitations of this study is its retrospective nature. Additionally, since diet is a cornerstone of diabetes subject's treatment, people with diabetes may have changed their diets after diagnosis or after a complication is diagnosed. We were aware of this potential limitation and we asked about diet changes in the last year and since diagnosis of diabetes. Preliminary data showed no great difference between groups, however changes in diet for cases could hinder a relation between dAGEs and complications in this study. It seems from our preliminary results that controls have a lower intake of AGEs and the preliminary analysis indicates an odds ratio of 7.4 with a p value of 0.057. Despite its limitations, this study could support a proposal for a larger scale epidemiological study to corroborate these results. The best study design for doing this could be a cohort study at different stages of diabetes, but with subjects without complications, and as endpoint any diabetes-related complication. This could allow studying the influence of dAGEs before and after the onset of diabetes.

In summary, this work found increased intake of dietary AGEs by subjects with diabetes with higher risk for cardiovascular disease and a possible negative association between dietary AGEs and diabetes-related cardiovascular complications. In addition, the development of the FFQ for dAGEs could help standardize data reported by other

researchers when measuring dAGEs in the diet. Recommendations regarding dAGEs still cannot be made because more research is needed. However, the health impact of dAGEs should not be ignored, especially in susceptible populations (subjects with diabetes and kidney disease).

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Appendix A
Additional Results for Chapter 4

This appendix includes data that were not part of the published manuscript, however it is important to report these results. Previously, it was shown that body mass index (BMI) was statistically different between Mexicans and non-Hispanic whites. An analysis by gender was also carried out to analyze if the differences in BMI were due to gender only (Table A.1). It was found that BMI in males is similar among groups, while BMI in females was different only between Mexicans and non-Hispanic whites.

Metformin intake and smoking could be confounders for this study; hence a brief description of its distribution among all participants is shown in Table A.2.

Participants in the moderate-high risk level for cardiovascular disease had slight lower levels of HbA1c (7.9±1.8%) when compared with those in the low risk level (8±1.9%), but not statistically significant.

Regarding the relationship of complications and dietary AGEs, only cardiovascular complications were associated with dietary AGEs (LogAGEs). The odds ratio found was 3.01 with the following confidence intervals (1.1, 8.4) (X^2=10.9, p=0.027 R^2=0.24).

Several variables have been reported as risk factors for cardiovascular disease, including smoking, BMI, hemoglobin a1c (HbA1c), and gender. In addition, metformin intake could have a role in decreasing levels of AGEs. For this reason, logistic regression was used to explore an independent relation for each of these variables with cardiovascular complications, but no associations were found.

Finally, the effect of gender was also of interested and for this reason logistic regression was used to look for an association between dAGEs after controlling for gender. Results are shown on Table A.3.

Additional results for dietary variables (Table A.4), health care variables (Table A.5) and the percentage of moderate-high risk cardiovascular disease, eye disease and peripheral disease (Figure A.1) are also shown.

Table A.1. BMI Results by Gender.

	Mexicans	*Mexicans-Americans*	*Non-Hispanic Whites*
Males	n=9	n=8	n=8
BMI (kg/m^2)	29.1 ± 7.1	31.6 ± 4.4	35.7 ± 11.8
Females	n=26	n=7	n=7
BMI (kg/m^2)	29 ± 4.1*	33.2 ± 9.6	36.8 ± 4.4*

Results are presented as mean±SD. One-way ANOVA used for differences among groups. Same symbols show differences between groups ($p<0.05$).

Table A.2. Metformin and Smoking Percentages.

	Frequency
Metformin	72%
Current or past smokers	34%
Current smokers:	12.3%
Less than 10 cigarettes per day	9.2%
More than 10 cigarettes per day	3.1%

Table A.3. Logistic Regressions for Cardiovascular Disease Complications.

	β (SE)	*Odds Ratio*	*95% Confidence intervals*
Constant	-8.352 (4.8)		
LogAGEs	1.07 (0.6)*	2.9	(1.01, 8.5)
Gender	0.12(0.7)	1.1	(0.3, 4.5)

$X^2=5.2$, $p=0.07$ $R^2=0.11$

Table A.4. Dietary Variables.

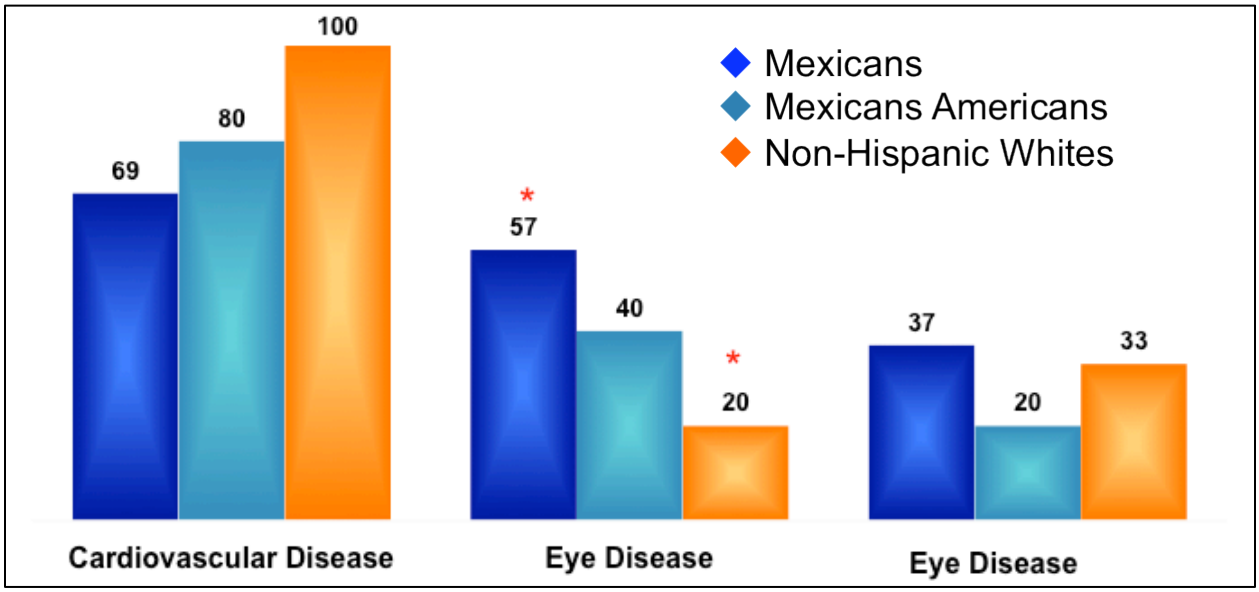
	Mexicans (n=35)	*Mexicans-Americans (n=15)*	*Non-Hispanic Whites (n=15)*
Dietary AGEs (KU)	6303 ± 2842 *	12947 ± 7940 †	19798 ± 8707 †
Log Dietary AGEs¶	8.6 ± 0.5 *	9.3 ± 0.5 †	9.8 ± 0.4 ‡
Energy intake (Kcal)	1575 ± 330 *	1821 800 *	1984 ± 582 †
Carbohydrates (g)	217.5 ± 53.1*	214 ± 80 *	226.2 ± 67.4*
Protein (g)	72.4 ± 22.2*	84 ± 41*	85.9 ± 21.1*
Fat (g)	54.1 ± 15.1 *	71 ± 40 *	83.2 ± 31.2 †
Saturated fat (g)	13.5 ± 4.2 *	22 ± 12 †	27.7 ± 11.2 †

Results are presented as mean±SD. ¶Natural logarithm transformation used because skewed data. One-way anova used for differences among groups. Different symbols show differences between groups ($p<0.05$).

Table A.5. Health Care Variables.

	Mexicans (n=35)	Non-Hispanic Whites (n=15)	Mexicans in Illinois (n=15)
Ophthalmologic visits			
Within the last 12 months (%)	37.1*	80*	60
More than 1 year (%)	31.4	13.3	20
Never (%)	31.4	6.7	20
Diabetes Educator visits			
Within the last 12 months (%)	68.6	33.3	26.7
More than 1 year (%)	5.7	60	33.3
Never (%)	25.7	6.7	40
Dietitian Visits			
Within the last 12 months (%)	65.7	33.3	33.3
More than 1 year (%)	8.6	53.3	40
Never (%)	25.7	13.3	26.7

Figure A.1. Percentage of Moderate-High Risk Cardiovascular Disease, Eye Disease and Peripheral Disease.



Appendix B
Questionnaires for Chapter 4

This appendix includes questionnaires in English and in Spanish used for the study "The Relationship Between Dietary Advanced Glycation end products and Indicators of Diabetes Severity in Mexicans and non-Hispanic Whites: A Pilot Study" presented on chapter 4. The material in this appendix includes Recruitment Material, Screening Tool, Inform Consent, Diabetes History Questionnaire, Vitamins and Supplement Intake and Food Records.

Recruitment material

Figure B.1. Recruitment Post on Eweek.

Diabetes, food-record study seeks participants

If you have diabetes, are 18 or older, and are willing to keep food records for 10 days, you could partipate in our study and receive $100. Especially seeking Hispanic adults from Mexico. Contact Claudia Luevano Contreras at luevano1@illinois.edu or 244-6281.

Figure B.2. Recruitment Flyer.

WE NEED PARTICIPANTS FOR OUR STUDY ON DIABETES MELLITUS COMPLICATIONS AND DIET

WE INVITE YOU TO PARTICIPATE:

- IF YOU HAVE DIABETES MELLITUS TYPE 2
- IF YOU ARE 18 YEARS OR OLDER,
- IF YOU ARE NON-HISPANIC WHITE OR MEXICAN AMERICAN OR MEXICAN, AND
- IF YOU ARE WILLING TO WRITE DOWN WHAT YOU EAT FOR 10 DAYS

YOU WILL GET A COMPENSATION OF 100 DOLLARS IF YOU WRITE DOWN FOR 10 DAYS WHAT YOU EAT.

If you are interested, please contact Claudia Luevano: luevano1@illinois.edu, 217-721-2757, 217-244-6281

Figure B.3. Recruitment Flyer in Spanish.

NECESITAMOS PARTICIPANTES PARA NUESTRO ESTUDIO DE COMPLICACIONES EN DIABETES MELLITUS Y EL TIPO DE ALIMENTACIÓN

TE INVITAMOS A PARTICIPAR SI:

- TIENES DIABETES MELLITUS TIPO 2
- ERES MAYOR DE 18 AÑOS
- SI ERES MEXICANO O MEXICO-AMERICANO

El estudio consiste en que registres tu consumo de alimentos por 10 días. Recibirás ***100 dólares como compensación*** por tu participación.

Si estas interesado contacta a Claudia Luevano: 217-721-2757 o 217-244-6281 , Luevano1@illinois.edu

Screening Tools

Figure B.4. Screening Tool.

Are you 18 or older?
____ yes [continue] ____No [thank but cannot continue]
Are you either non-Hispanic white or Mexican American or Mexican?
____ yes [continue] ____No [thank but cannot continue]
Do you have type 2 diabetes?
____ yes [continue] ____No [thank but cannot continue]
Have you had your blood glucose or hemoglobin A1c measured in the past 3 months?
____ yes [continue] ____No [thank but cannot continue]
Do you know what that number was, and will you share that number with us?
____ yes [continue] ____No [thank but cannot continue]
Are you a vegan?
____ yes [thank but cannot continue]____No [continue]
Are you following a protein restricted diet for any reason?
____ yes [thank but cannot continue]____No [continue]
Are you pregnant or breastfeeding?
____ yes [thank but cannot continue]____No [continue]
Are you willing to write down what you eat for a 10 day timespan?
____ yes [continue] ____No [thank but cannot continue]
Do you have a phone, can you meet with one of the investigators, or can you email your first day's record so we can review any problems with the completeness of the record?
____ yes [continue] ____No [thank but cannot continue]
Where can we send your information packet, or when can we meet to review? The information packet includes all the surveys and 2 copies of the informed consent. One copy is for you to keep. One copy needs to be mailed back to us or delivered to one of the investigators.

Figure B.5. Screening Tool in Spanish.

¿Eres mayor de 18 años?
Si___ No___ (gracias pero no puedes continuar)
¿Eres Mexicano o Mexico-Americano?
Si___ No___ (gracias pero no puedes continuar)
¿Tienes diabetes mellitus tipo 2?
Si___ No___ (gracias pero no puedes continuar)
¿Te han medido tu glucosa en ayunas o hemoglobina A1c en los últimos tres meses?
Si___ No___ (gracias pero no puedes continuar)
¿Sabes los resultados y los compartirías con nosotros?
Si___ No___ (gracias pero no puedes continuar)
¿Eres vegetariano?
Si___ No___ (gracias pero no puedes continuar)
¿Estás embarazada o amamantando?
Si___ No___ (gracias pero no puedes continuar)
¿Estás dispuesto a escribir todos los alimentos que consumas por un período de 10 días?
Si___ No___ (gracias pero no puedes continuar)
¿Tienes teléfono o un correo electrónico para que los investigadores puedan revisar el registro de alimentos del primer día para ver que este completo?
Si___ No___ (gracias pero no puedes continuar)

Inform Consents

Figure B.6. Inform Consent.

Title of Project: Relationship between certain food or parts of foods and diabetes

Responsible Principal Investigator: Dr Karen Chapman-Novakofski, University of Illinois, Urbana-Champaign; Dr Eugenia Garay, Institute of Medical Research, Leon, Mexico

1. **Purpose of the Study:** Certain foods, or their components like protein or fat, may be related to how well diabetes is controlled. We want to see if foods that people eat, or how they cook those foods, may be related to how well their blood glucose is controlled.
2. **Procedures to be followed:** After you have been screened for eligibility in this project, you will be briefly trained on how to complete a food record (5-10 minutes). This training can be in person, by phone or through email. Basically we will read through the food record forms with you to make sure you understand all parts. You will be given 10 days worth of food record forms, a survey about supplements, and a survey about yourr past health. You may receive these forms in person, mailed with a phone discussion, or via email. After you complete the first days' food record, you will be asked to send by email, regular mail, or fax that first day and to talk to an investigator about the record. This will be a review for completeness, and help you complete the forms in as much detail as we need for the additional 9 days. If the record is not reviewed with an investigator, you cannot be included in the study- your records will not be used and if you were to receive compensation you will not. All 10 days must be recorded but they do not need to be consecutive if a special occassion or an unusal eating day arisies.

3. **Discomforts and Risks and Costs:** You may participate in this project if you live outside of the area code (phone) of the investigator, but if you do not have email you may have to leave a message and be called back to review training and the first days' record. If you do not have access to the internet, you may have to use your phone. If you pay for internet service, you may have a charge to participate. If you choose to visit the investigator in person, you may have transportation costs. There are no other risks outside of those you may encounter in everyday life.

4. **Benefits:** There are no personal benefits to you participating in this study. However, participating may help us look at foods and cooking methods and whether they are linked to higher blood glucose or hemoglobin A1c levels or complications of diabetes. This information could eventually help many people achieve better blood glucose control and prevent or delay complications of diabetes.

5. **Statement of Confidentiality:** Your forms will be coded. A key linking your code to your name will be kept by the investigators in a locked cabinet until all the surveys have been completed. The identifying key will then be shredded. Your consent form will also be kept locked in the investigator's office. When the results of this research are published, no personally identifiable information will be shared. The results will be shared at professional meetings, and in professional journals.

Figure B.6 (cont.)

6. **Whom to contact:** Please contact Dr. Karen Chapman-Novakofski [217-244-2852, kmc@illinois.edu] if you are in Illinois or Dr. Eugenia Garay [marugaray_2000@yahoo.com] if you are in Mexico with any questions, or concerns about the research. You may also contact Dr. Karen Chapman-Novakofski or Dr. Eugenia Garay if you feel you have been injured or harmed by this research. If you have any questions about your rights as a participant in this study, please contact the University of Illinois Institutional Review Board at 217-333-2670 or via email at irb@uiuc.edu.

7. **Compensation:** If you complete all of the forms and records completely, you will receive $100.
8. **Voluntariness:** Your participation is voluntary. You may discontinue at anytime without any penalty. The decision to participate, decline, or withdraw from participation will have no effect on future relations with the University of Illinois.

