SERUM FATTY ACIDS AND RISK OF ISCHEMIC STROKE IN POSTMENOPAUSAL WOMEN

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ABSTRACT

SIRIN YAEMSIRI: Serum Fatty Acids and Risk of Ischemic Stroke in Postmenopausal Women
(Under the direction of Ka He)

Ischemic stroke is a major cause of death and disability among postmenopausal women. One of the risk factors of ischemic stroke is diet, including dietary fat intake.

It has been established that intake of trans and saturated fatty acids are positively associated with coronary heart disease, whereas monounsaturated and polyunsaturated fatty acids are inversely associated. Despite the expectation that these associations would be consistent for ischemic stroke, this has not been the case in studies using participant reported measures of fatty acid intake. Rather, studies using biomarkers of fatty acid intake, such as serum, have had more success in finding the expected associations. This dissertation attempts to explain these inconsistent results through two aims.

The first aim was to estimate the correlations between individual serum fatty acids and fatty acid intakes, which may partially explain previous inconsistent results from studies using different fatty acid assessment methods. The participants were 925 women from the Women's Health Initiative Observational Study (WHI-OS) of postmenopausal US women. Serum fatty acid composition was determined from a fasting serum sample collected at enrollment. Fatty acid intakes were measured using a 122-item food frequency questionnaire also at enrollment. Individual fatty acids with the highest Spearman rank correlations between serum and dietary intake were docosapentaenoic (DHA), eicosapentaenoic (EPA), and 18:1*t* fatty acid. Serum saturated, monounsaturated, and n6 polyunsaturated fatty acids were not correlated with the corresponding intake measures.

This work highlights the need for further research to find suitable biomarkers for intakes of individual fatty acids.

The second aim was to estimate the association between individual serum fatty acids and incidence of ischemic stroke and ischemic stroke subtypes. We conducted a case-control study nested in the WHI-OS. Incident ischemic stroke cases were centrally adjudicated, classified by etiologic subtype, and matched (1:1) to controls for a total of 964 case-control pairs. Serum linelaidic, palmitic, and oleic acids were associated with higher incidence of ischemic stroke, while serum EPA, docosapentaenoic (DPA), DHA, and arachidonic acids were associated with lower incidence. These associations were generally consistent for atherothrombotic and lacunar, but not cardioembolic, stroke. This work highlights the importance of individual fatty acids in the development of particular subtypes of ischemic stroke.

This dissertation brings to attention the areas for future research. First, there is a need to find suitable biomarkers for individual fatty acid intakes. Second, future studies should explore the associations between individual fatty acids and disease, not only focusing on fatty acid groups. Third, the heterogeneous etiology of ischemic stroke suggests that ischemic stroke subtypes should be examined separately. Lastly, public health initiatives to increase the intake of DHA and EPA and reduce intake of 18:1t may reduce the incidence of ischemic stroke among postmenopausal US women.

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95% CI	95% confidence interval
AHA	American Heart Association
ANOVA	analysis of variance
BMI	body mass index
CHD	coronary heart disease
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
N	omega
OR	odds ratio
US	United States
WHI-OS	Women’s Health Initiative Observational Study

I. INTRODUCTION

Ischemic stroke and coronary heart disease (CHD) are both atherosclerotic disorders and share many of the same risk factors, but surprisingly limited epidemiologic data indicate that dietary fat intake may have different associations with ischemic stroke than with CHD. It has been well established that trans and saturated fats are positively associated with coronary heart disease, whereas monounsaturated and polyunsaturated fats are inversely associated with CHD. Despite the expectation that these associations would be consistent for ischemic stroke, several large cohort studies using participant reported measures of fatty acid intake reported null or inverse associations between saturated fat intake and ischemic stroke. Rather, case-control studies using biomarkers of fatty acid intake, such as serum, have had more success in finding the expected associations with ischemic stroke. In this dissertation, we investigated possible explanations for the inconsistencies across (i) cohort studies of dietary fat intake and CHD versus ischemic stroke and (ii) studies measuring dietary fat intake through participant report versus fatty acid biomarkers.

The inconsistencies across cohort studies could be due to individual fatty acids having different associations with ischemic stroke. We hypothesized that fatty acids may play a role in the development of some ischemic stroke subtypes, but not others. Fatty acids have a powerful effect on serum cholesterol and there is evidence that serum cholesterol may play a role in the development of some ischemic stroke subtypes, but not others. Therefore, examining ischemic stroke as a composite outcome would not be appropriate and would attenuate expected associations. In addition, examining ischemic stroke subtypes based on etiology may provide insight on the mechanism of ischemic stroke development. Despite

these motivations, data on ischemic stroke subtypes were available in few studies. Statistical power was limited for the studies where such data were available.

The inconsistencies between studies measuring dietary fat intake through participant report versus fatty acid biomarkers could be due to their respective methods of measuring dietary fat intake. The cohort studies estimated fatty acid intake through participant report and food frequency questionnaires (FFQ), a measure that is subject to substantial error. Participants may be reluctant or unable to report their habitual food intake accurately. Further, nutritional databases that convert foods to nutrients may not be updated to reflect the continuously changing fatty acid content of food items. Measurement of the composition of fatty acids in serum in case-control studies has been proposed as an alternative to the FFQ. However, serum fatty acids reflect metabolic processes and endogenous synthesis of fatty acids after intake. Therefore, it is unclear which serum fatty acids correlate with long-term fat intake as measured by the FFQ among postmenopausal US women. Few studies have examined the correlations between serum fatty acids and fatty acid intakes in this population.

Another difference between the cohort and case-control studies is that the cohort studies focused on fat intake groups. However, individual fatty acids within the same group may have different, and sometimes opposing, effects on cardiovascular diseases. The effect of grouping these fatty acids may dilute possible associations for individual fatty acids and ischemic stroke. Previous studies that were able to examine individual fatty acids were limited by statistical power, with less than 150 cases of ischemic stroke.

To address these concerns, the aims of the proposed study were (1) to examine the correlations between serum fatty acids and fatty acid intakes and (2) to estimate the associations between individual serum fatty acids and ischemic stroke and its subtypes. We used data from the Women’s Health Initiative Observational Study (WHI-OS) of postmenopausal US women.

II. SPECIFIC AIMS

We hypothesized that serum fatty acids that are not endogenously synthesized in large amounts (polyunsaturated fatty acids, trans fatty acids, and odd-numbered saturated fatty acids) would have higher correlations with fatty acid intakes than other serum fatty acids.

To test this hypothesis, we estimated the correlations between serum fatty acids and fatty acid intakes (Aim 1).

We hypothesized that higher levels of serum saturated (myristic, 14:0; and palmitic, 16:0) and trans fatty acids (18:1*t*; and linelaidic, 18:2*tt*) were associated with increased incidence of ischemic stroke.

We hypothesized that higher levels of n3 (alpha-linolenic acid, 18:3n3; EPA, 20:5n3; DPA, 22:5n3; and DHA, 22:6n3) and n6 (linoleic acid, 18:2n6; and arachidonic acid, 20:4n6) polyunsaturated fatty acids were associated with decreased incidence of ischemic stroke.

We hypothesized that associations for total ischemic stroke would be consistent for atherothrombotic stroke, but not other types of ischemic stroke.

To test these hypotheses, we estimated the associations between individual serum fatty acids and ischemic stroke and ischemic stroke subtypes (Aim 2).

III. SIGNIFICANCE

Ischemic stroke is a major cause of death and disability among postmenopausal women. There are no reliable warning signs and treatment is difficult and time-limited emphasizing the importance of the primary prevention of ischemic stroke. In contrast to CHD, which is directly associated with dietary fat intake through its effect on blood cholesterol, there is no clear association between types of dietary fat intake and ischemic stroke. Cohort studies that have examined this association may be limited by their dietary fat intake assessment, their examination of types of fat as a composite exposure, and total ischemic stroke as a composite outcome. Case-control studies of biomarkers of dietary fat intake were limited by the small number of ischemic stroke events. There is an opportunity to examine the association between the composition of serum fatty acids and ischemic stroke subtypes among postmenopausal women to address these concerns.

A. High burden of stroke among postmenopausal women

In the US, stroke is the fourth leading cause of death, behind heart disease, cancer, and chronic lower respiratory disease, and is responsible for over 134 thousand deaths annually (Roger et al., 2012). One in five middle-aged individuals who experience a first stroke will die within one year. Of those who survive, a quarter will require institutional care and 20% to 50% will be permanently disabled. Therefore, the key to reducing stroke mortality and morbidity is not treatment, but primary prevention. This dissertation focuses on ischemic strokes, which make up 87% of incident strokes in the US.

Although age-specific stroke incidence and mortality rates are higher in men than women, stroke affects a greater number of women, a trend that is expected to continue (Reeves et al., 2008). Each year, 55 thousand more women than men have a

stroke, and women make up 60% of annual stroke mortality (Roger et al., 2012). Over the past half century, the 30-day stroke mortality rate has not improved in women (Carandang et al., 2006). After a stroke, a greater proportion of women than men have moderate to severe disability at discharge and women have a lower probability of achieving independence.

The primary reason that the burden of stroke is higher in women is that they live about 7 years longer than men. Stroke typically occurs at a later age, the majority occurring after menopause in women (Roger et al., 2012), its risk doubling every decade of life after age 55. However, even comparing women and men in the same age group, women still have higher risk of stroke than men. In a nationally representative survey, women age 45 to 54 years of age were more than twice as likely as men in the same age group to have suffered a stroke (Towfighi et al., 2007). In addition, factors other than age have been linked with increased risk of stroke in women, such as use of hormone replacement therapy (Viscoli et al., 2001; Rossouw et al., 2002; Wassertheil-Smoller et al., 2003; Hendrix et al., 2006), experiencing early menopause (Lisabeth et al., 2009), pregnancy (Kittner et al., 1996), and atrial fibrillation (a stronger stroke risk factor in women than men) (Wolf et al., 1991). Women and older women specifically are underrepresented in stroke studies. These statistics point to a need to study the etiology of stroke in older women, who share a disproportional burden of stroke risk.

B. Opposite trends in CHD and stroke mortality

Ischemic stroke and CHD are both atherosclerotic disorders and share many of the same risk factors, but they seem to differ in their associations with dietary fat intake. Trans and saturated fat intakes are positively associated with CHD and monounsaturated and polyunsaturated fat intakes are inversely associated with CHD through their respective effects on serum cholesterol and atherosclerosis. The associations between dietary fat intake and ischemic stroke are not as clear.

The association between dietary fat intake and ischemic stroke has been debated since

the 1950s when Tavia Gordon observed that CHD mortality rates were higher and vascular lesions affecting the central nervous system were lower among US whites than Japanese (Gordon, 1957). Reducing the possibility that these trends were due to genetic makeup, a similar gradient was found where Japanese living in the US had higher CHD mortality than those living in Japan, and again the inverse was true for vascular lesions (**Figure 1)**. Japanese living in Hawaii occupied the intermediate position in both cases. Concluding that the opposite trends were due to environmental or cultural differences, Gordon wrote, "We are faced with a serious medical puzzle…What mechanism would diminish the effect of atherosclerosis of the coronary artery while increasing its effect on the cerebrovascular system?"