- I am 18 years of age or older.
- I have read and understand the above consent form and voluntarily agree to participate in this study.
- You will be given a copy of this consent form for your records.

_____ _____

Participant Signature Date

Figure B.7. Inform Consent in Spanish.

Titulo del Proyecto: Relación entre ciertos alimentos o partes de alimentos y diabetes

Investigador Principal Responsable: Dra. Karen Chapman-Novakofski, Universidad de Illinois, Urbana-Champaign; Dra. Ma. Eugenia Garay, Instituto de Investigaciones Médicas, León, México.

1.- Propósito del estudio: Ciertos alimentos, o sus componentes como las proteínas o grasas, pueden estar relacionadas al buen control de la diabetes. Nosotros queremos ver si los alimentos que la gente come, o la manera en que cocinan sus alimentos, puede estar relacionados al buen control de la glucosa.

2.- Procedimientos a seguir: Después de que usted halla sido seleccionado en este proyecto, usted será brevemente entrenado en como completar el cuestionario de alimentos (5-10 minutos). Este entrenamiento puede ser en persona, por teléfono o por correo electrónico. Básicamente nosotros leeremos el cuestionario de alimentos con usted para asegurarnos que entendió todas las partes. Se le darán 10 días para valorar el cuestionario de alimentos, examine los suplementos que utiliza y examine sobre su estado de salud en el pasado. Usted puede recibir esta forma en persona, por correo con una platica vía telefónica o vía correo electrónico. Después usted completará los primeros días del cuestionario de alimentación. Se le pedirá que lo envié por correo electrónico, por correo regular o por fax el primer día y podrá hablar con un investigador acerca del cuestionario. Este se revisará para completarse y se le ayudará a completar el cuestionario en tantos detalles como sea necesario en los 9 días posteriores. Si el cuestionario no es revisado con un investigador, usted no podrá ser incluido en el estudio y su cuestionario no será usado. Los 10 días deben ser registrados, pero no necesitan ser consecutivos si hay una ocasión especial o un día de comida no habitual elimínelo.

3.-Molestias, riesgos y costos: Usted puede participar en el estudio si vive fuera del área (del teléfono) del investigador, si usted no tiene correo electrónico usted podrá dejar un mensaje y se le regresará la llamada para revisar el entrenamiento y el primer día del cuestionario. Si usted no tiene acceso a internet, usted puede usar el teléfono. Si usted paga por el servicio de internet, puede tener un cargo por participar. Si usted escoge visitar al investigador en persona, podrá tener un costo por la transportación. No hay otros riesgos fuera de esos que usted puede encontrar en la vida diaria.

4.- Beneficios: No hay beneficios personales por su participación en este estudio. Sin embargo participando puede ayudarnos a ver como los alimentos y los métodos de cocción están ligados a mayores niveles de glucosa o hemoglobina A1c o complicaciones de la diabetes. Esta información puede eventualmente ayudar a mucha gente a alcanzar un mejor control de glucosa y prevenir o retardar las complicaciones de la diabetes.

5.- Declaración de Confidencialidad: Su cuestionario será codificado. Una clave unirá su código con su nombre y será guardada por el investigador en un gabinete con llave hasta que todos los cuestionarios hallan sido completados. La clave de identificación será hasta entonces destruida. Su forma de consentimiento informado también será guardada en la oficina del investigador. Cuando los resultados de esta investigación sean publicados la

Figure B.7 (cont.)

información de su identificación personal no será proporcionada. Los resultados serán difundidos en reuniones de profesionales del área y en revistas profesionales.

6.- A quien contactar: Por favor contacte a la Dra. Karen Chapman-Novakofski (217-244-2852, kmc@illinois.edu) si usted esta en Illinois o con la Dra. Ma. Eugenia Garay (marugarau_2000@yahoo.com, 477 716-8354) si usted esta en México si tiene alguna pregunta o algún asunto relacionado con la investigación. Usted puede también contactar a la Dra. Karen Chapman-Novakofski y a la Dra. Ma. Eugenia Garay si usted siente que ha sido lastimado o dañado por esta investigación. Si usted tiene alguna pregunta acerca de sus derechos como participante en este estudio, por favor comuníquese a la Universidad de Illinois al Comité de Ética Institucional al 217-333-2670 o vía correo electrónico a irb@uiuc.edu

7.-Compensación: Si usted llena todos los cuestionarios y registros por completo usted recibirá $100.

8.- Voluntariedad: Su participación es voluntaria. Usted puede retirarse del estudio sin ninguna consecuencia. La decisión para participar, no aceptar o abandonar su participación no afectará la futura relación con el Instituto de Investigaciones Médicas.

.- Yo tengo 18 años de edad o mayor
.- Yo he leído y entendido la forma de consentimiento de arriba y voluntariamente estoy de acuerdo a participar en este estudio.
.- Se le dará una copia de este consentimiento para que usted lo guarde.

Firma del participante

Fecha

UNIVERSITY OF ILLINOIS
APPROVED CONSENT
VALID UNTIL

SEP 1 6 2010

Diabetes History Questionnaires

Figure B.8. Diabetes History Questionnaire.

CODE_____ Date _____

DIABETES HISTORY

First, we would like to ask you about the health care you have received recently.

Please answer every question by filling in the blank(s), circling the correct answer, or checking the correct box.

Section I – Resource Use

Q1. During the past 4 weeks, how many total visits to health care providers (doctors, nurse practitioners, etc.) did you make? (fill in the blanks)

____ visits in the past 4 weeks

Q2. During the past 12 months, how many total visits to health care providers did you make? (fill in the blanks)

____ visits in the past 12 months

Q3. When was your last visit with the following health care providers?

a. My last visit with an **ophthalmologist** was: (check one box)
(An ophthalmologist is a physician who specializes in the care and surgery of eye diseases, not an optometrist)

☐1 Within the last 12 months ☐2 1-2 years ago ☐3 2-3 years ago ☐4 More than 3 years ago ☐5 Never had a visit with an ophthalmologist

---

Figure B.8 (cont.)

b. My last visit with an **optometrist** was: (check one box)
(An optometrist is a person professionally trained to test the eyes and to detect and treat eye problems and some diseases, not an ophthalmologist)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a visit with an optometrist

c. My last visit with a **podiatrist** was: (check one box)
(A podiatrist is a physician who treats and takes care of people's feet)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a visit with a podiatrist

d. My last visit with a **dietitian** was: (check one box)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a visit with a dietitian

e. My last visit with a **diabetes educator** was: (check one box)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a visit with a diabetes educator

Q4. When was the last time that you had an eye exam during which the doctor put drops in your eyes that made your pupils large? (You may have been unable to see enough to drive or had to wear dark glasses afterward.) (check one box)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had this type of eye exam

Figure B.8 (cont.)

Q5. When was the last time that you had the following blood tests?

a. My last **Hemoglobin A1c test** was: (check one box)
(This is also known as glycohemoglobin or glycosylated hemoglobin, a test that measures your average blood sugar level over the past couple of months)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a Hemoglobin A1c test

b. My last **Cholesterol blood test** was: (check one box)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a cholesterol blood test

c. My last **Urine analysis** was: (check one box)
(Gave a urine sample to be tested by the health care provider, clinic, or laboratory)

☐1 Within the last 12 months	☐2 1-2 years ago	☐3 2-3 years ago	☐4 More than 3 years ago	☐5 Never had a urine analysis

Q6. Do you check your own blood sugar? (check one box)

☐1 No ↓

☐2 Yes → Q6a. During the past 7 days, how many times have you checked your own blood sugar?

__ __ times ↓

Q7. How often do you check your feet for signs of problems? (check one box)

☐1 Not at all
☐2 Monthly
☐3 Weekly
☐4 Daily

Figure B.8 (cont.)

Q8. During the past 12 months, were you a patient in a hospital overnight? (check one box)

☐1 No ☐2 Yes → Q8a. How many times in the past 12 months did you stay in a hospital overnight?

__ __ times

Q8b. How many nights altogether during the past 12 months did you stay in a hospital?

__ __ __ nights

Q9. Have you ever been hospitalized for diabetic ketoacidosis (DKA)? (check one box)

☐1 No
☐2 Yes
☐3 Don't Know

Q10. Who currently provides your main diabetes health care? (check only one box)

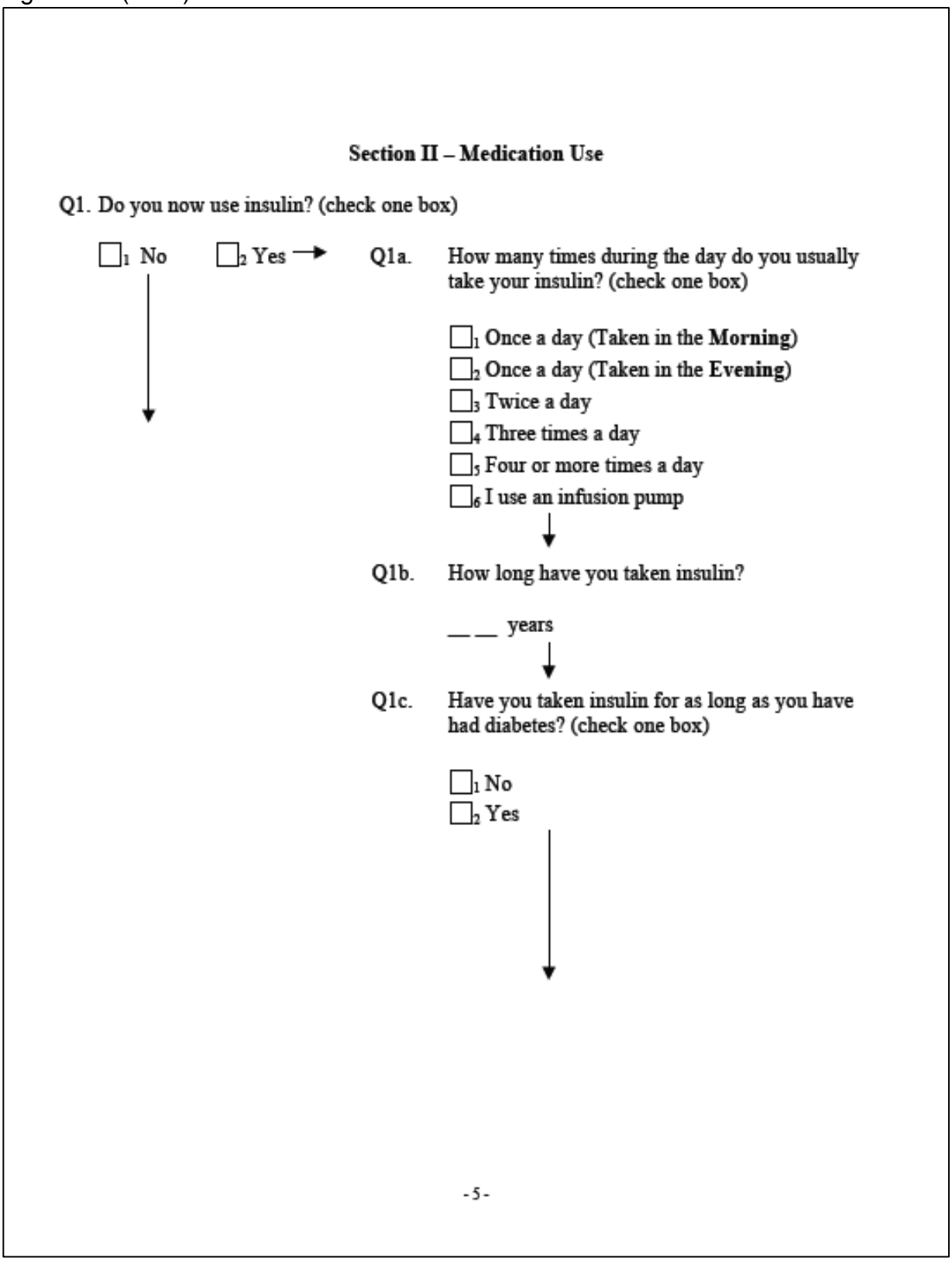
☐1 Generalist (general practitioner, family practitioner, internist, or nurse, nurse practitioner, physician assistant working with a generalist)

☐2 Specialist (diabetologist, endocrinologist, or nurse, nurse practitioner, physician assistant working with a diabetologist or endocrinologist)

☐3 Other (please specify): _____

☐4 No one, I do not have a regular health care provider who provides my diabetes care

Figure B.8 (cont.)



Section II – Medication Use

Q1. Do you now use insulin? (check one box)

☐1 No ☐2 Yes →

Q1a. How many times during the day do you usually take your insulin? (check one box)

☐1 Once a day (Taken in the **Morning**)
☐2 Once a day (Taken in the **Evening**)
☐3 Twice a day
☐4 Three times a day
☐5 Four or more times a day
☐6 I use an infusion pump

Q1b. How long have you taken insulin?

__ __ years

Q1c. Have you taken insulin for as long as you have had diabetes? (check one box)

☐1 No
☐2 Yes

- 5 -

Figure B.8 (cont.)

Q2. Are you currently taking any of the following diabetes pills?
(circle one answer on each line)

	No	Yes
1. Glucotrol (glipizide)	1	2
2. Micronase, Glynase, or Diabeta (glyburide)	1	2
3. Amaryl (glimepiride)	1	2
4. Tolinase (tolazamide)	1	2
5. Diabinese (chlorpropamide)	1	2
6. Glucophage (metformin)	1	2
7. Precose (acarbose)	1	2
8. Rezulin (troglitazone)	1	2
9. Prandin (repaglinide)	1	2
10. Other (please specify below): _____	1	2

Q3. In the past year, has **your health care provider** made changes in your insulin or pill dose on the basis of your home blood tests? (check one box)

- ☐1 No
- ☐2 Yes
- ☐3 Not using medications
- ☐4 Don't test

Q4. In the past year, have **you** made changes in your insulin or pill dose on the basis of your home blood tests? (check one box)

- ☐1 No
- ☐2 Yes
- ☐3 Not using medications
- ☐4 Don't test

Figure B.8 (cont.)

Q5. Do you change the timing/content of a meal on the basis of your home blood tests? (check one box)

☐1 No
☐2 Yes
☐3 Don't test

Q6. Have you been taught to change your insulin dose on the basis of your blood sugar tests? (check one box)

☐1 No
☐2 Yes
☐3 Not using insulin
☐4 Don't test

Q7. Are you currently taking medications for high cholesterol? (Check one box)

☐1 No
☐2 Yes
☐3 Don't know

Figure B.8 (cont.)

Section III - Comorbidities

Q1. Have you ever been told by a health care provider that you have any of the following problems with your eyes? (circle one answer on each line)

		No	Yes, on one eye	Yes, on both eyes
A.	Cataracts	1	2	3
B.	Glaucoma	1	2	3
C.	Detached retina	1	2	3
D.	Blurred vision (not correctable with eye glasses)	1	2	3
E.	Retinopathy (diabetic changes in the back of the eye)	1	2	3
F.	Blindness	1	2	3
G.	Macular degeneration (an aging change in the back of the eye)	1	2	3
H.	Macular Edema	1	2	3

Q2. Have you ever had any of the following operations on your eyes? (circle one answer on each line)

		No	Yes, on one eye	Yes, on both eyes
A.	Cataract Surgery	1	2	3
B.	Laser Treatment	1	2	3
C.	Other (please specify below):	1	2	3

Figure B.8 (cont.)

Q3. Have you ever been told by a health care provider that you have any of the following problems related to your heart or circulation? (circle one answer on each line)

		No	Yes
A.	Heart attack	1	2
B.	Heart failure	1	2
C.	High cholesterol	1	2
D.	Angina	1	2

Q4. Have you ever been told by a health care provider that you have high blood pressure?
(check one box)

☐1 No ☐2 Yes → Q4a. How many years ago were you told that you have high blood pressure?

__ __ years ago

Q4b. Do you now take medication for your high blood pressure? (Check one box)

☐1 No
☐2 Yes

Q5. Have you ever had any of the following operations or procedures related to your heart? (circle one answer on each line)

		No	Yes
A.	Coronary artery bypass surgery (open heart surgery)	1	2
B.	Coronary angioplasty ("balloon" heart procedure)	1	2
C.	Heart catheterization (angiogram)	1	2

Figure B.8 (cont.)

Q6. Have you ever been told by a health care provider that you have any of the following bladder, kidney, or urinary problems? (circle one answer on each line)

		No	Yes
A.	Kidney or bladder infections	1	2
B.	Kidney failure	1	2
C.	Protein in your urine	1	2
D.	Enlarged prostate (Men only)	1	2
E.	Vaginitis (Women only)	1	2

Q7. Have you ever been told by a health care provider that you have any of the following problems with your feet or legs? (circle one answer on each line)

		No	Yes
A.	Peripheral vascular disease (poor circulation in the legs)	1	2
B.	Intermittent claudication (cramping in the calves after exercise)	1	2
C.	Peripheral neuropathy (nerve problems causing numbness, tingling, or burning)	1	2
D.	Gangrene	1	2
E.	Foot ulcers	1	2
F.	Athlete's foot or fungus infection of the feet	1	2

Q8. Have you ever had an amputation of a toe, foot, part of a leg, or all of a leg **for a poorly healing sore or poor circulation**? (An amputation that is **not** due to an injury or accident [car crash, power tool injury, war injury, etc.])?

		No	Yes, right side only	Yes, left side only	Yes, both sides
A.	Toes	1	2	3	4
B.	Part of a foot (or feet)	1	2	3	4
C.	Leg, below the knee	1	2	3	4
D.	Leg, above the knee	1	2	3	4

Figure B.8 (cont.)

Q9. Have you ever been told by a health care provider that you have had any of the following problems?

		No	Yes
A.	Stroke	1	2
B.	Transient ischemic attacks (TIA or "mini-strokes")	1	2
C.	Epilepsy or seizure disorder	1	2
D.	Parkinson's Disease	1	2

Q10. During the **past 4 weeks**, how many days have you lost from school, work, or household activities due to illness or injury?

__ __ days

Q11. Have you ever been told by a health care provider that you have any of the following problems with your breathing? (circle one answer on each line)

		No	Yes
A.	Emphysema	1	2
B.	Chronic bronchitis	1	2
C.	Asthma	1	2

Q12. Have you ever been told by a health care provider that you may have any of the following problems? (circle one answer on each line)

		No	Yes
A.	Peptic or stomach ulcer	1	2
B.	Liver disease	1	2
C.	Ulcerative colitis (or Crohn's Disease)	1	2
D.	Irritable or functional bowel disease	1	2
E.	Gallstones or gallbladder disease	1	2

Figure B.8 (cont.)

Q13. Have you ever been told by a health care provider that you have:
(circle one answer on each line)

		No	Yes
A.	Osteoarthritis or degenerative joint disease	1	2
B.	Rheumatoid arthritis	1	2
C.	Slipped or herniated disc in your back	1	2
D.	Osteoporosis (or thinning bones)	1	2

Section IV – Background Information

Q1. How tall are you?

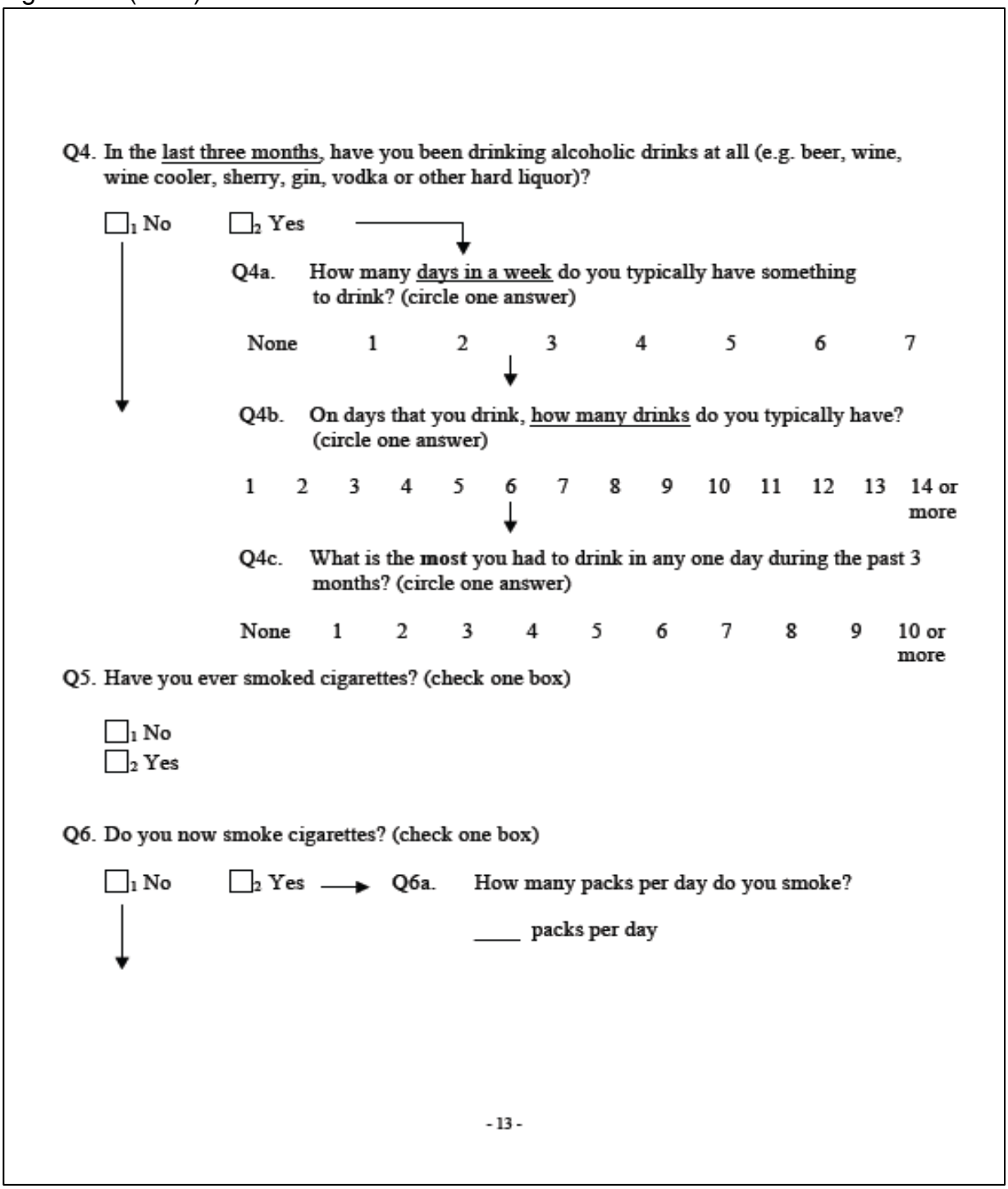
___ feet __ __ inches

Q2. How much do you currently weigh?

__ __ __ pounds

Q3. How long have you had diabetes?

Figure B.8 (cont.)



Q4. In the last three months, have you been drinking alcoholic drinks at all (e.g. beer, wine, wine cooler, sherry, gin, vodka or other hard liquor)?

☐1 No ☐2 Yes →

Q4a. How many days in a week do you typically have something to drink? (circle one answer)

None 1 2 3 4 5 6 7

Q4b. On days that you drink, how many drinks do you typically have? (circle one answer)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 or more

Q4c. What is the **most** you had to drink in any one day during the past 3 months? (circle one answer)

None 1 2 3 4 5 6 7 8 9 10 or more

Q5. Have you ever smoked cigarettes? (check one box)

☐1 No
☐2 Yes

Q6. Do you now smoke cigarettes? (check one box)

☐1 No ☐2 Yes → Q6a. How many packs per day do you smoke?

____ packs per day

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Adapted from Michigan Diabetes Research and Training Center DH2.0

Figure B.9. Diabetes History Questionnaire in Spanish.

Código _____

Fecha _____

Historia de Diabetes y Datos Demográficos

Por favor, responda todas las preguntas llenando los espacios en blanco, poniendo un círculo en la respuesta correcta, o marcando en la casilla correcta.

Sección I – Utilización de los recursos

Q1. Durante las últimas 4 semanas, ¿en total cuántos visitas a hecho a proveedores de servicios de salud (médicos, enfermera, profesionales de la salud, etc) (rellene los espacios en blanco)

____ Visitas en las últimas 4 semanas

Q2. ¿Durante los últimos 12 meses, en total cuántas visitas a los proveedores de salud ha hecho? (rellene los espacios en blanco)

____ Visitas en los últimos 12 meses

Q3. ¿Cuándo fue su última visita con los siguientes médicos ?

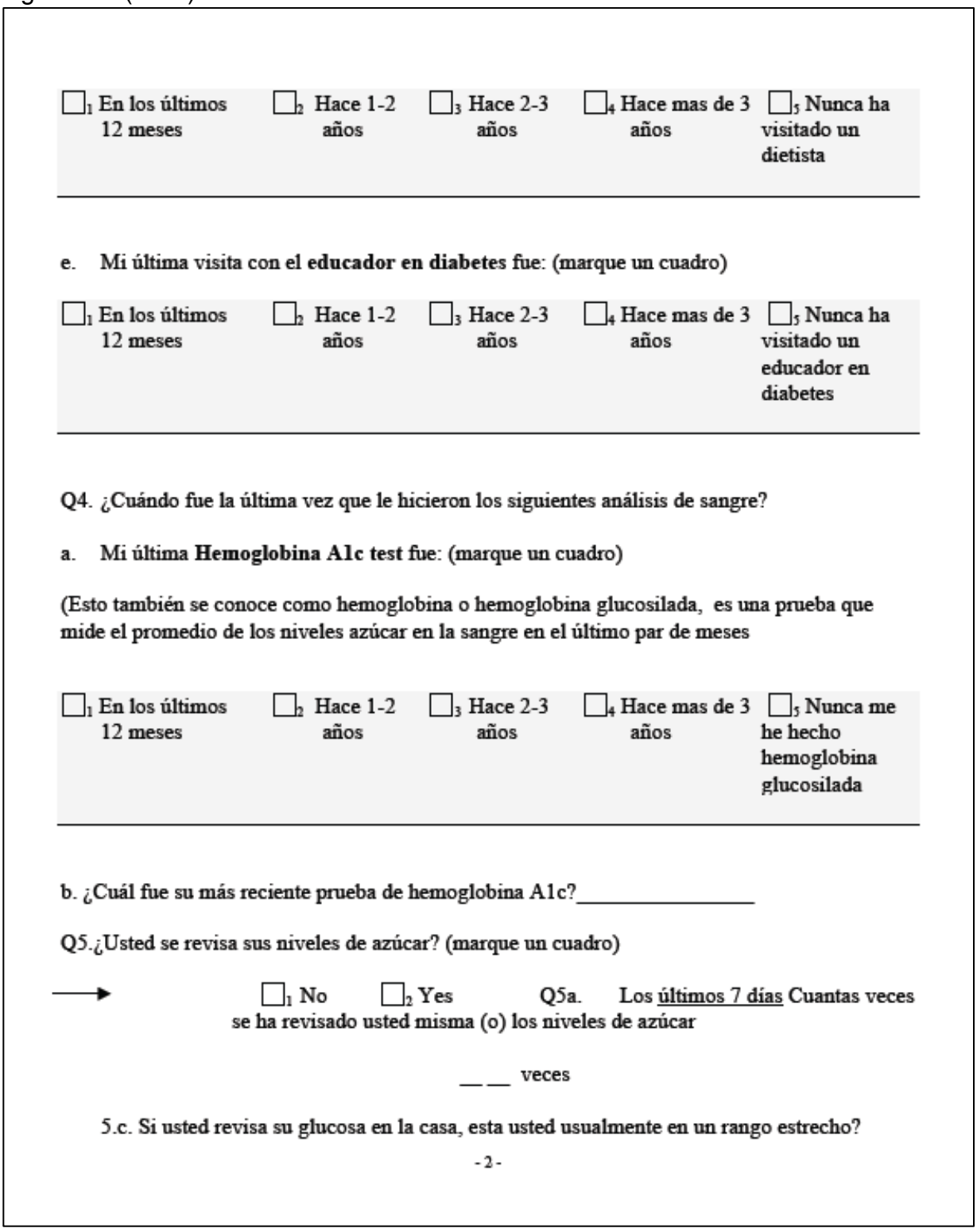
a. Mi última visita con un **oftalmólogo** fue: (marque un cuadro)
(Un oftalmólogo es un médico que se especializa en el cuidado y cirugía de enfermedades de los ojos, no un optometrista)

☐$_1$ En los últimos 12 meses	☐$_2$ Hace 1-2 años	☐$_3$ Hace 2-3 años	☐$_4$ Hace mas de 3 años	☐$_5$ Nunca ha visitado un oftalmólogo

---

b.. Mi última visita con el **dietista** fue: (marque un cuadro)

Figure B.9 (cont.)



☐1 En los últimos 12 meses ☐2 Hace 1-2 años ☐3 Hace 2-3 años ☐4 Hace mas de 3 años ☐5 Nunca ha visitado un dietista

e. Mi última visita con el **educador en diabetes** fue: (marque un cuadro)

☐1 En los últimos 12 meses ☐2 Hace 1-2 años ☐3 Hace 2-3 años ☐4 Hace mas de 3 años ☐5 Nunca ha visitado un educador en diabetes

Q4. ¿Cuándo fue la última vez que le hicieron los siguientes análisis de sangre?

a. Mi última **Hemoglobina A1c test** fue: (marque un cuadro)

(Esto también se conoce como hemoglobina o hemoglobina glucosilada, es una prueba que mide el promedio de los niveles azúcar en la sangre en el último par de meses

☐1 En los últimos 12 meses ☐2 Hace 1-2 años ☐3 Hace 2-3 años ☐4 Hace mas de 3 años ☐5 Nunca me he hecho hemoglobina glucosilada

b. ¿Cuál fue su más reciente prueba de hemoglobina A1c?_____

Q5.¿Usted se revisa sus niveles de azúcar? (marque un cuadro)

→ ☐1 No ☐2 Yes Q5a. Los <u>últimos 7 días</u> Cuantas veces se ha revisado usted misma (o) los niveles de azúcar

__ __ veces

5.c. Si usted revisa su glucosa en la casa, esta usted usualmente en un rango estrecho?

- 2 -

Figure B.9 (cont.)

☐1 No ☐2 Yes Q.5d. Si es si, cual es el rango? _____

5.e. Si no, ¿cuál es su nivel de glucosa en sangre más altos que suelen tener?_____
5.f. Si no, ¿cuál es su nivel de glucosa en sangre más bajo que suelen tener?_____

Q6. Durante los últimos 12 meses, estuvo usted hospitalizado algún día toda la noche? (marque un cuadro)

☐1 No ☐2 Si → Q6a. ¿Cuántas veces en los últimos 12 meses estuvo usted hospitalizado toda la noche?