Saturated fat intake was proposed to explain the opposite trends of CHD and stroke mortality found in Japan and the US. The Ni-Hon-San study confirmed Gordon's findings using standardized criteria to classify causes of death and noted that CHD mortality rates increased dramatically with higher saturated fat intake, while stroke rates followed the opposite pattern (Kato et al., 1973; Worth et al., 1975). Using data from 21 industrialized countries, Renaud summarized that dairy fat, which is rich in saturated fat, was positively related to CHD mortality. In the same countries, an inverse association between intake of dairy products and stroke mortality in men and women was observed (**Figure 2)** (Renaud, 2001). Nevertheless, a positive correlation between saturated fat intake and stroke mortality was reported in a survey performed in 17 countries (Sasaki et al., 1995). While ecologic studies can explore broad geographic and temporal differences in dietary habits and generate hypotheses, ecologic data are prone to be confounded by various factors.

C. Dietary fat intake and ischemic stroke

To address the possible confounding in the ecologic data, the associations between dietary fat intake and ischemic stroke were examined next in cohort studies. These cohort studies used FFQs, a participant reported measure of fatty acid intake. However, they did

not find the expected associations between saturated, monounsaturated, polyunsaturated fat intake and ischemic stroke. In addition, few studies examined trans fat intake.

Kagan and colleagues observed an inverse relation between total fat intake and incident ischemic stroke in 7,895 male Japanese or Japanese Americans living in Hawaii (154 thromboembolic strokes) over 10 years of follow-up (Kagan et al., 1985). In addition, Gillman and colleagues followed 832 middle-aged men (61 ischemic strokes) in the Framingham Heart Study for 20 years and found that intakes of total, saturated, and monounsaturated fat, but not polyunsaturated fat, were inversely associated with risk of ischemic stroke.

Subsequent cohort studies included a greater number of ischemic strokes, but did not detect significant associations. A 15-year follow-up study of 954 male and 1,329 female Japanese patients (75 cerebral infarctions) reported statistically nonsignificant associations for total fat and types of fat (Seino et al., 1997). However, the direction of the associations suggested that they were inverse for total, saturated, and monounsaturated fat and positive for polyunsaturated fat. Another cohort study of Japanese men and women (60 fatal cerebral infarction) observed that animal fat intake, mostly saturated fat, was inversely associated with cerebral infarction death, but found no significant associations for total, monounsaturated, or polyunsaturated fat intake (Sauvaget et al., 2004). Two large cohort studies of male and female healthcare professionals, the Health Professionals Follow-up Study and the Nurses' Health Study with 455 and 385 cases of ischemic stroke, respectively, did not find significant associations for total fat, cholesterol, or specific types of fat and ischemic stroke after adjustment for potential confounders and major lifestyle variables (Iso, Stampfer, et al., 2001; He et al., 2003). A large cohort study of postmenopausal US women also did not find significant associations for total, saturated, monounsaturated, or polyunsaturated fat intakes and ischemic stroke (Yaemsiri et al., 2012 [in press]). However, investigators did report a significant positive association between trans fat intake and ischemic stroke that was driven by lacunar stroke. Lacunar strokes are

strokes that occur in the smaller intracerebral arteries of the brain and are primarly caused by high blood pressure. A detailed description of lacunar strokes may be found in Section IV. D. 3.

The results of the cohort studies described here were generally not consistent with the expected associations based on CHD studies. We suspected that the inconsistent results were due to not examining ischemic stroke subtypes and not examining fatty acids individually. Assuming that serum cholesterol and development of atherosclerosis are the likely mediators of the association between dietary fat intake and ischemic stroke, then combining strokes with and without an atherogenic basis would not be appropriate. There is evidence that the associations between dietary fat intake and ischemic stroke are not consistent across ischemic stroke subtypes (Iso et al., 2002; Yaemsiri et al., 2012 [in press]). However, most of these cohort studies only examined ischemic stroke as a composite outcome. In addition, there is evidence that individual fatty acids, even within the same group, may have different effects on the risk of cardiovascular disease. However, these cohort studies only examined types of fat, not individual fatty acid intakes.

D. Biomarkers of Fatty Acid Intake and Ischemic Stroke

Case-control studies using biomarkers of fatty acid intake were able to examine fatty acids individually. These studies reported associations between fatty acid biomarkers and ischemic stroke that were generally consistent with CHD studies. However, these case-control studies were limited by statistical power. None included more than 125 cases of ischemic stroke. The low statistical power increased the chance that an association between a fatty acid and ischemic stroke would not be detected. In addition, none of these case-control studies examined trans fatty acids.

In the 1980s, Miettinen and colleagues conducted a nested case-control study to explore the difference in serum fatty acid concentration measured at baseline between ischemic stroke patients and healthy controls (Miettinen et al., 1986). They found that n6

polyunsaturated fatty acids, especially dihomo-gamma-linolenic acid (DGLA, 20:3n6), were lower in stroke patients than controls. DGLA This study included only 9 cases of cerebral artery thrombosis in males. To maximize statistical power, investigators did not control for confounding in multivariable models, but matched cases to controls on several factors: age, body weight, serum cholesterol and triglyceride concentrations, blood pressure, smoking, and glucose tolerance at baseline. Most, but not all, of subsequent case-control studies supported the inverse association between n6 polyunsaturated fatty acids and ischemic stroke.

In two studies (with 56 and 89 cases of ischemic stroke) that measured the fatty acid composition of red blood cell membranes, researchers found that linoleic acid, docosapenaenoic acid (DPA) and docosahexaenoic acid (DHA), and oleic acids were inversely associated with ischemic stroke (Ricci et al., 1987; Ricci et al., 1997). However, in another study of 18 cases of cerebral thrombosis, Bucalossi and Mori did not report a significant association for adipose tissue linoleic acid and cerebral thrombosis (Bucalossi & Mori, 1972). They did find significant positive associations for palmitic and stearic acids and an inverse association for 7-hexadecenoic acid.

Tilvis and colleagues found that age and the type of biomarker can modify the associations between fatty acids and ischemic stroke. Twenty-six middle-aged male cases of ischemic stroke were associated with higher concentrations of serum saturated, monounsaturated, and n3 polyunsaturated fatty acids and lower concentrations of n6 polyunsaturated fatty acids compared with controls. However, platelet fatty acid concentrations were not found to be associated with ischemic stroke. Among 14 younger male cases of ischemic stroke, none of the serum fatty acids were associated with ischemic stroke. However, higher concentrations of platelet linoleic acid and lower concentrations of platelet arachidonic acid were associated with ischemic stroke. These results suggest that the fatty acid biomarker used and the age of the study population are important variables to

consider in studying the association between fatty acids and ischemic stroke. None of the biomarker studies described here included more than 90 cases of ischemic stroke.

The largest study of serum fatty acids and ischemic strokes to date included 122 cases of ischemic stroke and 366 matched controls nested in a Japanese population-based cohort (Iso et al., 2002). To our knowledge, this is the only study of biomarkers of fatty acids that examined ischemic stroke subtypes. Iso and colleagues reported that serum saturated fatty acids were positively associated with incidence of ischemic stroke, while serum linoleic acid was associated with decreased incidence of ischemic stroke. These associations were largely driven by lacunar stroke, this subtype comprising 95 of the 122 ischemic strokes. Investigators did not find significant associations for marine n3 polyunsaturated fatty acids. However, they note that the Japanese have quite high levels of these fatty acids. Those with the lowest concentrations of marine n3 polyunsaturated fatty acids in this population may have already reached the level that provides cardioprotective effects, so that a significant association was not observed (Iso, Rexrode, et al., 2001; He et al., 2002). Investigators did not examine serum trans fat and acknowledged the uncertainty of the associations for other ischemic stroke subtypes. Further exploration is needed on the association between types of fat and ischemic stroke subtypes.

E. Gaps in the Literature

There are several gaps in the literature examining fatty acids and ischemic stroke. First, measurement of fatty acid intake though participant report in the cohort studies is subject to considerable systematic error. The effect of systematic error – if individuals with high fat intake are more likely to underreport fat intake (Millen et al., 2009) and are associated with high risk of stroke – is to bias associations toward the null. Measurement of fatty acid concentrations in tissue may have less systematic error, allowing us to detect associations with ischemic stroke. Second, most cohort studies examined fat intake groups, but not individual fatty acids. These studies were not able to examine fatty acids individually

because they measured fat intake through participant report (e.g. FFQ and 24-hour recall). These methods result in high correlations among individual fatty acids within the same group, making distinguishing their relations with cardiovascular disease risk difficult (Hu et al., 2001). Third, only three studies examined associations for ischemic stroke subtypes, despite the possibility that associations between fatty acids and ischemic stroke may not be consistent across all ischemic stroke subtypes (Iso, Stampfer, et al., 2001; Iso et al., 2002; Yaemsiri et al., 2012 [in press]). In the Nurses' Health Study, there were less than 50 cases for each ischemic stroke subtype (Iso, Stampfer, et al., 2001). Likely statistical power was too low to detect associations with ischemic stroke subtypes in this study. Fourth, only three studies have examined trans fatty acids in relation to ischemic stroke (Iso, Stampfer, et al., 2001; He et al., 2003; Yaemsiri et al., 2012 [in press]); none of these studies used a biomarker of fatty acid intake.

IV. INNOVATION

A. Introduction

Four changes in the approach of studying the associations between dietary fat intake and ischemic stroke may shed new light on these associations. First, previous studies using FFQs to measure dietary fat intake were subject to error in the self-report, underreporting, and changing fat content of foods. We proposed to use serum fatty acids as an objective biomarker for dietary fat intake. Second, previous studies reported null associations between dietary fat groups and ischemic stroke. It is possible that the grouping the fatty acids diluted the associations between individual fatty acids and ischemic stroke. We proposed to examine the associations for individual fatty acids, including individual trans fatty acids. Third, ischemic stroke is a heterogeneous condition; examining ischemic stroke as a composite outcome may not be appropriate and would attenuate expected associations. Previous studies did not have information on ischemic stroke subtypes or were underpowered to examine subtype specific associations. We proposed to examine ischemic stroke subtypes. Fourth, risk of stroke in women dramatically increases after menopause. Postmenopausal women have been underrepresented in ischemic stroke studies, even though stroke is a major health issue for older women. We proposed to examine these associations among a cohort of postmenopausal women.

B. Serum Fatty Acids

1. Limitations of self-reported measures of dietary intake

Fatty acid intake and metabolism play important roles in the development of cardiovascular disease (Hu et al., 2001). One of the challenges in studying individual fatty

acid intake is measurement (Arab, 2003). There are two primary methods of measuring fatty acid intake: directly through food chemistry or participant self-report (e.g., food frequency questionnaire [FFQ]) and indirectly through the use of a biological marker (Willett, 1998).

The food frequency questionnaire (FFQ) dietary assessment tool is widely used in large cohort studies for ease of administration and affordability, but has some limitations. The FFQ requires identification of detailed information, such as the frequency and portion size of intake of a particular food over a several months. Participants may be unable to accurately report their intake or likely to underreport intake of certain foods. Americans are large consumers of processed foods, but are not usually aware of the nutrient content of these foods (e.g. “hidden fats”). In addition, the fat content of food items has changed considerably over time (e.g. margarine). Databases used to derive nutrient information from foods may not reflect the actual nutrient content. Therefore, measurement of fatty acid concentrations in tissues (serum, adipose, and red blood cells) have been proposed as an objective method of indirectly measuring fatty acid intake (Freedman et al., 2010).

2. Biomarkers of dietary fat intake

Tissue biomarkers of dietary fat intake include serum/plasma, red blood cell membranes, and adipose tissue. Adipose tissue and red blood cells are considered better long-term biomarkers based on the slower turnover rate of fatty acids in these tissues compared with serum (Skeaff et al., 2006). However, adipose tissue aspiration is not widely acceptable to healthy subjects. Tissue aspiration is more invasive and painful than a blood draw. Healthy participants may be reluctant to undergo tissue aspiration without a therapeutic purpose. Epidemiologic studies using this procedure are open to the biases associated with limited participation. Compared with adipose tissue aspiration, serum samples are relatively easy to obtain (Skeaff et al., 2006). Compared with red blood cell fatty acids, serum fatty acids are less prone to degradation during storage (Glatz et al., 1989; Romon et al., 1995). In this dissertation, we examined serum fatty acids as a biomarker of fatty acid intake.

3. Serum Fatty Acids and CVD Risk Factors and Outcomes

Studies examining the correlation between serum fatty acids and cardiovascular risk factors and outcomes support the utility of serum fatty acids. Serum cholesteryl ester fatty acid concentrations are correlated with serum lipid levels (Knuiman et al., 1980; Sandker et al., 1993; Rosseneu et al., 1994; Mensink et al., 2003). Serum fatty acids have successfully predicted common health outcomes in observational studies, such as diabetes (Lovejoy et al., 2001; Wang et al., 2003; Lindgarde et al., 2006), cardiovascular disease (Miettinen et al., 1982; Simon et al., 1995; Laaksonen et al., 2005), premature death from cancer (Zureik et al., 1995), and Alzheimer's disease (Tully et al., 2003).

4. Correlations depend on the population under study

Correlations between serum fatty acid concentrations and fatty acid intakes may differ by the population under study (Baylin et al., 2002; Giltay et al., 2004; Kawabata et al., 2011). For example, one correlation study of adipose tissue conducted in Costa Rica reported high correlations for 18:1*t* and linelaidic acid due to the high intake of partially hydrogenated soybean oil in that population (Baylin et al., 2002). However, only a few studies have examined the correlations of fatty acids specifically among postmenopausal women. One study of serum fatty acids included a wide age range of both pre- and postmenpausal women (range: 43 to 69 years) (Sun et al., 2007). Another study of postmenopausal women examined adipose tissue fatty acids, but not serum (London et al., 1991). We can contribute to this literature by exploring the correlations between the serum fatty acids and dietary fat intakes among postmenopausal women.

5. Correlation between serum fatty acids and fatty acid intakes

Serum fatty acids differ from the direct measurement of fatty acid intake due to absorption, transport, uptake, metabolism, and excretion of fatty acids in diet (Arab & Akbar, 2002). Given these differences, comparisons between serum fatty acids and fatty acid intakes conducted even within the same population are not expected to yield high

correlations, although the fatty acids that cannot be endogenously synthesized are expected to yield higher correlations. They include polyunsaturated fatty acids (e.g., DHA and EPA), trans fatty acids, and odd-numbered saturated fatty acids (Willett, 1998).

a) Polyunsaturated Fatty Acids

Previous studies comparing serum fatty acids and fatty acid intakes focused on polyunsaturated fatty acids (e.g., DHA and EPA) (Ma et al., 1995; Andersen et al., 1996; Hjartaker et al., 1997; Andersen et al., 1999; Hodge et al., 2007). Among the polyunsaturated fatty acids, DHA and EPA generally have the highest correlations. Their correlations range from 0.29 to 0.78 and from 0.21 to 0.51, respectively. The correlations for DHA and EPA are generally good because they derive from a specific dietary source – fish or fish oil supplements – and are not endogenously synthesized in large amounts. Cold water oily fish (salmon, mackerel, and herring) have the most EPA and DHA (Kris-Etherton et al., 2002). A fatty acid precursor can be converted to DHA and EPA, but the extent of this conversion is modest (Kris-Etherton et al., 2002). Serum DPA, the intermediate fatty acid between EPA and DHA, and linoleic acids also have moderate correlations with intake.

b) Trans Fatty Acids

The three major trans fatty acids are: palmitelaidic acid (16:1t), 18:1t, and linolaidic acid (18:2tt). The dietary sources of these fatty acids are industrially manufactured partially hydrogenated oils (18:1t and 18:2tt) or meat and dairy products (16:1t and 18:1t). Linelaidic acid may be more atherogenic than 18:1t or palmitelaidic acids (Baylin et al., 2003). In contrast to polyunsaturated fatty acids, which have been well-characterized, only one study has examined correlations for serum trans fatty acids (Sun et al., 2007). This study reported moderate correlations for serum 18:1t and palmitelaidic acids, 0.29 and 0.27, respectively, and a lower correlation for linelaidic acid, r=0.19.

c) Odd-numbered Saturated Fatty Acids

The dietary source of odd-numbered saturated fatty acids is dairy fat. These serum fatty acids cannot be endogenously synthesized and are therefore hypothesized to have a higher correlation with fatty acid intake. However, with the exception of two studies, the correlations for odd-numbered saturated fatty acids have been uniformly low. Wolk and colleagues reported that the correlations between intake of dairy fat assessed by food records and dietary recalls for adipose tissue and serum 15:0 were 0.64 and 0.45, respectively (Wolk et al., 2001). Kroger and colleagues reported that the correlations for red blood cell 15:0 and 17:0 were 0.32 and 0.28, respectively (Kroger et al., 2011). Another study of adipose tissue fatty acids reported low correlations for both 15:0 and 17:0 fatty acids (Baylin et al., 2002). The authors attributed these low correlations to their nutrient database, which did not include complete data on these two fatty acids. However, authors did report a moderate correlation between dairy fat intake and adipose 15:0 and 17:0 (r=0.31 and 0.31, respectively).

Aim 1 of this dissertation addressed the lack of studies examining serum trans fatty acid correlations with fatty acid intakes and lack of correlation studies among postmenopausal women. To determine the serum fatty acids that correlate the best with intake, we have conducted a cross-sectional study to examine the correlations between individual serum fatty acids – including trans fatty acids – and (1) individual fatty acid intakes, (2) fatty acid intake groups, and (3) food groups rich in fat derived from a FFQ among a cohort of postmenopausal women.

C. Individual fatty acids

Individual fatty acid intakes, even those of the same type, are known to have different effects on the development of cardiovascular disease (Hu et al., 2001). However, few studies have examined individual fatty acid intakes and their role in the development of ischemic stroke. The nutrient information derived from participant reported measures used in

studies of dietary fat intake tend to be highly correlated. This makes distinguishing the associations between individual fatty acids and ischemic stroke difficult. Tissue biomarkers of fatty acid intake provide a less correlated measure so that individual fatty acids can be examined.

1. Trans fatty acids

No studies have examined the association between individual serum trans fatty acids and ischemic stroke. There is evidence that trans isomers 18:1t and 18:2tt have a stronger effect on cardiovascular disease than 16:1t (Mozaffarian et al., 2009). The risk of CHD death and nonfatal myocardial infarction were consistently associated with higher concentrations of biomarker 18:1t and 18:2tt fatty acids. These associations were less consistent for 16:1t.

2. Saturated fatty acids

Individual serum saturated fatty acids are of interest because they have heterogeneous effects on serum cholesterol. Compared with the oleic monounsaturated fatty acid, myristic and palmitic acids tend to increase serum total and LDL cholesterol concentrations, whereas stearic acid does not have a cholesterol-raising effect (Hu et al., 2001). These associations were reflected in one case-control study of serum fatty acids and ischemic stroke among Japanese (Iso et al., 2002). In this study, in unadjusted analyses, serum myristic and palmitic acids were positively associated with ischemic stroke, while no significant association was found for stearic acid. Despite the different associations found, investigators grouped myristic, palmitic, and stearic acids together in subsequent analyses that adjust for potential confounders.

Few ischemic stroke studies have examined serum 15:0 and 17:0 saturated fatty acids in relation to ischemic stroke. These serum fatty acids are present in small amounts and are thought to be markers of milk fat intake, since these fatty acids cannot be endogenously synthesized. A case-control study of stroke in Northern Sweden reported that that serum 15:0 and 17:0 were inversely associated with total stroke (ischemic +

hemorrhagic) (Warensjo et al., 2009). These associations have not been confirmed in studies examining ischemic stroke specifically.

3. Polyunsaturated fatty acids

Most telling of the importance of examining fatty acids individually are the associations between DHA and EPA intake versus total polyunsaturated fatty acids and ischemic stroke. Studies that examined total polyunsaturated fat intake reported null associations with ischemic stroke (Gillman et al., 1997; Seino et al., 1997; He et al., 2003; Sauvaget et al., 2004), while studies that examined DHA and EPA specifically reported significant inverse associations (Iso, Rexrode, et al., 2001; He et al., 2002). These results could be due to other fatty acids diluting the inverse association between DHA and EPA intake and ischemic stroke. The implication for future studies is that even longitudinal studies that measure fat intake through self-report should examine the associations between individual fatty acid intakes and disease, not fat intake groups. Combining fatty acid into groups, in particular combining n3 and n6 polyunsaturated fatty acids, could mask any potential association of individual fatty acids, depending on their relative contribution. However, few studies of fatty acid intakes were able to examine fatty acids individually.

D. Ischemic Stroke Subtypes

Previous studies of dietary fat intake and ischemic stroke were inconsistent with CHD studies, despite the fact that CHD and ischemic stroke share some major risk factors and are both thought to be atherosclerotic disorders. One possible explanation of the inconsistent findings is that ischemic stroke is a heterogeneous condition. Atherosclerosis may be a more proximate cause of some ischemic stroke subtypes, but not others. Given that fatty acid intakes have a strong effect on the effect of atherosclerosis development through serum cholesterol, fatty acid intakes could have a stronger effect on the development of some ischemic stroke subtypes. To investigate this possibility, we should

examine the association between fatty acids and ischemic stroke subtypes, rather than ischemic stroke as a composite outcome. This section describes the ischemic stroke subtypes, including atherothrombotic (large artery), lacunar (small artery), and cardioembolic strokes.