__ __ Veces

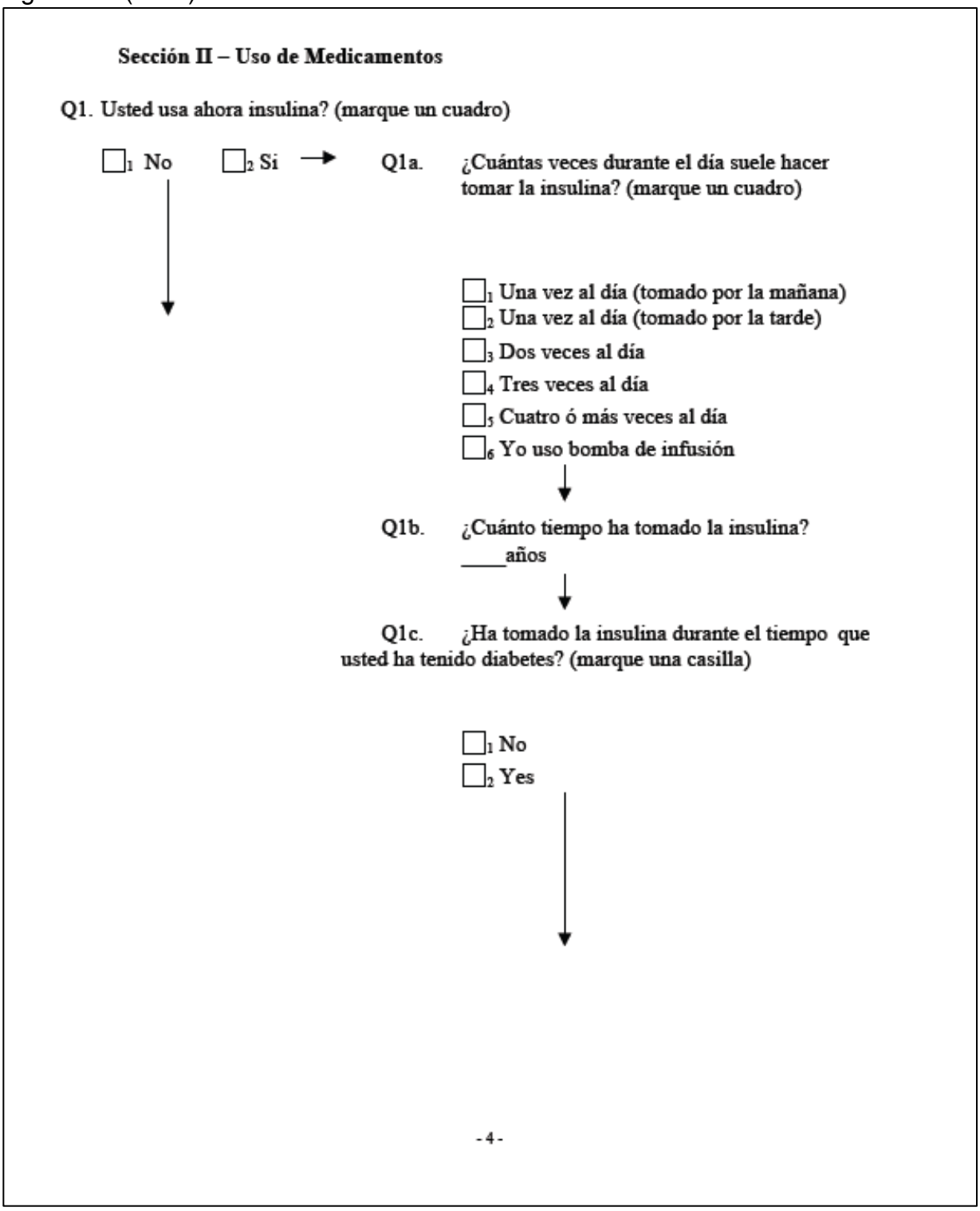
Q6b. ¿Cuántas noches en total durante los últimos 12 meses estuvo en un hospital?

__ __ __ Noches

Q7. ¿Alguna vez ha sido hospitalizado por cetoacidosis diabética (CAD)? (marque un cuadro)

☐1 No
☐2 Si
☐3 No sé

Figure B.9 (cont.)



Sección II – Uso de Medicamentos

Q1. Usted usa ahora insulina? (marque un cuadro)

☐1 No ☐2 Si → Q1a. ¿Cuántas veces durante el día suele hacer tomar la insulina? (marque un cuadro)

☐1 Una vez al día (tomado por la mañana)
☐2 Una vez al día (tomado por la tarde)
☐3 Dos veces al día
☐4 Tres veces al día
☐5 Cuatro ó más veces al día
☐6 Yo uso bomba de infusión

Q1b. ¿Cuánto tiempo ha tomado la insulina?
____años

Q1c. ¿Ha tomado la insulina durante el tiempo que usted ha tenido diabetes? (marque una casilla)

☐1 No
☐2 Yes

- 4 -

Figure B.9 (cont.)

Q2. ¿Está tomando actualmente cualquiera de los siguientes medicamentos para la diabetes? (marque una respuesta en cada línea)

	No	Si
1. Glucotrol (glipizide)	1	2
2. Micronase, Glinase, ó Diabeta (gliburide)	1	2
3. Amaryl (glimepirida)	1	2
4. Tolinase (tolazamida)	1	2
5. Diabinese (clorpropamida)	1	2
6. Glucophage (metformin)	1	2
7. Precose (acarbosa)	1	2
8. Rezulin (troglitazona)	1	2
9. Prandin (repaglinida)	1	2
10. Otros (por favor especifique abajo): _____	1	2

Q3. En el año pasado su **proveedor de servicios de salud** realizó cambios en su dosis de insulina o píldoras en base al análisis de sangre hecho en su casa? (marque una casilla)

- ☐ 1 No
- ☐ 2 Si
- ☐ 3 No uso medicamentos
- ☐ 4 No me hago la prueba

Q4. En el año pasado **usted** realizó cambios en su dosis de insulina o píldoras en base al análisis de sangre hecho en su casa? (marque una casilla)

- ☐ 1 No
- ☐ 2 Si
- ☐ 3 No uso medicamentos
- ☐ 4 No me hago la prueba

Figure B.9 (cont.)

Q5. ¿Cambia el horario y contenido de su comida en base del análisis de sangre realizado en su casa? (marque una casilla)

☐1 No
☐2 Si
☐3 No me hago la prueba

Q6. ¿Se le ha enseñado a cambiar su dosis de insulina en base de su prueba de azúcar en sangre? (marque una casilla)

☐1 No
☐2 Si
☐3 No uso insulina
☐4 No me hago la prueba

- 6 -

Figure B.9 (cont.)

Sección III - Comorbilidades

Q1. ¿Alguna vez le ha informado algún proveedor de servicios de salud que usted tiene alguno de los siguientes problemas con sus ojos? (marque una respuesta en cada línea)

		No	Si, en un ojo	Si, en Ambos ojos
A.	Catarata	1	2	3
B.	Glaucoma	1	2	3
C.	Desprendimiento de Retina	1	2	3
D.	Visión borrosa (no corregible con lentes)	1	2	3
E.	Retinopatía (cambios por la diabetes en la parte posterior del ojo)	1	2	3
F.	Seguera	1	2	3
G.	Degeneración macular (cambios por envejecimiento en la parte posterior del ojo)	1	2	3
H.	Edema macular	1	2	3

Q2. ¿Alguna vez ha tenido alguna de las siguientes operaciones en los ojos? (marque una respuesta en cada línea)

		No	Si, en un ojo	Si, en ambos ojos
A.	Cirugia de Catarata	1	2	3
B.	Tratamiento laser	1	2	3
C.	Otra (por favor especifique abajo):	1	2	3

Figure B.9 (cont.)

Q3. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted tiene alguno de los siguientes problemas relacionados con su corazón o la circulación? (marque una respuesta en cada línea)

		No	Si
A.	Ataque al corazón	1	2
B.	Falla cardiaca	1	2
C.	Colesterol alto	1	2
D.	Angina de pecho	1	2

Q4. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted tiene presión arterial alta? (marque una casilla)

☐1 No ☐2 Si → Q4a. ¿Cuántos años hace que le dijeron que usted tiene presión arterial alta?

__ __ años

↓

Q4b. ¿Usted esta tomando ahora medicamentos para su presión arterial alta? (Marque una casilla)

☐1 No
☐2 Yes

↓

Q5. ¿Alguna vez ha tenido alguna de las siguientes operaciones o procedimientos relacionados con su corazón? (Marque una respuesta en cada línea)

		No	Si
A.	Cirugía de revascularización coronaria (cirugía a corazón abierto)	1	2
B.	Angioplastia coronaria (procedimiento de colocación de "globo" en su corazón)	1	2
C.	Cateterismo cardiaco (angiograma)	1	2

Figure B.9 (cont.)

Q6. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted tiene alguno de los siguientes problemas en vejiga, riñones, o problemas urinarios? (marque una respuesta en cada línea)

		No	Si
A.	Infecciones Renales o de la vejiga	1	2
B.	Insuficiencia renal	1	2
C.	Proteínas en la orina	1	2
D.	Crecimiento de la próstata (Solo hombres)	1	2
E.	Vaginitis (Solo mujeres)	1	2

Q7. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted tiene alguno de los siguientes problemas con los pies o las piernas? (marque una respuesta en cada línea)

		No	Si
A.	Enfermedad vascular periférica (mala circulación en las piernas)	1	2
B.	Claudicación intermitente (calambres en las pantorrillas después del ejercicio)	1	2
C.	Neuropatía periférica (problemas en los nervios que causan adormecimiento, hormigueo, o ardor)	1	2
D.	Gangrena	1	2
E.	Ulceras en los pies	1	2
F.	Pie de atleta, ó hongos en los pies	1	2

Figure B.9 (cont.)

Q8. ¿Usted ha tenido alguna amputación de un dedo del pie, pie, parte de una pierna, o la totalidad de una pierna por una curación que no sano o mala circulación? (Una amputación que no es debido a una lesión o accidente [accidente de auto, herramienta eléctrica lesiones, lesiones de guerra, etc])?

		No	Si, del lado derecho solamente	Si, del lado izquierdo solamente	Si, de ambos lados
A.	Dedos de los pies	1	2	3	4
B.	Parte de un pie (o pies)	1	2	3	4
C.	Una pierna, por debajo de la rodilla	1	2	3	4
D.	Pierna, por encima de la rodilla	1	2	3	4

Q9. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted ha tenido alguno de los siguientes problemas?

		No	Si
A.	Ataque	1	2
B.	Ataques isquémicos transitorios (TIA o "mini-ataque")	1	2
C.	Epilepsia or crisis de epilepsia	1	2
D.	Enfermedad de Parkinson	1	2

Q10. Durante las últimas 4 semanas, ¿cuántos días has perdido de la escuela, el trabajo, o actividades domésticas debido a enfermedad o lesión?

__ __ días

Figure B.9 (cont.)

Q11. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted tiene alguno de los siguientes problemas con su respiración? (marque una respuesta en cada línea)

		No	Si
A.	Enfisema	1	2
B.	Bronquitis Crónica	1	2
C.	Asma	1	2

Q12. ¿Alguna vez ha sido informado por un proveedor de servicios de salud que usted puede tener cualquiera de los siguientes problemas? (marque una respuesta en cada línea)

		No	Si
A.	Úlcera Péptica o de estómago	1	2
B.	Enfermedad del higado	1	2
C.	Colitis Ulcerativa (Enfermedad de Crohn)	1	2
D.	Enfermedad intestinal Irritable o funcional	1	2
E.	Cálculos biliares o enfermedad de la vesícula biliar	1	2

Figure B.9 (cont.)

Q13. ¿Alguna vez ha sido informado por un proveedor de cuidado de la salud que usted tiene alguna de las siguientes enfermedades: (marque una respuesta en cada línea)

		No	Si
A.	Osteoartritis o enfermedad degenerativa de las articulaciones	1	2
B.	Artritis Reumatoide	1	2
C.	Deslizamiento o hernia discal en su espalda	1	2
D.	Osteoporosis (o adelgazamiento de huesos)	1	2

Figure B.9 (cont.)

Sección V – Antecedentes

Q1. ¿Cuánto mide?

___ Pies __ __ pulgadas

Q2. ¿Cuánto pesa actualmente?

__ __ __ libra

Q3. Usted es?

☐1 Hombre
☐2 Mujer

Q4. Si usted tiene 21 años de edad o más, por favor responda a esta pregunta. Si tiene menos de 21, por favor, pase.
En los últimos tres meses, ha estado tomando bebidas alcohólicas (por ejemplo, cerveza, vino, jerez, ginebra, vodka u otras bebidas alcohólicas)?

☐1 No ☐2 Si →

Q4a. ¿Cuántos días a la semana suele tener algo para beber? (marque una respuesta)

Ninguno 1 2 3 4 5 6 7

Q4b. En los días que usted bebe, ¿cuántas bebidas suele tomar usted? (marque una respuesta)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 o Mas

Q4c. ¿Cuál es lo máxima que ha bebido en cualquier día durante los últimos 3 meses? (marque una respuesta)

Figure B.9 (cont.)

Ning uno	1	2	3	4	5	6	7	8	9	10 o Mas

Q5. ¿Alguna vez ha fumado cigarrillos? (marque una casilla)

☐1 No
☐2 Si

Q6. Actualmente, ¿usted fuma cigarrillos? (marque una casilla)

☐1 No ☐2 Si ⟶ Q6a. ¿Cuántos paquetes por día Fuma?

____ paquetes por día

Adapted from
Michigan Diabetes
Research and Training Center
DH2.0

Vitamins and Supplement Intake Questionnaires

Figure B.10. Vitamins and Supplement Intake Questionnaire.

Code

Vitamin and Supplement Survey

Date

1. How often, if at all, do you take any vitamin or mineral supplement in pill or liquid form?

More than one supplement per day	(1.0)
Every day or almost everyday	(1.1)
Every so often	(1.2)
Not at all	(1.3)

If you checked 1.0, 1.1 or 1.2, please continue to 2.

2. Which types of supplements do you usually take (check all that apply)?

Multivitamin	(2.1)
Multivitamin with iron or other minerals	(2.2)
Antioxidants	(2.3)
Single vitamins/minerals	(2.4)

If you know the name of the supplement, please list

3. Which of these single vitamins and minerals do you usually take? If you know the milligrams of each, or the brand name, please provide that information.

Vitamin A	(3.1)
Vitamin B/B complex	(3.2)
Vitamin C	(3.3)
Vitamin D	(3.4)
Vitamin E	(3.5)
Calcium	(3.6)
Folacin	(3.7)
Fluoride	(3.8)
Iron	(3.9)
Zinc	(3.10)
Selenium	(3.11)
Chromium	(3.12)

Figure B.10 (cont.)

Something else (please specify) (3.13)_____

_____

4. How often, if at all, do you take a fish oil or omega three fatty acid supplement?

   Every day or almost everyday (4.1)___

   Number of capsules per day? ___

   Every so often (4.2)___

   Not at all (4.3)___

5. How often, if at all, do you take a fiber supplement?

   Every day or almost everyday (5.1)_

   Every so often (5.2)_

   Not at all (5.3)_

6. How often, if at all, do you take a nutrition supplement, (such as Ensure, Glucerna, Slimfast) in either liquid or solid form?

   Every day or almost everyday (6.1

   Every so often (6.2

   Not at all (6.3

   If you checked 6.1 or 6.2, please specify: _____

7. How often, if at all, do you take herbal remedies or supplements (examples: Echinacea, St. Johns wort, Gingko biloba)?

   Every day or almost everyday (7.1)_

   Every so often (7.2)_

   Not at all (7.3)_

   If you checked 7.1 or 7.2, please specify: _____

_____

8. Any other vitamins or supplements that you take?

Figure B.11. Vitamins and Supplement Intake Questionnaire in Spanish.

Código_____ Encuesta de vitaminas y suplementos
Fecha _____

1. ¿Con qué frecuencia, tomar cualquier vitamina o suplemento mineral en forma de píldoras o líquido?

   Mas de un suplemento por día (1.0))_____
   Todos los días ó casi todos los días (1.1)_____
   Muy frecuente (1.2)_____
   No en todos las anteriores (1.3)_____

   Si marcó 1.0, 1.1 o 1.2, por favor, siga 2
   .

2. ¿Que tipo de suplemento usted usualmente toma ? (marque todo los que apliquen)

   Multivitaminas (2.1)_____
   Multivitaminas con hierro u otro mineral (2.2)_____
   Antioxidantes (2.3)_____
   Vitaminas y minerales solos (2.4)_____

Si usted conoce el nombre del suplemento, por favor, márquelo

3. ¿Cuál de estas vitaminas y minerales normalmente toman? Si conoce los miligramos de cada uno, o la marca, sírvase proporcionar esa información.

   Vitamina A (3.1)_____
   Vitamina B/complejo B (3.2)_____
   Vitamina C (3.3)_____
   Vitamina D (3.4)_____
   Vitamina E (3.5)_____
   Calcio (3.6)_____
   Ácido fólico (3.7)_____
   Fluoruro (3.8)_____
   Hierro (3.9)_____

Figure B.11 (Cont.)

Zinc (3.10)_____

Selenio (3.11)_____

Cromo (3.12)_____

Alguna otra cosa (por favor especifique) (3.13)_____

_____

1. ¿Con qué frecuencia, toma aceite de pescado omega tres o suplemento de ácidos grasos ¿

   Todos los días o casi todos los días (4.1)_____

   Número de capsulas por día? _____

   Muy frecuente (4.2)_____

   Ninguna (4.3)_____

2. ¿ Con que frecuencia toma suplementos de fibra?

   Todos los días o casi todos los días (5.1)_____

   Muy frecuente (5.2)_____

   Ninguna (5.3)_____

3. ¿ Con qué frecuencia, usted toma un suplemento nutricio, (tales como Ensure, Glucerna, Slimfast), ya sea en forma líquida o sólida?

   Todos los días o casi todos los días (6.1)_____

   Muy frecuente (6.2)_____

   Ninguna (6.3)_____

   Si usted marco 6.1 o 6.2, por favor especifique: _____

   _____

4. ¿ Con qué frecuencia, usted toma remedios de hierbas o suplementos (ejemplos: Echinacea, mosto de St Johns, Gingko biloba)?

   Todos los días o casi todos los días (7.1)_____

   Muy frecuente (7.2)_____

   Ninguna (7.3)_____

   Si usted marco 7.1 o 7.2, por favor especifique: _____

8. ¿Alguna otra vitamina o suplemento que usted tome ?

Food Records

Figure B.12. Food Records.

Instructions for Recording the Food Record

1. Record everything you ate or drank during the 24-hour time period indicated (12:01 a.m. midnight)
2. To the best of you ability described combinations or mixed dishes that were eaten. For example, what ingredients were included on that piece of pizza? Was it thick or thin crust? Include brand names if known.
3. Describe the amounts consumed in terms appropriate to that item. For example: ounces (cup) of milk, tablespoon of French dressing, slices of bread, pieces of fruit, etc. If you had a piece of pizza, how big was it in inches or sections, etc.?
4. Describe how the food was cooked and for how long, if known or can be estimated. For example, 1 egg, scrambled in margarine; 1 breakfast biscuit microwaved on high or 2 minutes.
5. Remember to include beverages, and anything you may add to them, such as milk or sweetener.
6. Remember to include anything added to a food after it is prepared, such as margarine, salt, catsup, and the estimated amount.
7. If you need additional space, use the back of the paper or attach additional sheets.
8. Answer the question at the bottom of the day's record. (Does this day's record represent your usual food intake? Yes____ No___). If your answer is no, explain why it was not representative. Were you ill or are you on a special diet? Did you have unexpected guests and you took them out to dinner?

Breakfast	
Raisin bran	1 ounce o 30 ml
2% Milk	6 ounce o 180 ml
Orange	½ naranja
Toast with butter and	1 piece and 1 teaspoon
strawberry jelly	2 tablespoons
Coffee	1 taza (8 onzas o 240 ml)

Figure B.12 (Cont.)

Day of the Week	Code
Food or Beverage Item Consumed	**Amount Consumed**

Does this day's record represent your usual food intake? ___ Yes ___ No. If not, please explain.
Day of the Week _____Code_____

Food record follow-up survey
Please scan the food intake and answer these questions:
1) Do you know the specific brand names for the products consumed? Yes____ No ___
If yes, please include brands on the list
2) Were any of the foods microwaved? Yes ___ No ___
If yes, please list foods and how long the food was microwaved (approximately) and if the microwave power was high, medium, or low.
3) Were any of the meats broiled or grilled? Yes ___ No ___
If yes, please mark which foods.

Figure B.13. Food Records in Spanish.

INSTRUCCIONES PARA EL RECORDATORIO DE 24 HORAS

CODIGO_____

1.-Recuerde TODO lo que haya comido o bebido durante las 24 horas de tiempo indicado (12:01 a.m.-medianoche)
2.-Describa la combinación o mezclas de platillos que haya consumido. Por ejemplo, ¿cuales ingredientes se incluyeron en ese pedazo de pizza? ¿La pasta de la pizza es gruesa o delgada? Incluya el nombre del pan si lo sabe.
3.-Describa la cantidad consumida en términos apropiados para este caso. Por ejemplo: onzas (tazas) de leche, cucharadas de aderezo, rebanadas de pan, piezas de frutas, etc. Si tiene un trozo de pizza que tan grande es en centímetros o rebanadas. Recuerde exactamente las cantidades, lo mejor que Ud. pueda.
4.-Describa como se cocinó la comida y por cuánto tiempo si puede calcularlo. Por ejemplo: 1 huevo frito en margarina, 1 bisquet en el microondas a potencia alta por 2 minutos.
5.-Recuerde las bebidas y cada cosa que agregó a ellas, como leche y endulzante.
6.-Recuerda incluir cualquier cosa que se agregue a la comida después de ser preparada, como margarina, sal, cátsup y la cantidad calculada.
7.-Si necesita espacio adicional, utilice la parte posterior de la hoja o agregue otras hojas adicionales.
8.-Responda las preguntas en la parte inferior del cuestionario.

¿Este recordatorio representa su alimentación usual? Si _____No_____
Si no es así, explique porque no es representativo.
¿Está en una dieta especial?_____
¿Tuvo invitados inesperados y salió a comer fuera?_____

EJEMPLO DE DESAYUNO	
Cereal Raisin bran	1 onza o 30 ml
2% leche	6 onzas o 180 ml
Naranja	½ naranja
Pan integral tostado con mantequilla	1 porción(de mantequilla)
Y con mermelada de fresa	2 cucharadas cafeteras
Café negro	1 taza (8 onzas o 240 ml)

Figure B.13 (Cont.)

DIA DE LA SEMANA	CÓDIGO
COMIDA O BEBIDA CONSUMIDA	CANTIDAD CONSUMIDA

¿Este día de recordatorio representa su comida usual? Si_____ No_____
Si es no explique porque_____
Por favor revisa los alimentos consumidos y contesta las siguientes preguntas
1.- ¿Sabes lo nombres específicos de las marcas incluidas en los productos consumidos?
Si_____ No_____
Si es si incluya el listado
¿Alguna de las comidas fue horneada en microondas?
Si es si, que poder tuvo: alto, medio o bajo y tiempo (aproximadamente).
3.- ¿Algún alimento fue cocinado a la parrilla o asado?
Si_____ No_____
Si es si describa cual de los alimentos.

Appendix C
Questionnaires for Chapter 5

This appendix includes questionnaires used for the study "Development, Relative Validity, and Reliability of a Food Frequency Questionnaire for a Case-Control Study on Dietary Advanced Glycation End Products and Diabetes Complications" presented on chapter 5. The material in this appendix includes Recruitment Material, Screening Tool, Inform Consent, Food Frequency Questionnaire and Food Records.

Figure C.1. Recruitment Material.

WE NEED PARTICIPANTS FOR TESTING A FOOD QUESTIONNAIRE.

We invite you to participate:

- If you have Diabetes Mellitus type 2,
- If you are 18 years or older,

and

- If you are willing to write down what you eat for 7 days and complete 2 food frequency surveys.

You will get $50 dollars.

If you are interested, please contact Claudia Luevano: luevano1@illinois.edu, 217-974-0710.

Figure C.2. Screening Tool

Screening Tool

Name:_____ **Date:**_____

Cognitive Evaluation:

1. What is the day of the week?
2. Do you know where you are?
3. What is your name?

If person **does not know** the answer to any of the questions, thank them for their time and tell them they are not eligible.

Inclusion criteria:

1. Are you 18 years old and older?
2. Do you have type 2 diabetes?
3. Can you read?

If person responds **no** to any questions above, thank them for their time and tell them they are not eligible.

Exclusion criteria:

1. Are you blind?
2. Are you a vegan?
3. Are you following a protein restricted diet for any reason?
4. Are you willing to write down what you eat for a 7 day timespan?
5. Do you have a phone, can you meet with one of the investigators, or can you email your first day's record so we can review any problems with the completeness of the record?

If person responds **yes** to any questions above, thank them for their time and tell them they are not eligible.

Patient is eligible to participate in study
Patient is ineligible to participate in study

Figure C.3. Inform Consent

Title of Project: Relative validity of a food frequency questionnaire for assessing dietary advanced glycation end products.

Responsible Principal Investigator: Dr. Karen Chapman-Novakofski, University of Illinois at Urbana-Champaign

1. **Purpose of the study**: This is a research study looking at some food components found in foods rich in protein or fat that when are fried, broiled or grilled could be associated to diabetes complications. We need a good questionnaire to measure these components in the diet of individuals. For this reason this research is looking to validate a food frequency questionnaire.

2. **Procedures to be followed**: After you have been screened for eligibility in this project (able to read and write and answer a couple basic questions), you will be briefly trained on how to complete a food frequency questionnaire (10 minutes). This training can be completed at the investigator's office or at your place of convenience. We will read with you the instructions for completing the food frequency questionnaire. In addition, we will provide you with food models or pictures of foods' portion size that could help you to answer the questionnaire. You will fill out the food frequency questionnaire in this first meeting (approximate time is 40 minutes). At the end of this first meeting we will set an appointment to see you again in two weeks. The total time for the first meeting is around 1 hour.
   At this second meeting you will again fill out the food frequency questionnaire. We will read with you the instructions and we will provide you with food models and pictures of foods' portion size again. At the end of this meeting we will give you a closed package with food records that you fill out for 7 days. We will give you a brief training on how to fill out these food records. The total time for the second meeting is also around 1 hour.
   We will ask you to start filling out the food records two weeks after our second meeting. We will send you a reminder email or leave a phone message. After you fill out the first food record, one of the investigators will review it with you to make sure you understand all the information about the food we need. This can be by e-mail, by fax or by telephone. All seven days must be recorded, but they do not need to be consecutive in case a special occasion arises. Each food record will take around 15 minutes to fill out.
   At the end of the study you need to meet again with the investigator by phone or in person, to review the completeness of the food records. This last meeting will take around 10 to 15 minutes.
   Therefore, for this research your total time commitment will be around 4 hours.

3. **Discomfort and Risks and Costs**: If you choose to visit the investigator in person, you may have transportation costs. If you use your telephone or internet service to send food records, you could have a charge to participate. There are not risks outside of those you may encounter in everyday life.

Figure C.3 (Cont.)

4. **Benefits**: There are not personal benefits to you for participating in this study. However, your participation may help us to find a good questionnaire to asses some components in foods that could be linked to diabetes complications.

5. **Statement of confidentiality:** All the questionnaires and food records will be coded. A key with your code and your name will be kept in a locked cabinet at the investigators office until all the questionnaires are completed. The identifying key will then be shredded. We will use screening forms for evaluating eligibility for this study. Your consent form and the screening form will also be kept locked in the investigator's office. At the end of the study the screening form will also be shredded. The results of this research could be published in professional meeting or journals, but no personal information will be shared.

6. **Whom to contact:** Please contact Dr. Karen Chapman-Novakofski (217-244-2852, kmc@illinois.edu) or Claudia Luevano-Contreras (217-974-0710, luevano1@illinois.edu) if you have any question or concerns. You may also contact us if you feel you have been injured or harmed by this research. If you have any question about your rights as a participant in this study, please contact the University of Illinois Institutional Review Board at 217-333-2670 or via email at irb@illinois.edu.

7. **Compensation**: If you complete the food frequency questionnaires and the food records you will receive $50. If you complete only the food frequency, you will receive $10. For completing both food frequencies and not completing the 7 days of food records, you will receive $20.

8. **Voluntariness:** Your participation is voluntary. You may discontinue at anytime. The decision to participate, decline, or withdrawn from participation will have no effect on future relations with the University of Illinois.

- I am 18 years of age or older
- I have read and understand the above consent form and voluntarily agree to participate in this study.
- You will be given a copy of this consent form for your records.

_____ _____

Participant signature Date

Figure C.4. Food frequency questionnaire.

Food frequency questionnaire CODE:_____

Years with Diabetes _____ Age: _____
Weight: _____ Height: _____

Have you changed your diet significantly lately? Yes No
If yes, since when? Last month Last 6 months Last year Last 2 years

Could you explain briefly the changes?

Since you have been diagnosed with diabetes have you changed significantly your diet? ? Yes No
Could you explain briefly?

Instructions
1. Please answer each question to the best of your knowledge. If you do not know the correct answer you could guess, but please do not leave questions unanswered.
2. We are interested in your diet during the LAST YEAR. Put a (X) in the answer that best describes your average intake of that specific food during the past year. Besides each food write the number of portions that you usually have each time that you eat this food.
3. There are a lot of brands and types of a specific food. For example, for mayonnaise you could use regular or low fat or an imitation, like a salad dressing. For this reason, next to the food there is a blank column where you can write the TYPE or the BRAND of the food. If you know this information, please add to the food.
4. For meat, poultry and fish, please choose among the different cooking options. To help you identify, think about these methods:
Pan-fried: Cooking in a preheated pan or griddle (with or without added fat).
Grilled/BBQ: Cooking by placing it in a grill over coal, open fire, or ceramic briquettes heated by gas.
Oven-Broiled: Cooking by placing it below the heat source, such as in an oven with the setting it on broil.
Boiled: Cooking by placing in a boiling liquid.
Baked. Cooking by placing in a oven using dry heat.
Note: If you reheated your foods in a microwave specify the cooking method that was used for preparing the food.

EXAMPLES:
If you eat cookies twice per week and you eat 3 pieces then you should answer as follows:

	TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day
Cookie OREO	Low fat	3 pieces							X				

If you use olive oil for cooking, try to count how many tablespoons you use per occasion. If you use it every day, and on average you use 2 tablespoons, then you should answer as follow:

	TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day
Oil	Olive	2 Tbs										X	

Figure C.4 (Cont.)

Food frequency questionnaire CODE:_____

Foods, type and amounts				**AVERAGE USE LAST YEAR**											
		TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day	
Breakfast foods/ Cereals															
Rice Krispies/ Cinnamon Toast															
Total/Life/ Fiber One/Cheerios															
Other:															
Waffle	mix / Frozen														
Pancake	mix/ Restaurant														
Toast															
Cookies/pastries/Snacks															
Cookie	Type:														
Cracker	Wheat/Saltine/Honey														
Cracker	Cheddar/sandwich														
Bar	Nutrigrain/Rice														
Bar,granola	Peanut/Chocolate														
Donut															
Biscotti,															
Sweet roll, cinnamon															
Scone,															
Danish,															
Muffin,															
Pie															
Cake	Frosting/ NO														
Croissant	Butter/chocolate														
Biscuit	Restaurant/Homemade														
Chips Potato															
Cheetos															
Chips Corn															
Popcorn,	Type:														
Pretzel															
Other:															
Fat	**Circle one**														
Almonds	raw/roasted														
Cashews	raw/roasted														
Walnuts	raw/roasted														
Pecans	raw/roasted														
Peanuts,	raw/roasted														
Pistachios															
Other:															
Peanut butter															
Cream cheese	Reg./Soft														
Mayonnaise	Reg./Low fat														
Cream,	Heav/Whip/Sour														
Olive															
Salad dressing:															
Avocado															

Figure C.4 (Cont.)

Food frequency questionnaire CODE:_____

What fat do you use for cooking: a)None b) Oil c) Margarine d) Butter e) Non-Stick Spray f)Shortening

Foods, type and amounts			**AVERAGE USE LAST YEAR**											
	TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day
Margarine														
Butter														
Oil														
Deli Meat or Lunchmeats														
Beef, bologna														
Beef, corned brisket,														
Beef, frankfurter, Boiled/ Broiled/Grilled														
Ham, deli														
Sausage, (beef / pork links) Pan fried/BBQ/ Microwaved/Raw														
Beef, roast														
Beef, salami,														
Other:														
Beef														
Beef, ground,														
Pan-fried/ Broiled														
Grilled/ Oven														
Other:														
Beef, meatball,														
Beef, meatloaf,														
Beef, hamburger Homemade														
Pan-fried /Grilled														
Oven/ Broiled														
Other														
Beef, steak,														
Pan-fried														
Grilled														
Oven														
Broiled														
Other:														
Beef Stewed														
Pork														
Bacon, Fried/microwaved														
Bacon bits imitation														
Pork, roast,														
Pork, chop,														
Pork, ribs,														

Figure C.4 (Cont.)