1. Classification of ischemic stroke

Usually strokes are classified into two groups: ischemic and hemorrhagic. The etiologies of these two types of stroke are entirely different. Ischemic stroke is caused by obstruction within a blood vessel supplying blood to the brain. Hemorrhagic stroke, on the other hand, is caused by bleeding into the brain when a weakened blood vessel ruptures. In the US, approximately 87% of strokes are ischemic (Roger et al., 2012). The focus of this dissertation is ischemic strokes.

Ischemic strokes may be further classified by their etiology, or the origin of the obstruction, as thrombotic or embolic. A thrombotic stroke occurs when an obstructing blood clot (thrombus) forms locally within an artery of the brain. Atherosclerosis is the most prevalent cause of thrombotic strokes occurring in the large arteries of the brain. Mechanisms related to hypertension are common causes of thrombotic stroke occurring in the small vessels of the intracerebral arterial system. Embolic strokes are the result of a traveling blood clot to the brain, usually from a cardiac, aortic, or arterial source. Each of these subtypes has distinguishing prognosis, outcomes, and treatment, emphasizing the need to examine ischemic stroke subtypes separately.

The Trial of Org 10172 in Acute Stroke Treatment (TOAST) formalized this etiologic classification scheme for use in clinical trials and population studies, striving for a uniformity in diagnoses and greater interphysician consistency (Adams et al., 1993). Ischemic strokes may be classified according to five TOAST categories (**Table 1**).

This classification is based on a combination of clinical features and diagnostic tests to confirm etiology, since clinical features used to rapidly determine course of action alone are

often nonspecific (Madden et al., 1995). The classification showed moderate to good interphysician reliability, which improved with the use of standardized medical abstraction procedures and a computerized algorithm (Lindley et al., 1993; Madden et al., 1995; Goldstein et al., 2001). One limitation of the TOAST classification scheme is the large proportion of strokes classified as having undetermined etiology (Kolominsky-Rabas et al., 2001; Wassertheil-Smoller et al., 2003). Advances in stroke imaging and evaluation diagnostics have led to an increasing proportion of stroke to be classified as “undetermined” (e.g. two or more causes identified). The strict TOAST guidelines improve the specificity of the first 3 categories, but they also limit statistical power to examine those categories. Another limitation is that the TOAST classification uses a history of diabetes mellitus and hypertension in its lacunar stroke definition (Adams et al., 1993). Some studies suggest that blood pressure causes lacunar stroke more than other ischemic stroke subtypes, but this may be due to the TOAST classification. One study using “risk factor-free classification” found that the population attributable fraction for hypertension was approximately 35% for all ischemic stroke subtypes (Ohira et al., 2006). This study advocated the use of a “risk factor-free classification” to avoid classification bias. Still, the TOAST classification is the best available for etiologic studies of ischemic stroke (Wassertheil-Smoller et al., 2003).

2. Atherothrombotic stroke (large artery)

Atherosclerotic strokes are occlusions that occur in the extracranial (common and internal carotids and vertebral) and intracranial (Circle of Willis and proximal branches) arteries. The neurologic deficit that occurs with this type of stroke often fluctuates and may occur over a short or long period of time. Compared with patients with lacunar strokes, these large-artery infarcts are more likely to develop over a long period of time. Symptoms of a blocked left or right internal carotid arteries or middle cerebral arteries, which supply blood to the opposite side of the brain, include trouble communicating, or right or left body weakness, numbness, or loss of vision, respectively. Blockage of the left or right vertebral arteries or

basilar arteries, which supply blood to the brainstem and the back of the brain causes weakness or numbness on either side of the body, vertigo, nausea, vomiting, unsteadiness, or coma. Atherosclerotic infarcts block arteries that supply blood to large portions of the brain and may result in a large amount of brain damage. Angiography, a relatively invasive procedure with inherent risks, is the gold standard for diagnosing atherothrombotic stroke (Madden et al., 1995). Treatment options for atherosclerotic strokes include antiplatelet therapy, revascularization, and anticoagulation.

In the US, atherothrombotic strokes occur less frequently than lacunar or cardioembolic stroke making these types of strokes particularly difficult to accumulate in epidemiologic studies (**Figure 3)**. Still, intracranial stenosis causes approximately 50,000 ischemic strokes each year. Approximately 5% to 10% of ischemic strokes among whites are caused by atherothrombotic stroke, a proportion that is higher among blacks (14%), Hispanics (20%), Japanese (21%), and South Asians (27%) (R. L. Sacco et al., 1995; Syed et al., 2003; Turin et al., 2010). These racial differences are due to the proportions of intracranial, rather than extracranial, atherothrombotic strokes. Of all the ischemic strokes in the Northern Manhattan Stroke Study, 8% and 8% (52 and 50 of 661 ischemic strokes, respectively) were caused by intracranial and extracranial atherothrombotic strokes, respectively. In combination, this study had a higher proportion of atherothrombotic stroke than US whites because of the racial diversity among study participants (Rincon et al., 2009). The higher prevalence of diabetes and hypercholesterolemia among blacks and Hispanics accounted for much of the increased frequency of intracranial atherosclerotic stroke (R. L. Sacco et al., 1995; Ohira et al., 2006). Individuals with both type I and type II diabetes are associated with substantially increased risks of thrombotic stroke, including atherothrombotic stroke (Janghorbani et al., 2007).

Kuller and Reisler developed the hypothesis that serum cholesterol played a larger role in the development of atherothrombotic stroke than other subtypes of ischemic stroke.

These investigators examined the incidence of ischemic stroke subtypes in populations with high and low serum cholesterol levels (Kuller & Reisler, 1971). They found the ischemic strokes that occurred among populations with high serum cholesterol levels were mainly intracranial and extracranial. Intracranial and extracranial ischemic strokes define atherothrombotic stroke. Based on this observation, high serum cholesterol levels seemed to be related to atherothrombotic stroke, but not other subtypes of ischemic stroke. Given the strong effect of fatty acid intakes on serum cholesterol, we hypothesized that fatty acid intakes would have an effect on atherothrombotic stroke, but not other subtypes of ischemic stroke. The Honolulu Heart Program reported evidence for the effect of dietary fat on subtypes of ischemic stroke (D. M. Reed, 1990). In 198 men, the levels of atherosclerosis were greater in the large arteries in the Circle of Willis than in the small arteries in those who reported low intake of fat and animal protein. These studies suggest that dietary fat intake and atherosclerosis may only be involved in atherothrombotic stroke. However, there have been no confirmatory reports on the associations of types of fat (both dietary or fatty acid biomarkers) and types of ischemic stroke. Studies that have attempted to examine the associations between fatty acids and atherothrombotic stroke have been limited by low statistical power (Iso et al., 2002; Yaemsiri et al., 2012 [in press]).

3. Lacunar stroke (Small vessel)

Lacunar infarcts are occlusions that are 0.2-15mm in diameter occurring in the arteries that branch off from large cerebral arteries and supply blood to deep in the brain. Occlusion of these smaller arteries is most often caused by lipohyalinosis (small vessel disease) or microatheroma (atherosclerosis), both consequences of systemic hypertension (Fisher, 1982). A lacunar infarct can cause any of the symptoms of ischemic stroke, notably weakness or numbness on one side of the body. Compared with non-lacunar strokes, small artery infarcts have a better short-term (<1yr) prognosis (Bamford et al., 1987; S. E. Sacco

et al., 1991; The Publications Committee for the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) Investigators, 1998).

In the US, the annual incidence of lacunar infarcts is 25/100,000 overall and 52/100,000 among blacks (Petty et al., 1999; Woo et al., 1999). Lacunar stroke incidence is higher among US blacks than whites, probably due to having a higher prevalence of hypertension and diabetes. Lacunar infarcts make up 16-22% of all ischemic strokes in the US, but comprise a much larger proportion of all ischemic strokes among the Japanese; approximately 80% of ischemic strokes in Japanese are lacunar (D. Reed et al., 1994; Iso et al., 2002). Over the last 40 years, the incidence of lacunar stroke, but not other types of ischemic stroke, has steadily declined in Japan probably due to better hypertension control and decrease of other risk factors, namely diabetes and smoking (Kubo et al., 2006). In fact, treatment of systolic hypertension reduced risk of lacunar and possibly embolic strokes, but not atherosclerotic strokes (Perry et al., 2000). Results of this study contrast with others that suggest lacunar infarcts are not more likely to occur from hypertension (Lodder et al., 1990; Petty et al., 1999).

Serum fatty acids and fatty acid intakes have been shown to be associated with lacunar stroke. Investigators of a case control study of serum fatty acids and ischemic stroke subtypes reported a positive association between serum saturated fatty acids and ischemic stroke, and an inverse association for serum linoleic acid (Iso et al., 2002). These associations were driven by lacunar stroke, this subtype making up 95 of the 122 ischemic strokes. A more recent study of dietary fat intake and ischemic stroke reported a positive association between trans fat intake and ischemic stroke that was consistent for lacunar stroke, but not other subtypes of ischemic stroke (Yaemsiri et al., 2012 [in press]). Polyunsaturated fatty acids may be associated with lower incidence of lacunar stroke through their antihypertensive effect (Geleijnse et al., 2002). Trans fatty acids are not thought to have an effect on blood pressure, but may raise the incidence of lacunar stroke

through inflammatory mechanisms (Mozaffarian et al., 2009). However, we hypothesize that the main mechanism by which fatty acid intakes increase risk of ischemic stroke is through serum cholesterol's effect on increasing risk of atherothrombotic stroke.

4. Cardioembolic stroke

Cardioembolic stroke is caused by a blood clot (embolism) that forms in the heart, travels through the bloodstream, and blocks an artery supplying blood to the brain. Common sources of embolism include arrhythmias, especially atrial fibrillation, valve disorder, and heart failure. These diseases may cause normally flowing blood to pool, increasing the possibility of clot formation. Atrial fibrillation may develop following cardiac surgery, increasing concern for cardioembolic stroke. Compared with thrombotic strokes, the neurologic deficit associated with cardioembolic stroke is large. Onset of the deficit, as well as recovery after clearing the embolus, occurs quickly.

Treatment for cardioembolic stroke differs from other ischemic strokes in that treatment of both the local infarct and the cardiac source of the thrombus are important to prevent recurrence. Further distinguishing this subtype of ischemic stroke, long-term prevention of cardioembolic stroke includes anticoagulants therapy (warfarin); antiplatelets alone are not sufficient to prevent recurrence (R. L. Sacco et al., 2006). To prevent recurrence with proper treatment, all patients with possible stroke must undergo a thorough cardiac evaluation.