Food frequency questionnaire CODE:_____

Foods, type and amounts			**AVERAGE USE LAST YEAR**										
	TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day
Chicken													
Grilled													
Oven													
Broiled													
Breaded													
Fried													
Roasted													
Boiled/stewed													
Other:													
Turkey													
Fish or seafood													
Shrimp													
Crabmeat													
Trout													
Salmon													
Other:													
Tuna, canned													
Other:													
Other meat:													
Cheese,													
American/American low fat													
Cheddar/cheddar 2%													
Swiss													
Mozarrella													
Cottage													
Parmesan													
Brie/feta													
Other:													
Eggs													
Scrambled/ omelet/ fried/ boiled margarine/butter/oil/spray													
Combination foods													
Pasta													
Ziti, baked													
Macaroni and cheese													
Salad, Italian pasta													
Potatoes roasted/hash browns													
Hot pocket													

Figure C.4 (Cont.)

Food frequency questionnaire CODE:_____

Foods, type and amounts			**AVERAGE USE LAST YEAR**										
	TYPE	AMOUNT	Never	1-6 per year	7-11 per year	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2 -3 per day
Chicken													
Grilled													
Oven													
Broiled													
Breaded													
Fried													
Roasted													
Boiled/stewed													
Other:													
Turkey													
Fish or seafood													
Shrimp													
Crabmeat													
Trout													
Salmon													
Other:													
Tuna, canned													
Other:													
Other meat:													
Cheese,													
American/American low fat													
Cheddar/cheddar 2%													
Swiss													
Mozarrella													
Cottage													
Parmesan													
Brie/feta													
Other:													
Eggs													
Scrambled/ omelet/ fried/ boiled margarine/butter/oil/spray													
Combination foods													
Pasta													
Ziti, baked													
Macaroni and cheese													
Salad, Italian pasta													
Potatoes roasted/hash browns													
Hot pocket													

Figure C.5. Food record.

Instructions for Recording the Food Records

1. Please eat as you usually eat, and record everything you ate or drank during the 24-hour time period indicated. Include place and time of meal when possible.
2. Describe the amounts consumed in terms appropriate to that item. For example: ounces (cup) of milk, tablespoon of French dressing, slices of bread (regular, small or large), pieces of fruit, etc. If you have ***measuring cups and measuring table/teaspoons*** use them when appropriate. For other foods like meat, cake, or pizza please use the ***FOOD PORTION SIZE BOOKLET*** (attached). For example, for slices of pizza or pie used the page for ***wedges***, for cake use the page with ***squares*** and for pancakes, cookies or tortillas use the page with ***circles***.
3. Describe how the food was cooked if known or can be estimated. For example, 1 egg, scrambled in margarine, 1 breakfast biscuit microwaved on high or 2 minutes, 6 oz. steak grilled, 3 oz. breast chicken fried and breaded.
4. Remember to include beverages, and anything you may add to them, and to include anything added to a food after it is prepared, such as margarine, catsup.
5. To the best of you ability described combinations or mixed dishes that were eaten. See examples below.
6. If you need additional space, use the back of the paper or attach additional sheets, and answer the questions at the bottom of the day's record.
7. You need to send by email (luevano1@illinois) or fax (217-333-9368) your first day of food record.

How to Record Each Food

Include:	For example:
How prepared	Fried, breaded, grilled, etc
Added Fats	Fried in butter, oil or pam
Brand names	Margarine (I can't believe it's not butter)
Describe each ingredient: Spaghetti & Meat Balls	1½ cups spaghetti, 4 meatballs (1" diam.), ½ cup Ragu spaghetti sauce, 1 Tbsp. parmesan cheese

Breakfast/Home /7:30 am	
Raisin bran with 2% Milk	1 measuring cup and 6 ounces milk
Toast with butter and jelly	1 piece and 1 teaspoon of regular butter, 2 Tbsp jelly
Coffee	1 Cup (8 oz.) and 2 teaspoon of cream
Lunch/Caulvert's/12:30	
1 small Cheeseburger and fries	3 oz. patty grilled, 1 slice of american cheese, 1 small package mayo, and 1 pac. Mostaza. Small order of fries.

Use the following guide to better estimate the amount of food you eat each time.

How to measure different portions using your hand	
1 FIST =	
	**1 cup** pasta, cereal, green salad, broccoli, cut-up fruit, mashed potato/baked potato

Figure C.5 (Cont.)

1 CUPPED HAND =

1 ounce of nuts or chips/pretzels, **½ cup** pasta, rice, or fruit salad, berries, applesauce, or coleslaw, potato salad, mashed potatoes, or cottage cheese.

1 PALM OF THE HAND =

3 ounces of cooked meat or canned fish.

The following pics could help you to estimate your intake when you have dinner out.

½ Cup Vegetables

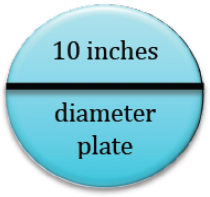
1 Cup Vegetables

½ Cup Corn

½ Cup Spaghetti

Q t

1 Cup Spaghetti



3 oz Beef

6 oz Beef

3 oz Pork loins

3 oz Chicken Breast

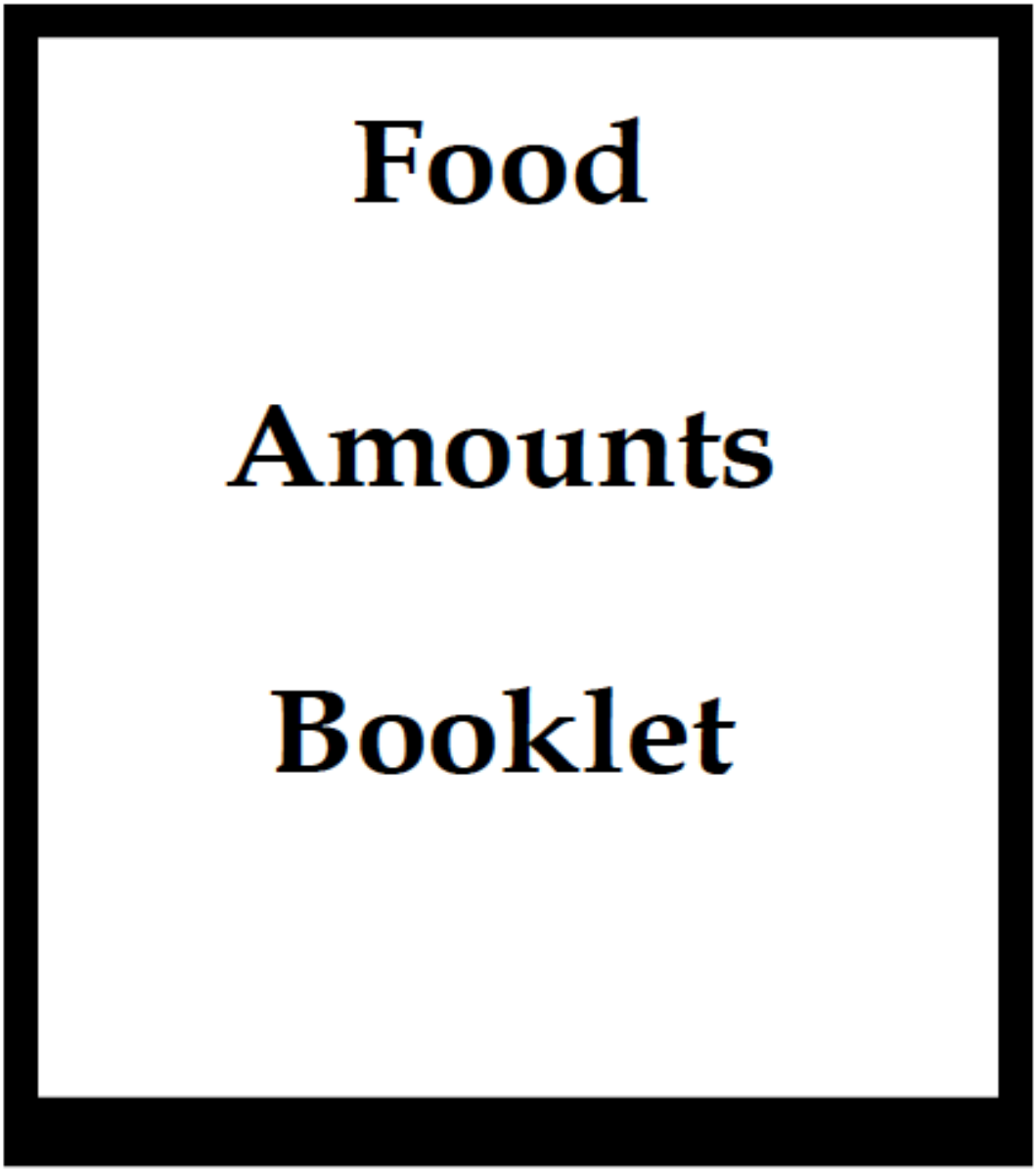
6 oz Chicken breast

Figure C.5 (Cont.)

Food or Beverage consumed / Place / Time *Please remember to include brands of foods and cooking type.*	Amount
Does this day's record represent your usual food intake? ___ Yes ___ No. If not, please explain.	

Figure C.6. Food Portion Booklet

Adapted from: Van Horn LV, Stumbo P, Moag-Stahlberg A, Obarzanek E, Hartmuller VW, Farris RP, Kimm SY, Frederick M, Snetselaar L, Liu K. The Dietary Intervention Study in Children (DISC): dietary assessment methods for 8- to 10-year-olds. J Am Diet Assoc. 1993 Dec;93(12):1396-403.
Modified by Alejandra Valencia, Mary Stevens, Nutrition Coordinating Center, University of Minnesota for the Hispanic Community Health Study, Study of Latinos, 2007.



Remember to use the food portion size booklet as a guide to calculate your portion size.

Adapted from modified version of Van Horn LV, et al. Nutrition Coordinating Center University of Minnesota.

Figure C.6 (Cont.)

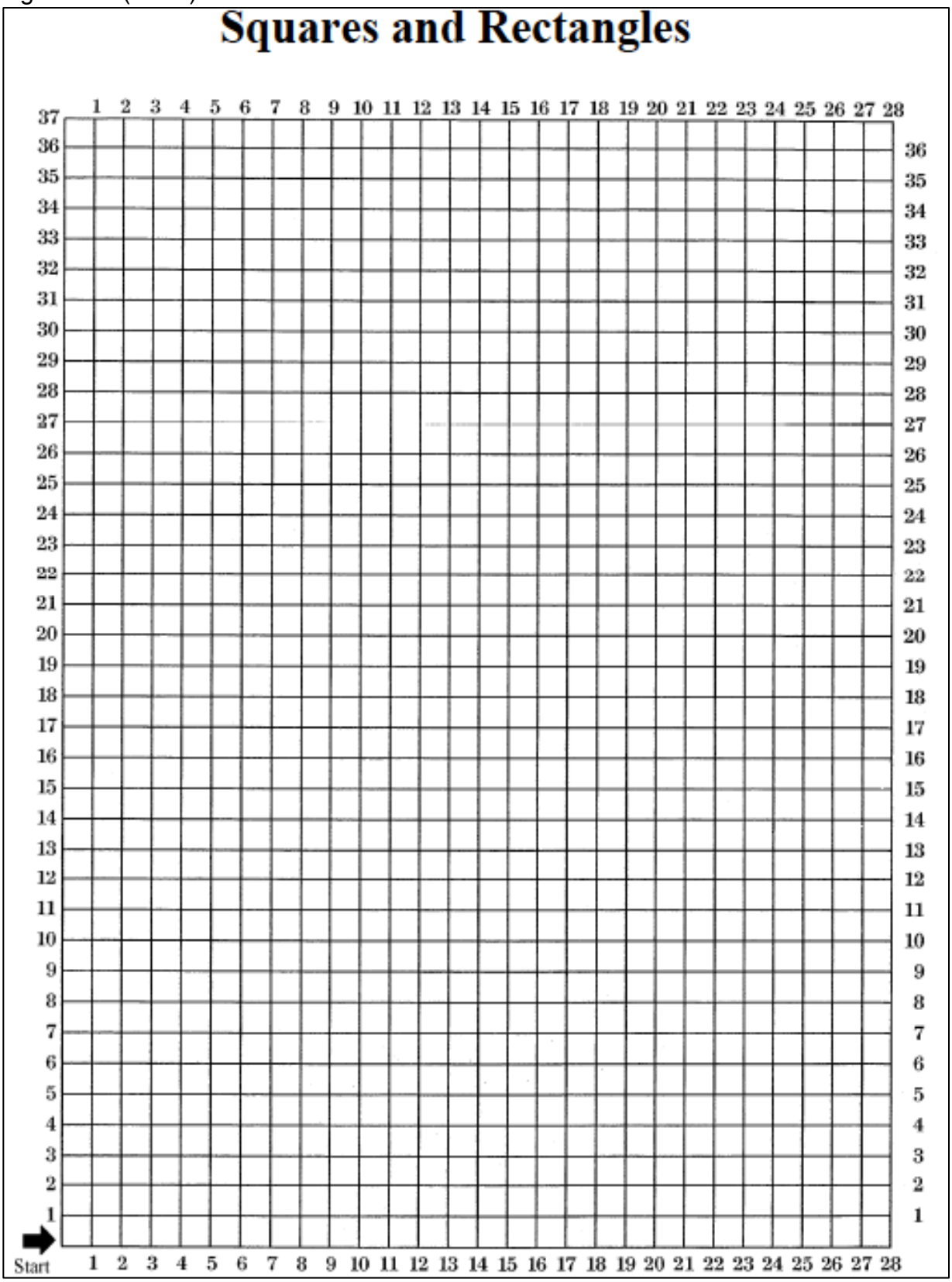


Figure C.6 (Cont.)

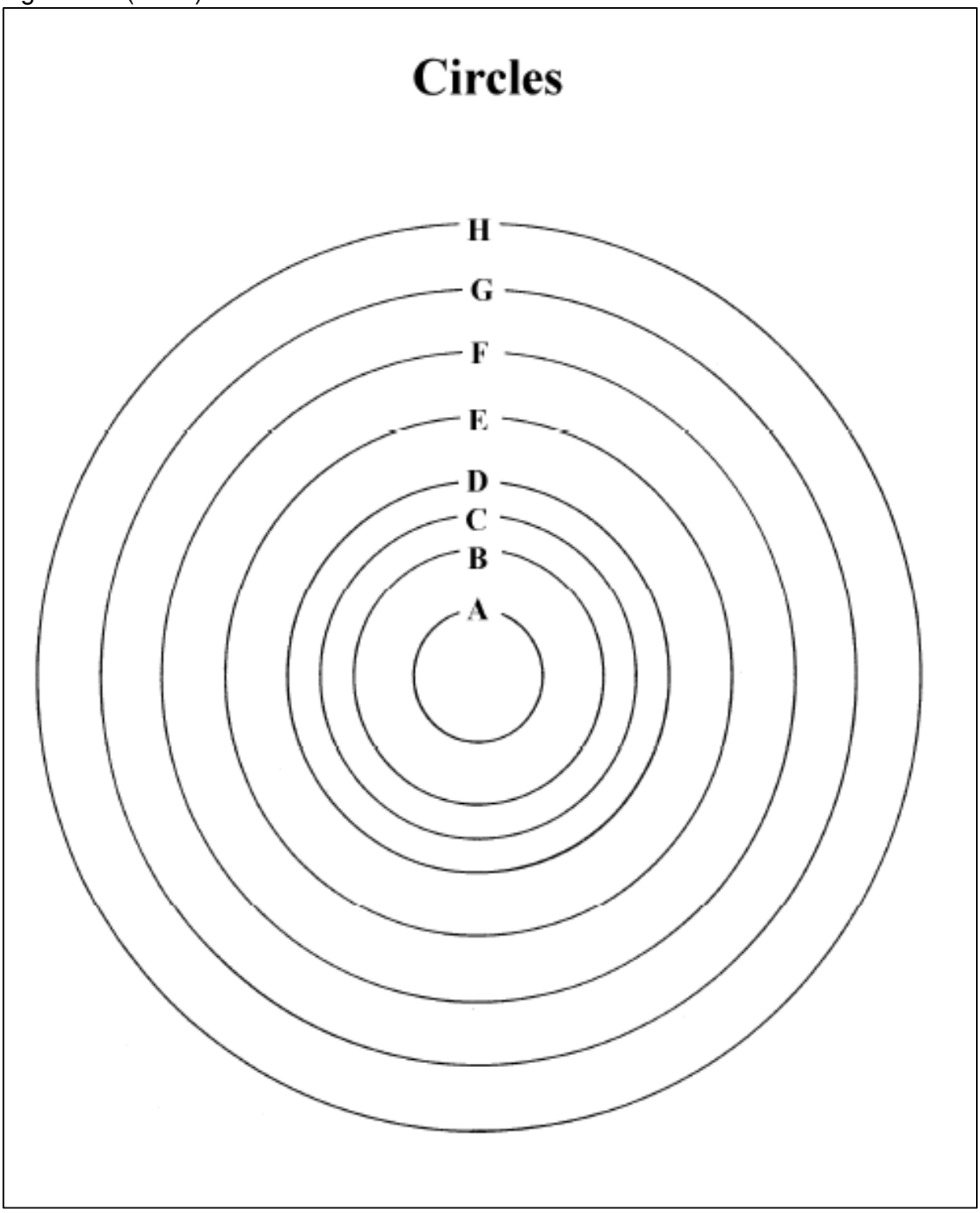


Figure C.6 (Cont.)