Cardioembolic stroke accounts for approximately 20% of all ischemic strokes (R. L. Sacco et al., 2006). They occur more frequently among US whites, than US blacks or Hispanics (R. L. Sacco et al., 1995), but these differences are attributable to prevalent risk factors, such as age, adiposity, systolic blood pressure, and HDL cholesterol (Ohira et al., 2006). The most important risk factor for cardioembolic stroke is atrial fibrillation. Atrial fibrillation increases risk of stroke five-fold. One in four strokes among the elderly are attributable to atrial fibrillation (Roger et al., 2012). In addition, younger patients with heart disease are more likely to have cardioembolic stroke compared with other ischemic strokes.

Cardiac surgery is also a major risk factor for cardioembolic strokes. Adverse cerebral outcomes occur in 6.1% of patients who undergo a coronary bypass surgery (Roach et al., 1996). Approximately half of patients who experience a cerebral outcome have focal neurologic damage and the other half experience a deterioration of intellectual function or seizures.

Fatty acids may play a distal role in cardioembolic strokes through their effect on serum cholesterol, the development of atherosclerosis, and coronary heart disease. However, their effect on atherothrombotic stroke, or even lacunar stroke, should be more apparent than their effect on cardioembolic stroke.

V. METHODS

The aims of this dissertation were (1) to examine the correlations between serum fatty acids and fatty acid intakes and (2) to examine the associations between individual fatty acids and ischemic stroke and its subtypes. We increased statistical power to meet these objectives by using data from the WHI-OS, a large cohort of postmenopausal women. We will address the first aim using a cross-sectional study design and calculating Spearman rank correlation coefficients between serum fatty acids and fatty acid intakes. We will address the second aim using a matched case-control study design. Given this study design, we will use conditional logistic regression, conditioning on the matched factors, to examine the associations between fatty acids and ischemic stroke. This dissertation involves the analysis of extant data. Data from this dissertation were derived from two Women's Health Initiative Observational Study ancillary studies: (1) Stroke risk factors and molecular markers in postmenopausal women (AS126, PI: Dr. Sylvia Wassertheil-Smoller) and (2) Serum fatty acids and incidence of ischemic stroke in women (AS187, PI: Dr. Ka He). This section provides a description of the data and the analytic procedures.

A. The Women's Health Initiative Observational Study

The WHI-OS is a long-term prospective cohort study to identify and assess the impact of biological, lifestyle, biochemical, and genetic factors on the risk of heart disease, cancer, osteoporosis, and other major health events. Between 1993 and 1998, the WHI-OS recruited women age 50 to 79 years from areas surrounding 40 clinical centers in 24 states and the District of Columbia and enrolled 93,676 women (Hays et al., 2003). The cohort is closed, so no additional recruitment or enrollment occurred since baseline. The design of the WHI-OS has been described previously (Prentice et al., 1998).

The baseline characteristics of the participants have been published (Langer et al., 2003). The WHI-OS cohort is somewhat healthier than a probability sample of US women age 55-74 in the NHANES III cohort (1988-1994). Among women aged 50 to 79 in the NHANES III, 48% were hypertensive, 7% reported a history of physician-diagnosed heart attack, and 5% reported a physician-diagnosed stroke. The prevalence of these conditions in the WHI-OS at baseline was 33.5%, 2.5%, and 1.5%, respectively. **Table 2** compares other characteristics of WHI-OS women to that of US women ages 50-79 from the NHANES 1999-2000 (the NHANES was not conducted during WHI enrollment between 1993 and 1998). Compared with US women, WHI women were more likely to have higher levels of formal education and have higher household incomes. WHI women were less likely to be obese or be a current smoker. While the WHI cohort is not representative of postmenopausal US women, much less adults worldwide in the sense of a probability sample, its nonrepresentativeness is assumed to be unrelated to the biologic mechanism linking serum fatty acids and the development of ischemic stroke. In fact, limiting the study population to postmenopausal US women without safety or retention concerns maximizes the internal validity and aids generalizability to unstudied groups (Rothman et al., 2008).

B. Case identification and classification

Of the 93,676 participants in the WHI-OS, we excluded 9,831 women for not meeting study eligibility criteria (having no previous history of myocardial infarction or stroke and adequate blood sample available for the biomarker assays) and 627 women with a locally adjudicated ischemic stroke that was not confirmed by central adjudication (Wassertheil-Smoller et al., 2008). These exclusions left a remaining cohort of 82,591 women from among whom the stroke case and control subjects were selected.

Incident ischemic strokes during the follow-up period were identified through self-report during annual medical history updates (annual response rate 94%) (Curb et al., 2003). Using additional details from medical charts, brain imaging, or death certificates, the

potential outcomes were subject to local adjudication by physicians, then central adjudication by trained neurologists, according to standard criteria. Over 95% of participant-reported stroke cases were classified based on brain imaging (Chen et al., 2008). There were 972 confirmed incident ischemic stroke cases between 1993 and 2003.

Central adjudicators further classified ischemic strokes by subtypes according to the Trial of ORG 10172 Acute Stroke Trial (TOAST) Classification, based on the presumed underlying stroke etiology (**Table 1**) (Goldstein et al., 2001). The TOAST classification system includes five categories: atherothrombotic stroke, lacunar stroke, cardioembolic stroke, stroke of other determined etiology, and stroke of undetermined etiology. The classification is based on clinical features, brain imaging (CT/MRI), cardiac imaging (echocardiography, etc.), duplex imaging of extracranial arteries, arteriography, and laboratory assessments for a pro-thrombotic state. Probable and possible subtypes determinations were combined in this report. Transient ischemic attack, hemorrhagic stroke, strokes not requiring hospitalization, and strokes not confirmed by central adjudication were not included as a stroke outcome.

C. Control selection

Controls were sampled from the risk set when ischemic stroke events occurred during the follow-up period (incidence density matching) and matched 1:1 to case subjects on age at screening (±2 years), race/ethnicity (white, black, Hispanic, Asian, American Indian, or other/unspecified), date of study enrollment (±3 months), and follow-up time (control follow-up time greater than case follow-up time).

D. Baseline data collection

Baseline data were collected by trained and certified clinical center staff using self-administered forms and interviews and making clinical measurements (**Table 3**) (Anderson et al., 2003).

1. Blood specimen and serum fatty acid assessment

Fasting blood samples were collected from women at study baseline by clinic staff members following a standardized protocol for venipuncture (Anderson et al., 2003). Samples were centrifuged, separated by layers, frozen onsite at -70℃, and shipped to the central WHI repository for long-term storage. Blood samples from the selected cases and controls were sent to Dr. Rhobert Evans' laboratory at the University of Pittsburgh for serum fatty acid assessment.

Fatty acids (**Table 4**) were extracted according to the general technique of Bligh and Dyer (Bligh & Dyer, 1959) using 1, 2-dinonadecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL) (50 µg of 19:0) as an internal standard. The lipid extracts were resuspended in 1.5 ml 14% boron trifluoride methanol and derivatized according to the procedure of Morrison and Smith (Morrison & Smith, 1964). The extracts were analyzed by capillary GC:column (SP-2380, 105 m x 53 mm ID, 0.20 um film thickness). The gas chromatograph was a Perkin Elmer Clarus 500 equipped with a flame ionization detector. Operating conditions: the oven temperature was 140℃ for 35 min, then ramped at 8℃/min to 220℃, and held for 12 min. Injector and detecto r temperatures were both at 260℃ and helium, the carrier gas, was at 15 psi. Identification of components was by comparison of retention times with those of authentic standards (Sigma). Duplicate samples and control pools were analyzed with each batch of samples.

Lipoprotein profiles were assayed at Liposcience using nuclear magnetic resonance. Low-density lipoprotein (LDL) cholesterol was calculated from high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglyceride concentrations among women who had a

triglyceride value <400 mg/dL using the Friedewald equation (National Cholesterol Education Program Expert Panel on Detection, 2002). LDL cholesterol values were set to missing for those women whose triglyceride value was >400 mg/dL (n=35) or who were missing HDL cholesterol, total cholesterol, or triglyceride values (n=7).

Fasting glucose was assayed by the hexokinase method (Anderson et al., 2003).

2. Demographics

Participants reported their age, race, level of formal education, and household income. Participants could report their race/ethnicity as American Indian or Alaskan Native, Asian or Pacific Islander, Black or African-American, Hispanic/Latino, White (not of Hispanic origin), or other. Because of the relatively low number of non-white participants, we dichotomized race/ethnicity as white and non-white for Aim 1. All six categories of race/ethnicity were used as matching factors in Aim 2. Levels of formal education were collapsed into three categories (completed high school or obtained General Equivalency Diploma or less, some college or training, and college graduate). Levels of household income were collapsed into three categories, as well (<$35,000, $35,000 to $74,999, and ≥$75,000).

3. Medical history

Participants reported their history of treatment for diabetes, atrial fibrillation, angina, and revascularization. Using additional information from blood biomarkers, diabetes (yes, no) was defined as being on treatment for diabetes by self-report or having a fasting glucose level ≥126 mg/dL.

4. Physical activity, alcohol consumption, smoking

Participants reported their recreational physical activity, alcohol intake, and smoking status. Recreational physical activity was assessed by asking about the frequencies and duration of walking at various intensities and other types of recreational physical activity classified by intensity (strenuous, moderate, or light). These data were summarized into total

metabolic equivalent task (MET)-hours per week and categorized by episodes per week of moderate or strenuous activity of 20 minutes or more duration (as defined by a metabolic equivalent score of at least 4.0) (Ainsworth et al., 1993). Participants were queried about their past and current alcohol consumption and were categorized as past drinker, current drinker, <7 drinks/wk, or ≥7 drinks/wk. Smoking status was dichotomized as current smoker (yes, no).

5. Food frequency questionnaire

The WHI used a self-administered food frequency questionnaire (FFQ) to assess diet (Patterson et al., 1999). The FFQ asked about usual frequency of intake and portion size for 122 foods and food groups over the previous 3-month period, including questions about fats in meat and dairy and reduced-fat foods. It also included 19 adjustment questions that permitted a more refined analysis of fat intake by asking about food preparation practices and types of added fats. In addition, 4 summary questions asked about usual intake of fruits, vegetables, and fats added to foods and used in cooking. Participants received a sheet with portion size pictures to standardize portion size. The nutrient database was derived from the University of Minnesota Nutrition Coding Center nutrient database (2005 version, Nutrition Coordinating Center, Minneapolis, MN). We compared food groups that are rich in fat and nutrient variables derived from the WHI FFQ with serum fatty acid concentrations for Aim 1 (**Table 5**).