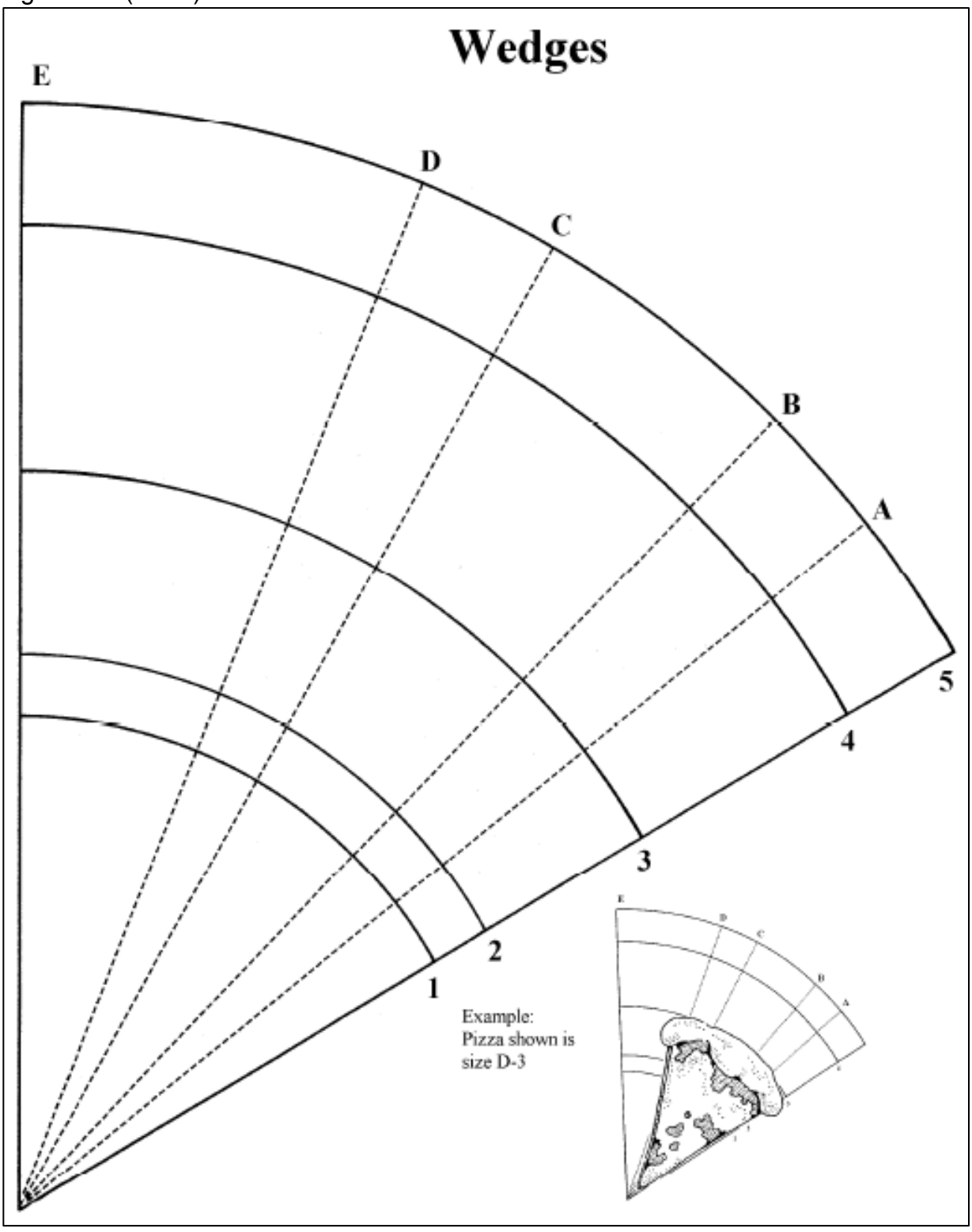


Figure C.6 (Cont.)

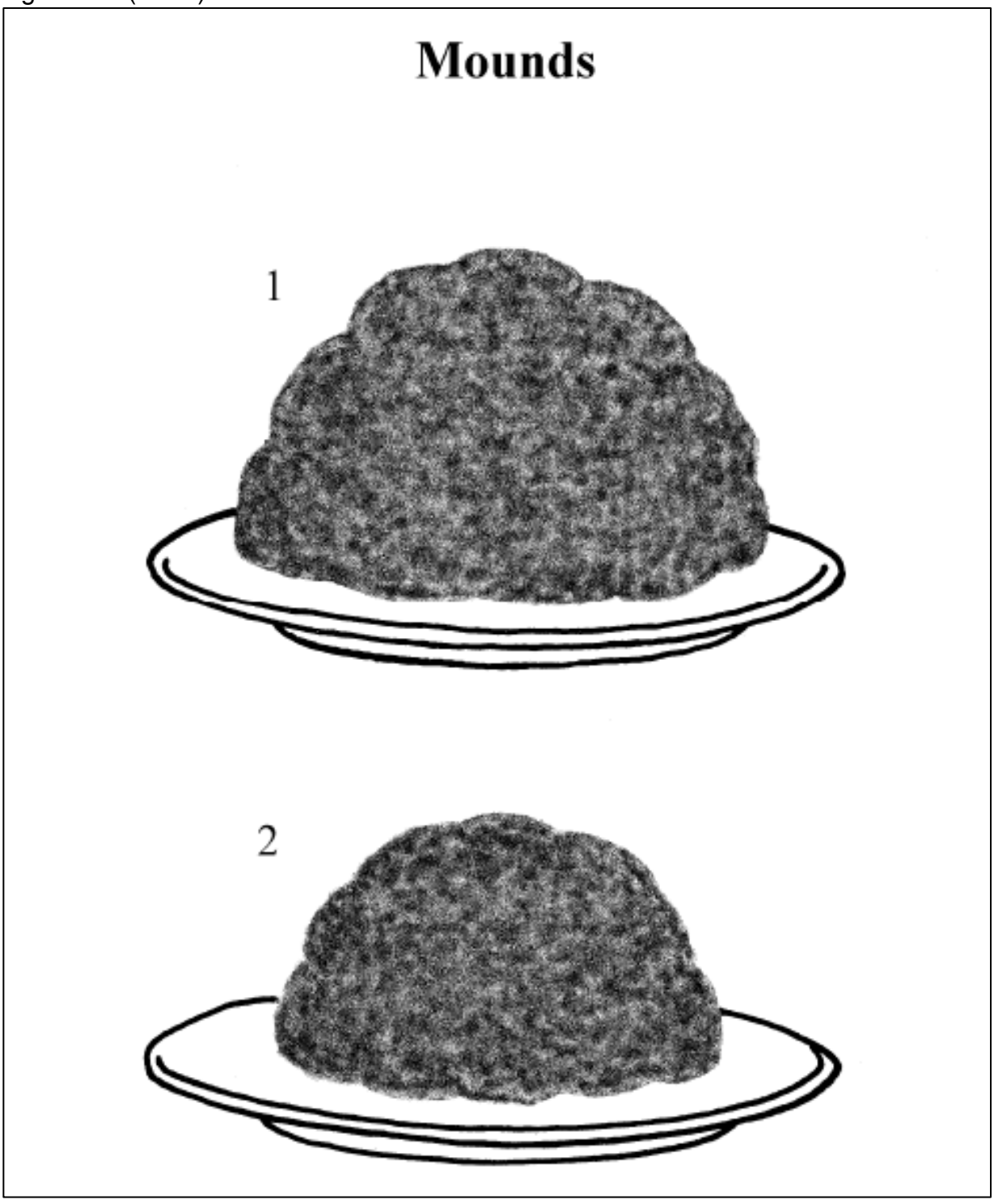


Figure C.6 (Cont.)

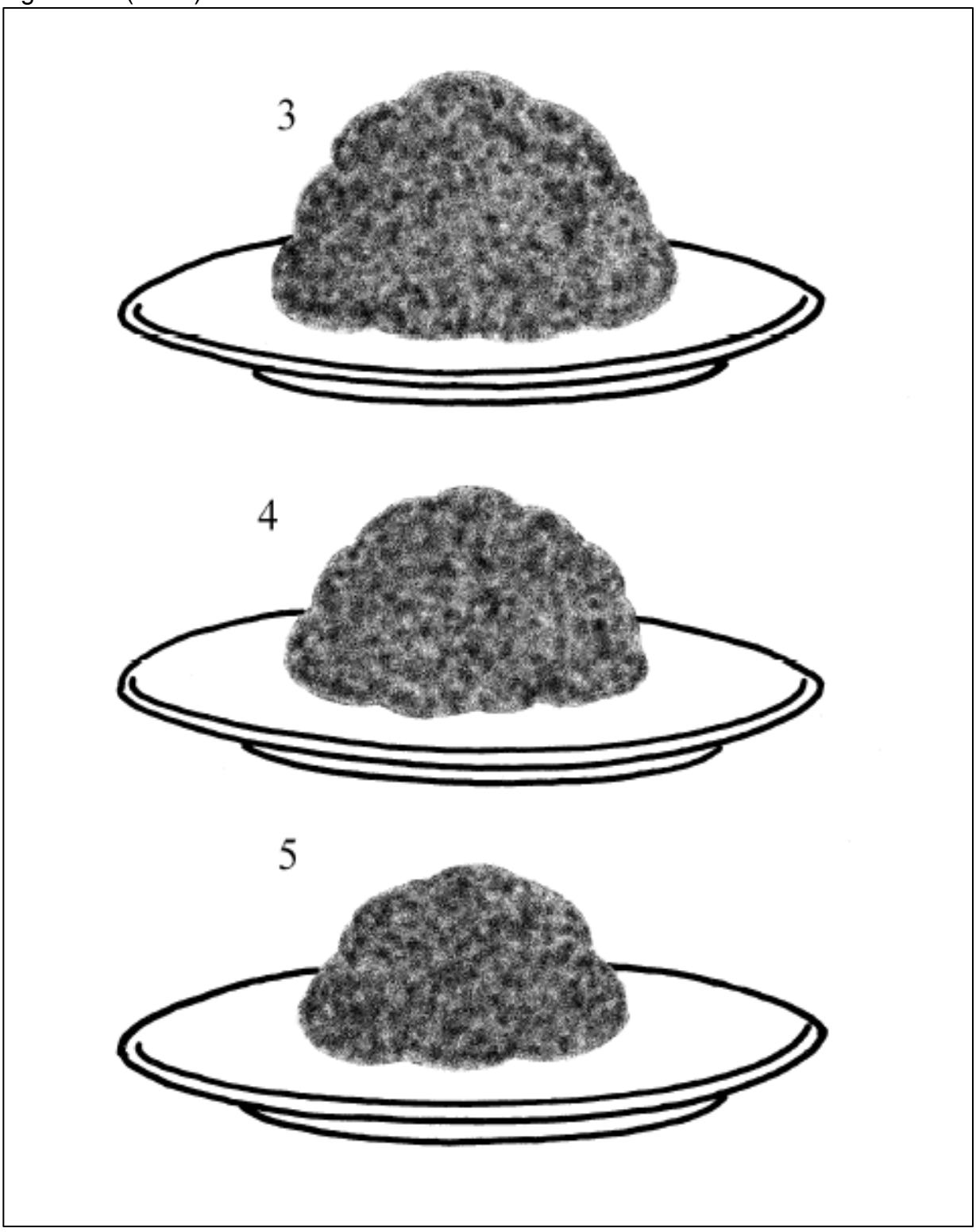


Figure C.6 (Cont.)

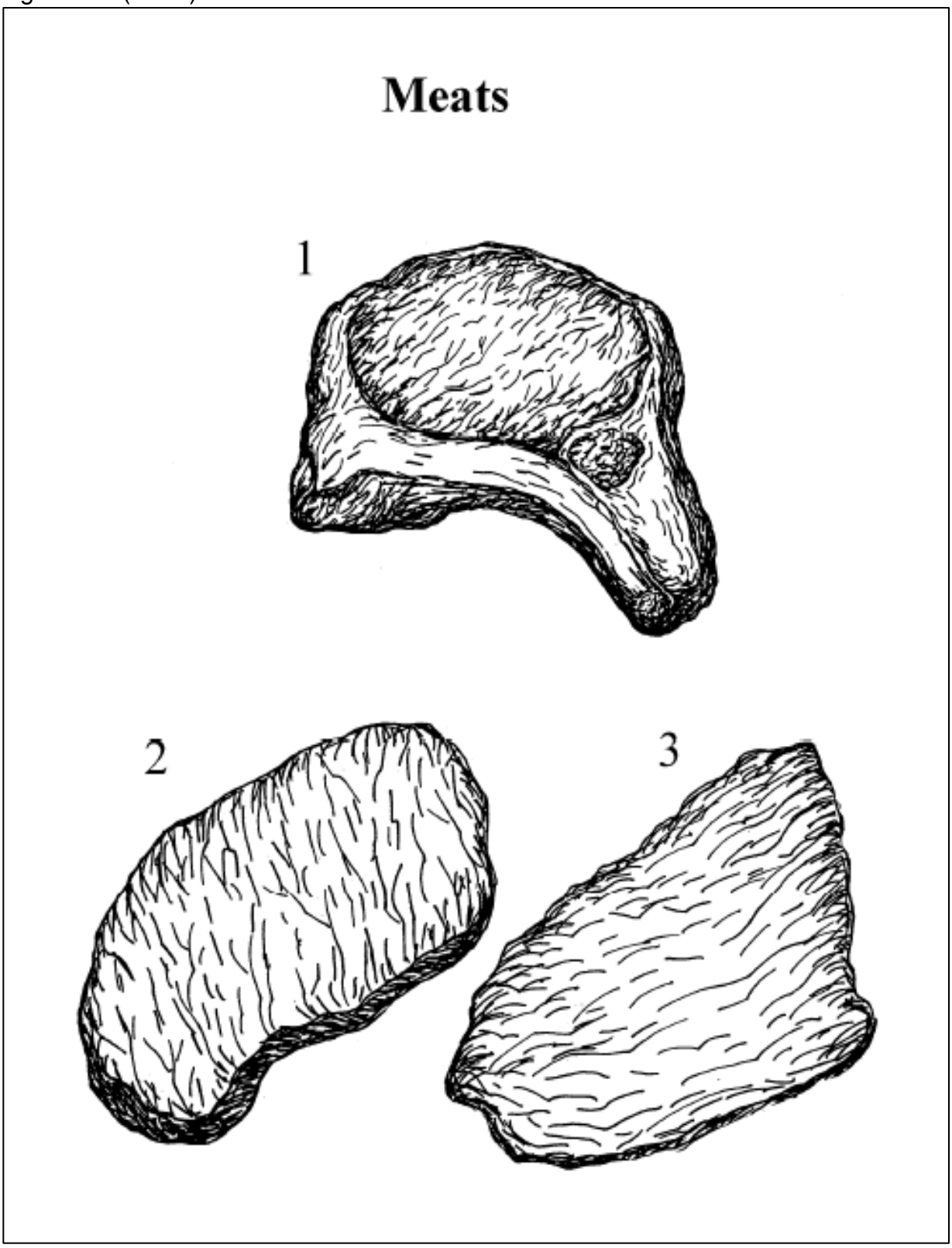


Figure C.6 (Cont.)