The measurement properties of the WHI FFQ were evaluated in a sub-sample of the WHI and were found to be similar to other FFQs in studies of older women (Patterson et al., 1999). The Pearson correlation coefficient between the FFQ and 8 days of dietary recalls and food records were 0.64 for total fat, 0.63 for saturated fat, 0.64 for monounsaturated fat, and 0.54 for polyunsaturated fat. The correlation coefficient for trans fat was not reported in the evaluation study. However, in the WHI Dietary Modification Clinical Trial, a trend was observed toward CHD reduction among the intervention group who reached the lowest

levels of trans fat and saturated fat intakes, as measured by the WHI FFQ (Howard et al., 2006). These results demonstrate that the WHI FFQ dietary trans fat intake assessment is appropriate and can successfully predict health endpoints including ischemic stroke by ranking participants according to intake.

6. Hormone replacement therapy use

To capture hormone use prior to WHI enrollment, an in-person interview was conducted with each woman to determine her entire history of postmenopausal hormone use (Anderson et al., 2003). We categorized women as being current, past, or never users of hormone replacement therapy (estrogen plus progesterone or unopposed estrogen).

7. Current medications

Use of medications and dietary supplements was captured directly from pill bottles that participants were asked to bring to the clinic. The medications were recorded by an interviewer and matched to a Medication Therapeutic Class. Participants who reported taking pills for hypertension or medication in the antihypertensive or diuretic therapeutic classes were classified as antihypertension medication users. Those who reported having high cholesterol requiring pills or taking medication in the antihyperlipidemic therapeutic classes were classified as cholesterol-lowering medication users. Participants who reported taking medications that contained aspirin as an ingredient for at least 14 days were classified aspirin users.

8. Blood pressure

Blood pressure was measured at a baseline clinic visit by certified staff using standardized procedures after a 5-minute seated rest. Two systolic and diastolic blood pressure measurements were taken with a 30-second rest in between. The average of the first and second systolic and diastolic blood pressure readings were averaged. Since systolic and diastolic blood pressures are highly correlated, only systolic blood pressure was used in analysis.

9. Height and weight

Certified clinic staff measured participant height, weight, and blood pressure. Height and weight were measured without shoes or heavy clothing and with pocket contents removed. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Participants were categorized as having BMI <25.0, 25.0 to 29.9, or ≥ 30.0 kg/m^2.

Following standardized procedures, blood pressure was measured twice after a 5-minute seated rest with a 30-second rest in between. The average of two blood pressure measurements was used in analysis. Hypertension was defined as self-report of antihypertensive medication use or systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg.

E. Statistical analysis

All analyses were performed using SAS statistical software (version 9.2, SAS Institute Inc., Cary, NC, USA).

1. Aim 1 analyses

The objective of aim 1 was to calculate the correlation coefficients between individual serum fatty acids and fatty acid intakes. In addition, we calculated the correlation coefficients for intakes of fatty acid groups and selected food groups rich in fat. Aim 1 analyses excluded women who developed ischemic stroke during follow-up and women who reported implausible daily energy intake (<600 or >5000 kcal), leaving 925 women in this analysis. Even though women with a history of myocardial infarction or stroke were excluded from being selected as a case or control, we further excluded women who developed ischemic stroke during follow-up since they may have had another condition at baseline that may have caused their fat intake or metabolism to be substantially different from the women who did not develop ischemic stroke.

We calculated Spearman, rather than Pearson, correlation coefficients to account for the highly skewed serum fatty acid and fatty acid intake variables. Unlike Pearson correlation coefficients, Spearman rank correlation coefficients do not need to be normally distributed since they convert the values to ranks. We considered correlations $r \geq 0.20$ with $P \leq 0.001$ to be statistically significant to account for multiple comparisons. $P \leq 0.001$ is more conservative than the Bonferroni correction for $P \leq 0.05$ for 25 comparisons.

Our primary analysis was unadjusted, rather than adjusted or partial, correlation coefficients between serum fatty acids and fatty acid intakes and food groups. Since our objective was to estimate the correlations *in this population of postmenopausal US women*, adjustment for other variables would decrease the correlations' relevance to this study population. We adjusted the correlation coefficients for age (continuous), race (white, non-white), BMI group (<25, 25 to <30, ≥30 kg/m^2), and current smoking status (no, yes) in secondary analyses. These variables are markers of major lifestyle factors and biological processes that can influence fatty acid intakes and serum fatty acid concentration.

2. Aim 2 analyses

The objective of Aim 2 was to examine the associations between individual serum fatty acids and ischemic stroke and its subtypes. Aim 2 analyses included 964 matched ischemic stroke case and control pairs with sufficient serum samples.

To account for the matched design, we used conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for ischemic stroke and its subtypes, conditioning on matching factors in all models. We estimated ORs and 95% CIs comparing the odds of ischemic stroke among women in the higher quintiles of serum fatty acids with the odds of ischemic stroke among women in the first quintile. Linear trend tests were performed by assigning the median value of the quintile of the fatty acid to each category and entering this variable into the model as a continuous variable. Since trends were

generally linear, unadjusted ORs were calculated per 1-standard deviation (SD) increment in each serum fatty acid (**Model 1, Table 6**).

Potential confounders were identified by determining which variables were related both to serum fatty acids and to ischemic stroke (**Figure 4**). In a matched case-control study where the inclusion of a case-control pair depends on both having complete covariate data, we were careful to add only variables that are justified and necessary to maximize statistical power. Cases and controls were already matched on age and race/ethnicity. Therefore, we considered other comorbidities and lifestyle factors that would affect serum fatty acid concentrations and ischemic stroke incidence: BMI, smoking status, diabetes, and aspirin use. The model controlling for these variables was our primary model (**Model 2, Table 6**). These variables were associated with ischemic stroke in our study and are markers of comorbidities and lifestyle that could affect serum fatty acid concentrations and ischemic stroke incidence. We additionally adjusted for two potential mediators, blood pressure and serum cholesterol, in secondary analyses (**Models 3 and 4, Table 6**). Associations with two-sided $P \leq 0.001$ was considered significant to account for multiple comparisons. This is a more conservative P than the Bonferroni correction of $P \leq 0.05$ for 25 comparisons.

F. IRB/Human subjects

Approval for this project was obtained through the Institutional Review Board of the University of North Carolina Gillings School of Global Public Health on 6/29/2011 for Aim 1 and 7/8/2008 for Aim 2. No additional contact was made with study participants, as all analyses were of secondary data. The WHI study coordinators at each clinic site obtained approval for data collection.

G. WHI Publications and Presentations (P&P) Committee

The WHI P&P Committee approved the paper proposals related to Aims 1 and 2 of this dissertation.

VI. Correlations Between Serum Fatty Acids and Fatty Acid Intakes Among Postmenopausal Women

This manuscript presents the results and discusses the implications of Aim 1. This manuscript addresses the correlations between individual serum fatty acids with (1) fatty acid intakes, (2) fatty acid groups, and (3) food groups. We examined correlations for individual trans fatty acids, which few studies have examined previously.

A. SUMMARY

Background: Serum fatty acid concentrations have been proposed as a useful method of indirectly measuring fatty acid intake. It is unclear which serum fatty acids are valid biomarkers of fatty acid intake among postmenopausal US women.

Objective: We examined the correlations between serum fatty acids and intakes of fatty acids and food groups rich in fat among postmenopausal women in the Women's Health Initiative Observational Study.

Design: Participants were 925 women aged 50 to 79 years. Serum fatty acids were assessed using gas chromatography from a fasting serum sample. Intakes of fatty acids and food groups were derived from a 122-item food frequency questionnaire. Spearman correlation coefficients and measures of agreement were calculated.

Results: The major components of total serum fatty acids were linoleic (18:2n6, 27%), palmitic (16:0, 24%), oleic (18:1n9, 18%), stearic (18:0, 9%), and arachidonic (20:4n6, 8%) acids. Serum docosahexaenoic acid (DHA, 22:6n3, r=0.53), eicosapentaenoic acid (EPA, 20:5n3, r=0.42), and 18:1*t* (r=0.39) had the strongest correlations with corresponding intakes. There was fair agreement between quintiles of serum and intake for DHA, EPA, and 18:1*t* (weighted kappa= 0.35, 0.26, and 0.25, respectively). Serum DHA and EPA were

correlated with fish intake (r=0.39 and 0.35, respectively). Serum 18:1*t* was correlated with intakes of added fats, butter, and pastries (r=0.22, 0.26, and 0.30, respectively).

Conclusions: Serum DHA, EPA, and 18:1*t* concentrations are suitable biomarkers of their corresponding fatty acid intakes as assessed by an FFQ in this cohort of postmenopausal US women.

B. INTRODUCTION

Fatty acid intake and metabolism play important roles in the development of cardiovascular disease (Hu et al., 2001). One of the challenges in studying fatty acid intake is measurement (Arab, 2003). There are two primary methods of measuring fatty acid intake: directly through food chemistry or participant report (e.g., food frequency questionnaire [FFQ]) and indirectly through the use of a biological marker (Willett, 1998).

Most large-scale observational studies use the FFQ, a tool that aims to measure usual food intake in relation to chronic disease. Its usefulness is limited by participants who may be reluctant or unable to report food intake accurately (Willett, 1998). Further, nutritional databases that convert foods to fatty acids may not be updated to reflect the continuously changing fatty acid content of food items. Therefore, measurement of fatty acid concentrations in tissues (serum, adipose, and red blood cells) have been proposed as an objective method of indirectly measuring intake of foods rich in fat and fatty acid intake. Whereas foods and fatty acids derived from the FFQ reflect intake but not metabolic processes, serum fatty acids reflect intake after absorption, transport, uptake, metabolism, and excretion of dietary fatty acids and endogenous synthesis of fatty acids (Arab & Akbar, 2002).

Given these differences between serum fatty acids and intake, comparisons conducted even within the same population are not expected to yield high correlations. However, fatty acids from diet that cannot be endogenously synthesized and do not undergo significant changes upon ingestion are expected to yield higher correlations with intake than other fatty

acids (Willett, 1998). These fatty acids include n3 polyunsaturated fatty acids, trans fatty acids, and odd-numbered fatty acids. While previous studies comparing fatty acid biomarkers have focused on n3 polyunsaturated fatty acids (e.g., DHA and EPA) (Ma et al., 1995; Andersen et al., 1996; Hjartaker et al., 1997; Andersen et al., 1999; Hodge et al., 2007; Sun et al., 2007; Patel et al., 2010), few studies have examined correlations for serum trans (Sun et al., 2007) or odd-numbered fatty acids. These three classes of fatty acids are also of interest because of their effects on a host of issues that affect the health of older women: ischemic stroke, osteoporosis, cognition, cancer, and autoimmune disease. However, few studies have examined the correlations among postmenopausal women (London et al., 1991; Sun et al., 2007).

To determine the serum fatty acids that best correlate with intake among postmenopausal women, we have conducted a cross-sectional study to examine the correlations between individual serum fatty acids and FFQ-derived i) individual fatty acid intakes, ii) fatty acid intake groups, and iii) food groups rich in fat.

C. SUBJECTS AND METHODS

1. Study Population

Study participants were the control participants of a case-control study nested within the Women's Health Initiative Observational Study (WHI-OS). The WHI-OS is a long-term prospective cohort study to identify and to assess the impact of biological, lifestyle, biochemical, and genetic factors on the risk of heart disease, cancer, osteoporosis, and other major health events. Between 1993 and 1998, the WHI-OS recruited women age 50 to 79 years from areas surrounding 40 clinical centers in 24 states and the District of Columbia and enrolled over 90,000 postmenopausal women. The design of the WHI-OS has been described previously ("Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group," 1998).

The present analysis used controls from a nested case-control study of ischemic stroke. Controls were matched to 972 ischemic stroke cases based on age and race/ethnicity (Wassertheil-Smoller et al., 2008). We excluded controls whose blood quantity was not sufficient to analyze fatty acid content (n=4) or reported implausible daily energy intake (<600 or >5000 kcal, n=43), leaving 925 women in this analysis.

2. Data collection

Participants completed sociodemographic and lifestyle questionnaires during baseline visits (Anderson et al., 2003). Anthropometric measurements were measured by certified clinic staff. Height and weight were measured without shoes or heavy clothing and pocket contents removed. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (National Cholesterol Education Program Expert Panel on Detection, 2002). Blood pressure was measured using standardized procedures after a 5-minute seated rest. The average of two blood pressure measurements taken with a 30-second rest in between was used in the analysis. Hypertension was defined as having a systolic or diastolic blood pressure equal to or greater than 140 and 90 mm Hg, respectively, or report of use of antihypertensive medication (National Cholesterol Education Program Expert Panel on Detection, 2002).

Fasting blood samples were collected from women at study baseline by clinic staff members following a standardized protocol for venipuncture (Anderson et al., 2003). Samples were centrifuged, separated by layers, frozen onsite at -70℃, and shipped to the central WHI repository for long-term storage.

3. Serum fatty acid analysis

Fatty acids were extracted according to the general technique of Bligh and Dyer (Bligh & Dyer, 1959) using 1, 2-dinonadecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL) (50 μg of 19:0) as an internal standard. The lipid extracts were resuspended in 1.5 ml 14% boron trifluoride methanol and derivatized according to the

procedure of Morrison and Smith (Morrison & Smith, 1964). The extracts were analyzed by capillary GC:column (SP-2380, 105 m x 53 mm ID, 0.20 um film thickness). The gas chromatograph was a Perkin Elmer Clarus 500 equipped with a flame ionization detector. Operating conditions: the oven temperature was 140℃ for 35 min, then ramped at 8℃/min to 220℃, and held for 12 min. Injector and detecto r temperatures were both at 260℃ and helium, the carrier gas, was at 15 psi. Identification of components was by comparison of retention times with those of authentic standards (Sigma). Duplicate samples and control pools were analyzed with each batch of samples.

4. Diet assessment

The WHI used a self-administered FFQ to assess diet at baseline. The FFQ asked about usual frequency of intake and portion size for 122 foods and food groups over the previous 3-month period. The nutrient database was derived from the University of Minnesota Nutrition Coding Center nutrient database (2005 version, Nutrition Coordinating Center, Minneapolis, MN). The measurement properties of the WHI FFQ were evaluated against four 24-hour recalls and 4-day food records in a subcohort of 113 WHI participants (Patterson et al., 1999). The Pearson correlation coefficients were 0.64 for total fat, 0.63 for saturated fat, 0.64 for monounsaturated fat, and 0.54 for polyunsaturated fat. The correlation coefficient for trans fat was not reported in the evaluation study.

5. Statistical analysis

Fatty acid intakes were calculated as a percent of total fat intake in order to be comparable to serum fatty acids, which were assessed as a percent of total serum fatty acids. The distributions of serum fatty acids and fatty acid intakes were highly skewed to the right. Therefore, we calculated the median as the measure of central tendency. For both serum fatty acids and fatty acid intakes, fatty acid groups (trans, saturated, monounsaturated, n3 polyunsaturated, and n6 polyunsaturated) were calculated by summing the concentrations of individual fatty acids in that group.

To determine which serum fatty acids may be viable indirect measures of intake, unadjusted Spearman rank correlation coefficients were calculated between serum fatty acids and i) their corresponding individual fatty acid intake, ii) their corresponding fat intake groups, and iii) select food groups rich in fat. Partial correlations were calculated between serum fatty acids and fatty acid intake adjusting for age (continuous), race (white, non-white), BMI group (<25, 25 to <30, ≥30 kg/m^2), and current smoking status (no, yes). These variables are considered markers of major lifestyle factors and biological processes that can influence report of fatty acid intakes and serum fatty acid concentration. Correlations that were ≥ 0.20 at a *P*-value <0.003 (Bonferroni correlation for 16 comparisons) were considered statistically significant.

Two measures of agreement were calculated between quintiles of women defined by serum fatty acids and their corresponding fatty acid intake: percent agreement and weighted Kappa statistic. Percent agreement is the percent of women who fell into the same quintile on the basis of serum and intake. The weighted Kappa is a chance-corrected agreement rate. It is used for ordered categories and assigns less weight to agreement as categories are further apart. Kappa <0.00, 0.00 to 0.20, and 0.21 to 0.40 represents less than chance agreement, slight agreement, and fair agreement, respectively (Viera & Garrett, 2005). All analyses were performed using SAS statistical software (version 9.2, SAS Institute Inc., Cary, NC, USA).

D. RESULTS

1. Characteristics of the study population

The study population included 925 postmenopausal women age 50 to 79 years old at baseline (**Table 7**). Half of the women in the study population were age 70 to 79 years old at baseline. One in five of the women were obese and one in two had hypertension. Few were

current smokers. Women in the study population were predominately white and not of Hispanic origin.

2. Composition of serum fatty acids and fatty acid intakes

Twenty-five individual serum fatty acids were examined (**Table 4**). Linoleic acid represented the largest proportion of serum fatty acids (27%, **Figure 5**). The major components (>5%) of serum fatty acids were linoleic acid (27%), palmitic acid (24%), oleic acid (18%), stearic acid (9%), and arachidonic acid (8%).

Sixteen individual fatty acid intakes were examined. Oleic acid represented the largest proportion of fatty acid intake (34%). The major components (>5%) of fatty acid intakes were oleic acid (34%), palmitic acid (18%), linoleic acid (18%), stearic acid (9%), and 18:1*t* (5%).

3. Correlations between serum fatty acids and fatty acid intakes

The highest correlations between serum measures and intake were observed for DHA (r=0.53), EPA (r=0.42), and 18:1*t* (r=0.38, **Figure 6**). Serum saturated, monounsaturated, and n6 polyunsaturated fatty acids yielded low correlations with intake. There were no substantial differences between the unadjusted and adjusted correlation coefficients.

4. Further investigation of serum DHA, EPA, and 18:1*t*

We examined the correlations between serum DHA, EPA, and 18:1*t* with their fatty acid intakes in various subgroups (**Table 8**). Correlations for serum DHA and 18:1*t* were lower among black women than white women. Also, correlations were lower among women with lower levels of formal education, though the differences were not statistically significant. However, correlations remained relatively consistent by household income. Correlations for serum DHA and 18:1*t* were lower among women with higher BMI.

We calculated the percent of agreement between quintiles defined by the three serum fatty acids and those defined by their corresponding fatty acid intake (**Table 9)**. Fair agreement was observed (% agreement = 34% for DHA, 28% for EPA, and 30% for 18:1*t*; weighted Kappa statistics = 0.35 for DHA, 0.26 for EPA, and 0.25 for 18:1*t*). We also

examined the correlations between the three serum fatty acids measured at baseline with their fatty acid intakes measured 3 years after baseline. The correlation coefficients were 0.47 for DHA, 0.34 for EPA, and 0.34 for 18:1*t*.

5. Correlations between serum fatty acids and fat intake groups

The correlations for serum DHA and EPA and total n3 polyunsaturated fat intake were 0.33 and 0.29 respectively, **Table 10**). Serum DHA and EPA were not correlated with total polyunsaturated fat intake. The correlation between serum 18:1*t* and trans fat intake was 0.39. The correlation between serum pentanoic acid (15:0) was 0.24. The remaining serum fatty acids were not correlated with their fat intake groups.

6. Correlations between serum fatty acids and food groups

Serum DHA and EPA were correlated with overall fish intake (r=0.39 and 0.35, respectively, **Table 11**). These correlations were the highest for dark fish and low-to-moderate for white fish, tuna, and shellfish. Serum DHA and EPA were not correlated with fried fish. In general, serum DHA had higher correlations with fish intake than serum EPA. The correlation between serum DHA+EPA and overall fish intake was 0.41. Serum 18:1*t* was correlated with intake of added fat, butter, and pastries (r=0.22, 0.26, and 0.30, respectively). We also observed a modest correlation between serum myristic acid (14:0) and dairy intake (r=0.20). Correlations $\geq$ 0.20 were not observed for intake of red meat, poultry, eggs, milk, nuts, or chips.

We examined the correlations between DHA and EPA with fish intake in greater detail. The correlations between serum DHA and EPA and fish intake were lower among women who ate $\leq$ 3 medium servings of fish per month (0.12 and 0.09, respectively, n=240) and higher among women who ate > 3 medium servings of fish per month (0.34 and 0.29, respectively, n=685). Fair agreement was observed between quintiles defined by serum DHA and DPA and those defined by fish intake (% agreement = 28% for DHA and 26% for

EPA; weighted Kappa = 0.25 for DHA and 0.22 for EPA). The correlations between serum DHA and EPA measured at baseline with fish intake measured 3 years after baseline were 0.35 and 0.30, respectively.

E. DISCUSSION

We compared serum fatty acid concentrations with intake of individual fatty acids, fat groups, and food groups assessed by an FFQ. Serum DHA, EPA, and 18:1*t* concentrations were moderately correlated with their corresponding fatty acid intakes in this cohort of postmenopausal US women. Serum DHA and EPA were correlated with n3 polyunsaturated fat intake, and serum 18:1t was correlated with trans fat intake. In regards to food groups, serum DHA and EPA concentrations were correlated with fish intake, and serum 18:1*t* was correlated with intakes of added fat, butter, and pastries. Serum saturated, monounsaturated, and n6 polyunsaturated fatty acids were not correlated with fatty acid or food group intake.

As expected, we found higher correlations for fatty acids that could not be endogenously synthesized from carbohydrates (Willett, 1998). These fatty acids included n3 polyunsaturated, trans, and odd-numbered fatty acids. In our study, two n3 polyunsaturated fatty acids, DHA and EPA, and one trans fatty acid, 18:1*t*, had moderate correlations. Consistent with the results of our study, correlations for DHA, EPA, and 18:1*t* were generally the highest of those reported in studies comparing serum/plasma fatty acids and fatty acid intake (Ma et al., 1995; Andersen et al., 1999; Hodge et al., 2007; Sun et al., 2007; Patel et al., 2010). These correlations ranged from 0.29 to 0.78 for DHA, 0.21 to 0.51 for EPA, and 0.29 to 0.38 for 18:1*t*.