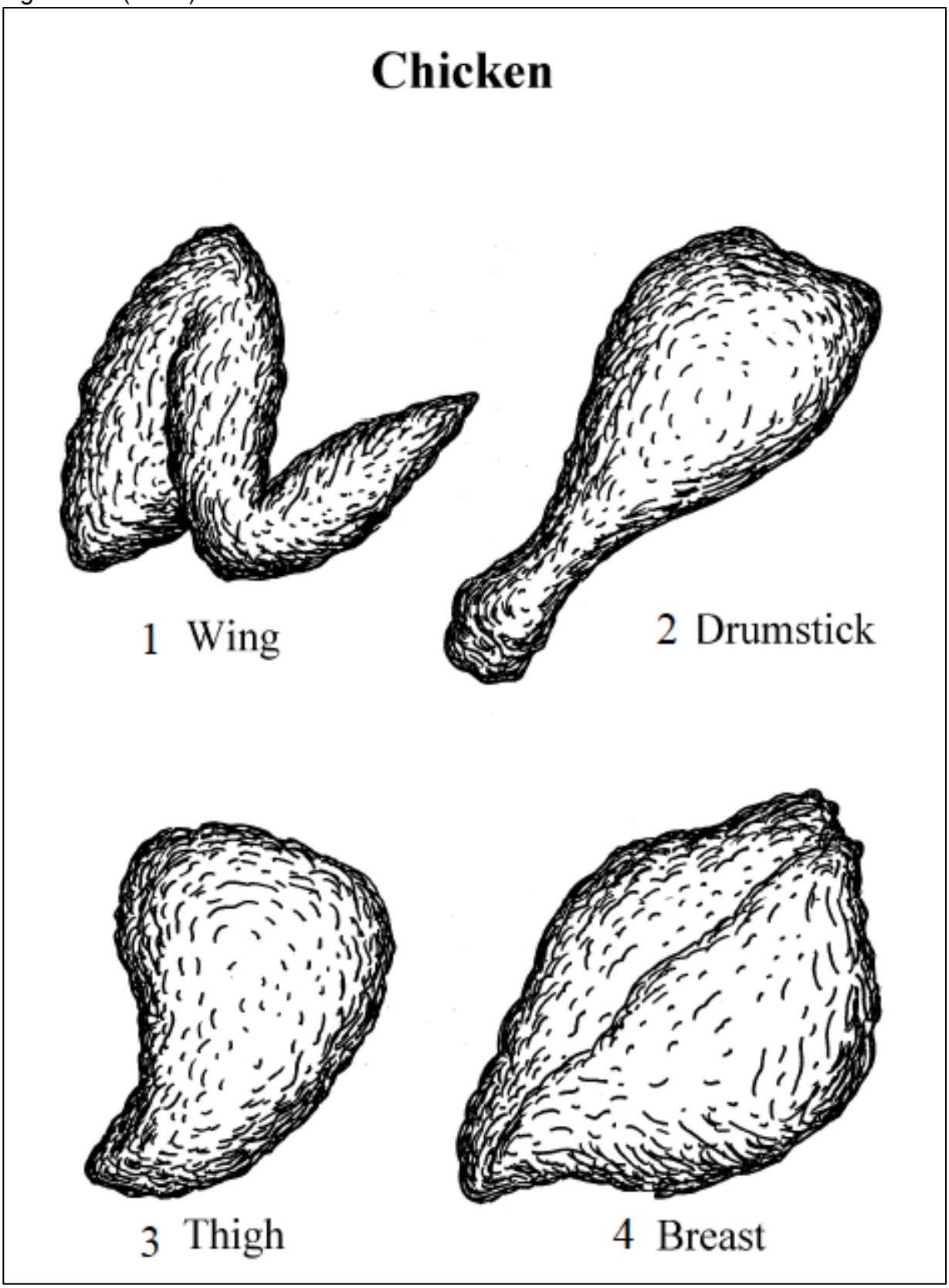
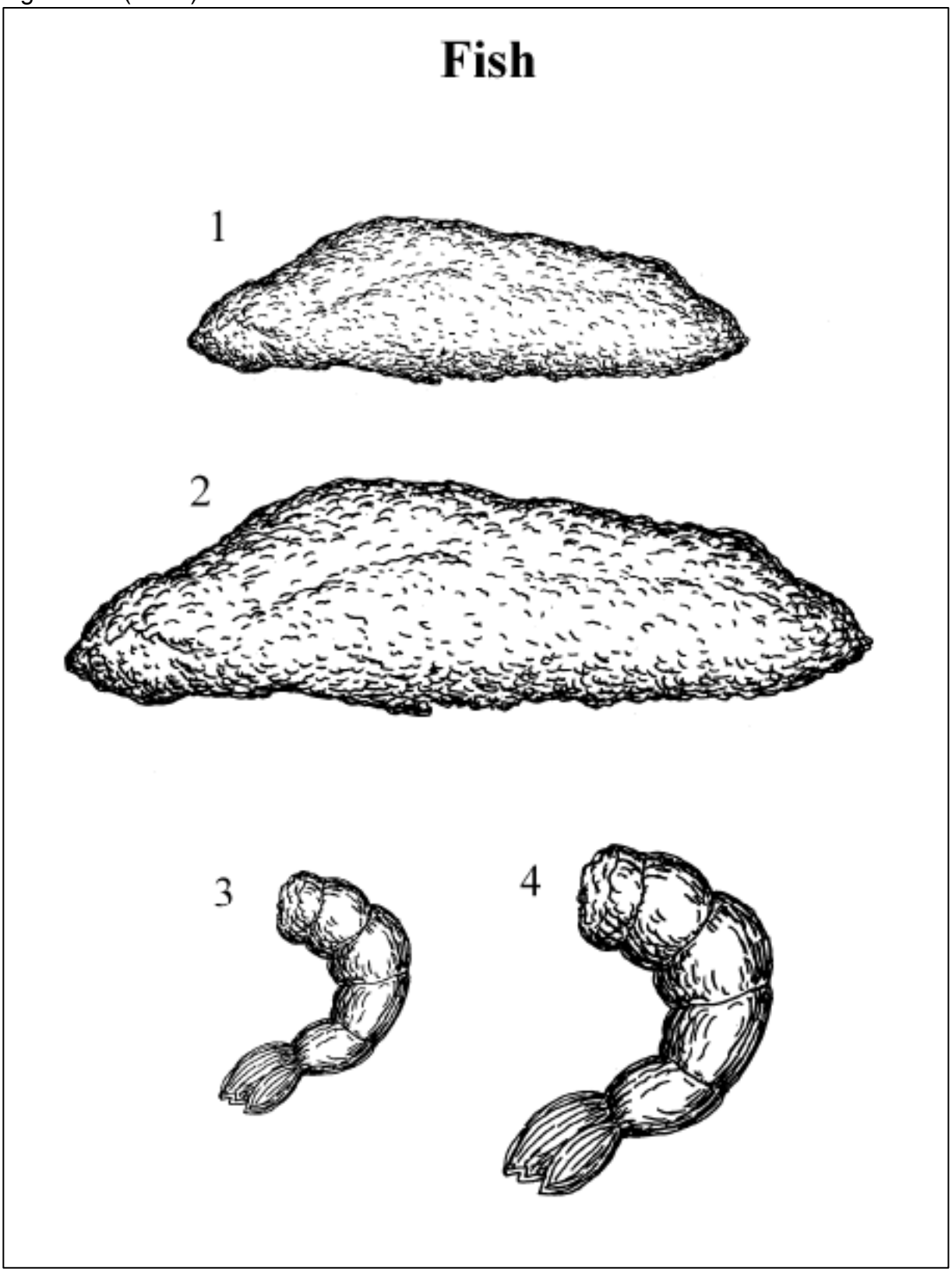


Figure C.6 (Cont.)



Appendix D
Questionnaires for Chapter 6

This appendix includes questionnaires used for the study "A Case-Control Study of Complications from Diabetes and Intake of Advanced Glycated End Products" presented on chapter 6. The material in this appendix includes Invitation Letter, Screening Tool, Inform Consent, and Diabetes Complications Data.

Figure D.1. Invitation Letter

Dear Patient,

We want to invite you to participate in a research project that is a joint project between the University of Illinois and Carle. The research study is called *A Case-Control Study of Complications from Diabetes and Intake of Advanced Glycated End Products*.

Advanced Glycation End Products are found in some foods rich in proteins and fat and that are fried, broiled or grilled. We want to see if eating very high or very low levels of these foods is associated with complications of diabetes.

To be in the study you must be over 18 years old, have diabetes, to not be a smoker, and be willing to keep a food intake record for 7 days. The expected duration of the study will be ten days in which you have to record your entire food intake.

You will receive $50 as compensation for keeping the food records and for allowing indicators of your health to be released to the investigators. Those indicators include your hemoglobin A1c, your blood pressure, if you have albumin in your urine, your blood lipid levels, whether you have indications of eye, kidney or nervous tissue damage that may be caused by your diabetes.

If you would like to participate or have questions about participating, please call or email Dr. Karen Chapman-Novakofski at 217-244-2852, kmc@illinois.edu or Claudia Contreras at luevano1@illinois.edu.

Thank you

[physician name]

Figure D.2. Screening Tool.

Screening Tool

Name:_____ **Date:**_____

Cognitive Evaluation:

4. What is the day of the week?
5. Do you know where you are?
6. What is your name?

If person **does not know** the answer to any of the questions, thank them for their time and tell them they are not eligible.

Inclusion criteria:

4. Are you 18 years old and older?
5. Do you have type 2 diabetes?
6. Have you had type 2 diabetes for 3 to 10 years?
7. Are you willing to write down what you eat for a 7-day timespan?
8. Do you have a phone, can you meet with one of the investigators, or can you email your first day's record so we can review any problems with the completeness of the record?
9. Can you read?

If person responds **no** to any questions above, thank them for their time and tell them they are not eligible.

Exclusion criteria:

1. Are you a vegan or vegetarian?
2. Are you following a protein-restricted diet for any reason?
3. Are you blind?
4. Do you smoke?
5. Are you pregnant?
6. Are you breastfeeding?

If person responds **yes** to any questions above, thank them for their time and tell them they are not eligible.

Patient is eligible to participate in study
Patient is ineligible to participate in study

Figure D.3. Inform Consent.

Title of Project: A Case-Control Study of Complications from Diabetes and Intake of Advanced Glycated End Products.

Responsible Principal Investigator: Dr. Karen Chapman-Novakofski, University of Illinois at Urbana-Champaign

1. **Purpose of the study**: This is a research study looking at some food components found in foods rich in protein or fat that when are fried, broiled or grilled could be associated to diabetes complications. We will measure these components on foods with a food frequency questionnaire and with 7-days of food records. In addition, we will need information from your physician regarding diabetes related cardiovascular complications, as well as a list of diabetes-related medications, including those related to cardiovascular, renal, eye, or peripheral conditions. Specifically we will need information regarding presence of myocardial infarction, angina, coronary angioplasty, heart failure, cardiovascular bypass, stroke, transient ischemic attack, claudication, and peripheral vascular disease. In addition, results of a complete lipid profile (total cholesterol, LDL-cholesterol, VLDL- cholesterol and triglycerides), and urine albumin and proteins lab results will be requested, if available, to assess for renal disease complications. The purpose is to find if there is an association between the diabetes-related complications and the food components that we are measuring.

2. **Procedures to be followed**: After you have been screened for eligibility in this project (able to read and write and answer a couple basic questions) and you consent to participate, you will be briefly trained on how to complete a food frequency questionnaire (5 minutes). This training can be completed at the investigator's office. We will read with you the instructions for completing the food frequency questionnaire. In addition, we will provide you with food models or pictures of foods' portion size that could help you to answer the questionnaire. You will fill out the food frequency questionnaire in this first meeting (approximate time is 35 minutes). Then, you will receive training about how to fill out the food records (5 minute). You will be given written instructions, a food portion booklet and food records forms for 7 days. You also will receive two additional surveys to fill out at home (Diabetes History and Vitamins Intake). At the end of this first meeting we will set an appointment to see you again in two weeks. The total time for the first meeting is around 50 minutes. We will ask you to start filling out the food records a day after our first meeting. After you fill out the first food record, one of the investigators will review it with you to make sure you understand all the information about the food we need. This can be by e-mail, by fax or by telephone. All seven days must be recorded, but they do not need to be consecutive in case a special occasion arises. Each food record will take around 15 minutes to fill out, and the additional surveys (Diabetes History and Vitamins Intake) around 10 minutes each. When you finish your food records and surveys you need to meet again with the investigator in person, to review the completeness of the food records. This last meeting will take around 10 minutes. Therefore, for this research your total time commitment will be around 3 hours.

3. **Discomfort and Risks and Costs**: If you choose to visit the investigator in person, you may have transportation costs. If you use your telephone or internet service to send food records, you could have a charge to participate. There are not risks outside of those you may encounter in everyday life.

Figure D.3 (Cont.)

4. **Benefits**: There are not personal benefits to you for participating in this study. However, your participation may help us to determine if the food components that we are studying are associated with increased complications secondary to diabetes.

5. **Statement of confidentiality**: All the questionnaires and food records will be coded. A key with your code and your name will be kept in a locked cabinet at the investigators office until all the questionnaires are completed. The identifying key will then be shredded. We will use screening forms for evaluating eligibility for this study. Your consent form and the screening form will also be kept locked in the investigator's office. At the end of the study the screening form will also be shredded. The results of this research could be published in professional meeting or journals, but no personal information will be shared.

6. **Whom to contact:** Please contact Dr. Karen Chapman-Novakofski (217-244-2852, kmc@illinois.edu) or Claudia Luevano (217-974-0710, luevano1@illinois.edu) if you have any question or concerns. You may also contact us if you feel you have been injured or harmed by this research. If you have any question about your rights as a participant in this study, please contact the University of Illinois Institutional Review Board at 217-333-2670 or via email at irb@illinois.edu.

7. **Compensation**: If you complete the food frequency, the food records and the additional surveys you will receive $50. If you complete only the food frequency and additional surveys you will receive $25, and at the last meeting after you turn in the 7-day of food record, you will received another $25.

8. **Voluntariness:** Your participation is voluntary. You may discontinue at anytime. The decision to participate, decline, or withdrawn from participation will have no effect on future relations with the University of Illinois.

- I am 18 years of age or older.
- I have read and understand the above consent form and voluntarily agree to participate in this study.
- You will be given a copy of this consent form for your records.

____I will initial here if my physician can share information regarding diabetes-related complication with the investigators. I understand that they will not have direct access to my medical records.

_____ _____

Participant signature Date

Figure D.4. Diabetes complications data.

CODE:_____			
			Values:
HbA1c			
Glucose levels			
Triglycerides			
Cholesterol			
LDL, low-density lipoprotein cholesterol level			
Serum Creatinine			
Albumin			
Actual Medication			
Weight			
Height			
Arterial Pressure			
	Yes	No	
Cardiovascular disease			
Angina			
CABG, coronary artery bypass graft;			
CAD, coronary artery disease;			
CHF, congestive heart failure;			
CVA, cerebrovascular accident;			
CVD, cardiovascular disease;			
HTN, hypertension;			
LVH, left ventricular hypertrophy;			
MI, myocardial infarction;			
PVD/peripheral vascular diseases			
Amputation			
Ulcer or infection			
Gangrene			
Acute ischemic foot			
Bypass for PVD			
Diminished sensation			
Intermittent claudication			
Abnormal NIVS, noninvasive vascular studies;			
Eye disease complications:			
Renal disease complications:			
Proteinuria			
Microalbuminuria			