Further investigation of the correlations for DHA, EPA, and 18:1t revealed that the correlations varied by race, education, and body weight status. These varying correlations are likely the result of inaccurate reporting of intake by participants. In a study of energy consumption using recovery biomarkers among 544 participants in the WHI Dietary

Modification Trial, investigators reported that Blacks and Hispanics (vs. Caucasians) and those with a higher BMI were more likely to underreport total energy intake than their counterparts (Neuhouser et al., 2008). It is unlikely that measurement of serum fatty acids were systematically influenced by race, education, and BMI. Rather, the varying correlations were likely the result of inaccurate reporting of intake related to social desirability or ability to recall intake of foods by the participants.

We investigated the correlations between serum fatty acids and intake of foods because foods are more relevant to communicating dietary recommendations than individual fatty acids. The correlations found between DHA, EPA, and 18:1*t* and food groups were reflected by the sources of these fatty acids. The major sources of serum DHA and EPA are fish and fish oil (Kris-Etherton et al., 2002). The major source of 18:1*t* between 1993 and 1998 were foods made with industrially produced partially hydrogenated vegetable oil, such as butter and pastries (Micha et al., 2010). These findings precede the 2006 mandated labeling of trans fatty acids in foods and subsequent reformulation of many foods that contained trans fatty acids (Harnack et al., 2003).

Serum DHA may be a more reliable marker of fish intake than serum EPA. These findings are of interest because prospective studies have reported that fish consumption is related to lower incidence of coronary heart disease mortality and ischemic stroke (Kris-Etherton et al., 2002; He, Song, Daviglus, Liu, Van Horn, Dyer, Goldbourt, et al., 2004; He, Song, Daviglus, Liu, Van Horn, Dyer, & Greenland, 2004). The specific nutrients in fish hypothesized to be responsible for the lower incidences were thought to be DHA and EPA through a variety of beneficial effects on cardiovascular risk factors (Kris-Etherton et al., 2002). Our study and others' report stronger correlations between fish intake and serum/plasma DHA than EPA, suggesting that DHA may be the more reliable marker of fish intake than EPA (Ma et al., 1995; Hjartaker et al., 1997; Andersen et al., 1999; Hodge et al., 2007; Sun et al., 2007; Patel et al., 2010). Similar results were described in studies using

adipose tissue biomarkers (Tjonneland et al., 1993; Andersen et al., 1999; Baylin et al., 2002).

Odd-numbered saturated fatty acids, 15:0 and 17:0, plus 14:0 were hypothesized to be markers of dairy fat intake in a study conducted by Wolk and colleagues (Wolk et al., 2001). We found a low-moderate correlation between serum 14:0 and dairy intake and only low correlations between serum 15:0 and 17:0 and dairy and milk intakes. Our study population had very low concentrations of serum 15:0 and 17:0, which could have resulted in the low correlations. Also, it is possible that the multiple 24-hour recalls and food records used by Wolk and colleagues were more sensitive to dairy intake than the FFQ used in the present study.

Adipose tissue and erythrocyte fatty acids are often thought to be better biomarkers of long-term fatty acid intake than serum based on the longer half-lives of fatty acids in these tissues (Sun et al., 2007). However, our results and that of others' suggest that serum DHA, EPA, and 18:1*t* have correlations with long-term intake that are similar to or better than those reported for adipose tissue (Hunter et al., 1992; Tjonneland et al., 1993; Garland et al., 1998; Lemaitre et al., 1998; Andersen et al., 1999; Baylin et al., 2002). In addition, we obtained higher correlations for serum DHA, EPA, and 18:1*t* than others have obtained for erythrocytes (Andersen et al., 1999; Hodge et al., 2007), with the exception of one study (Sun et al., 2007). It is possible that intake of these fatty acids remains relatively consistent over time in subjects eating a Western diet, so that it is reflected in the shorter half-life associated with serum. The moderate correlations we found between serum DHA, EPA, and 18:1*t* measured at baseline and intake measured at the year 3 follow-up visit also support this hypothesis. Other factors to consider are ease of collection and degradation during storage. Serum fatty acids are relatively easy to collect compared with adipose tissue and are less prone to degradation during storage than red blood cell fatty acids (Glatz et al., 1989; Romon et al., 1995). In addition, determining the half-life of fatty acids in various

tissues is very complicated. The half-life commonly cited for adipose tissue fatty acids is based on linoleic acid only. Individual fatty acids in different tissues have different half-lives. The correlations we found for serum fatty acids suggest that selecting a fatty acid biomarker – serum, adipose tissue, or erythrocyte – should be based on more than half-life alone.

This correlation study has several strengths. First, this study included more than 900 postmenopausal women, which provided sufficient statistical power to examine correlations between serum and intake. However, statistical power to examine correlation among non-whites was limited. Second, we examined the correlations for serum trans fatty acids, which only one study had examined previously (Sun et al., 2007). Third, we examined the measurement characteristics of the WHI FFQ against serum fatty acids, a biomarker of fatty acid intake that has measurement errors that are uncorrelated with errors in the FFQ. Previously, fatty acid intakes derived from the WHI FFQ were compared against other self-reported measures (Patterson et al., 1999). Since these measures shared major sources of error (e.g. memory, perception of serving sizes, and nutrient database) and those errors were likely correlated in both measures, it is possible that a large proportion of the correlation was due to these errors.

Our study also has limitations. One concern is the stability of serum fatty acids during storage and freeze/thaw procedures. However, serum storage for up to 12 years at -80℃ protected polyunsaturated fatty acids from oxidation (Zeleniuch-Jacquotte et al., 2000). We conducted an analysis of 15 samples that demonstrated that there were no substantial changes in the composition of the serum fatty acids analyzed originally in 2005, one year later in 2006, and then after an additional freeze/thaw procedure. No statistically significant differences were detected between the mean values of the fatty acids from the original and one-year or freeze/thaw samples. Polyunsaturated fatty acids, the most susceptible fatty acids to storage and freeze/thaw, gave the best results according to this analysis.

Another concern is that our study population of mostly non-Hispanic Caucasian, postmenopausal US women is relatively homogenous, limiting generalizability to other populations. One correlation study of adipose tissue conducted in Costa Rica reported high correlations for 18:1*t* and linelaidic acid due to the high intake of partially hydrogenated soybean oil in that population (Baylin et al., 2002). Our results suggest that the correlations between serum DHA, EPA, and 18:1*t* and intake may vary by race, education, and body weight status. These varying correlations are likely the result of inaccurate reporting of intake by participants. These results emphasize the importance of examining the correlations of fatty acids biomarkers and intake in various populations.

Finally, the WHI did not collect information on intake of fish oil supplements. Correlations for DHA and EPA may have been underestimated by not accounting for fish oil in the intake in EPA and DHA. Despite the lack of data on fish oil supplementation use, our results indicate that serum DHA and EPA can rank participants according to intake of DHA, EPA, and fish.

In summary, in this cohort of postmenopausal US women, serum DHA, EPA, and 18:1*t* are suitable biomarkers of their corresponding dietary fatty acid intakes as assessed by an FFQ. Based on these moderate correlations, future studies that find associations between serum DHA, EPA, or 18:1*t* and disease may make cautious dietary recommendations. Future studies that find associations between dietary intake of DHA, EPA, 18:1*t*, fish, added fats, and pastries and disease are strengthened by the moderate correlations with the objective serum fatty acid measurements reported in this study.

VII. Serum Fatty Acids and the Risk of Ischemic Stroke Among Postmenopausal Women

This manuscript presents the results and discusses the implications of Aim 2. It addresses two possibilities that could explain inconsistent findings in previous studies of fatty acids and ischemic stroke risk: combining fatty acids with opposing effects on CVD risk and examining ischemic stroke as a composite outcome despite its heterogeneous etiology. The few studies that had data on ischemic stoke subtypes lacked statistical power to examine those subtypes. Few of the previous studies included women, despite the higher burden of ischemic stroke among postmenopausal women. Therefore, this manuscript examines the associations between individual serum fatty acids and total ischemic stroke and its etiologic subtypes among a cohort of postmenopausal women, who have elevated risk of ischemic stroke.

A. SUMMARY

Context: While epidemiologic studies have linked trans and saturated fatty acids with increased risk of coronary heart disease, and polyunsaturated fatty acids with decreased risk, few epidemiologic studies have examined associations between serum fatty acids and ischemic stroke.

Objective: To examine the associations between serum fatty acid concentrations and incidence of ischemic stroke and its subtypes.

Design and Setting: Prospective case-control study nested in the Women's Health Initiative Observational Study cohort of postmenopausal US women aged 50 to 79 years.

Participants: Between 1993 and 2003, incident cases of ischemic stroke were matched 1:1 to controls on age, race, and length of follow-up (964 matched pairs).

Main Outcome Measure: Incident ischemic stroke and ischemic stroke subtypes.

Results: The multivariable odds ratio (OR) and 95% confidence interval (CI) of ischemic stroke associated with a 1-standard deviation (SD) increment in serum fatty acid concentration was 1.38 (1.17, 1.63) for linelaidic acid (18:2*tt*, SD=0.04%); 1.27 (1.14, 1.41) for palmitic acid (16:0, SD=2.74%); 1.20 (1.08, 1.33) for oleic acid (18:1n9, SD=2.32%); 0.84 (0.75, 0.93) for eicosapentaenoic acid (20:5n3, SD=0.54%); 0.72 (0.64, 0.80) for docosapentaenoic acid (22:5n3, SD=0.18%); 0.72 (0.64, 0.80) for docosahexaenoic acid (22:6n3, SD=0.91%); and 0.81 (0.73, 0.91) for arachidonic acid (20:4n6, SD=2.02%). These associations were generally consistent for atherothrombotic and lacunar stroke, but not cardioembolic stroke.

Conclusions: These findings suggest that individual serum trans, saturated, and monounsaturated fatty acids are positively associated with particular ischemic stroke subtypes, while individual n3 and n6 polyunsaturated fatty acids are inversely associated.

B. INTRODUCTION

Few epidemiologic studies have examined the associations between serum fatty acids, a biomarker of fatty acid intakes, and ischemic stroke risk (He et al., 2007). Thus far, the results have been inconsistent. One study conducted in Japan found that serum saturated fatty acids were associated with increased risk of ischemic stroke, while serum linoleic acid (an n6 polyunsaturated fatty acid) was associated with decreased risk (Iso et al., 2002). Another study conducted in Finland found similar associations as the Japan study, but additionally reported that serum monounsaturated and n3 polyunsaturated fatty acids were associated with increased risk of ischemic stroke (Tilvis et al., 1987). However, a third study found no significant associations for serum saturated or monounsaturated fatty acids (Miettinen et al., 1986).

Two possibilities have been raised that could explain these inconsistent findings. First, the associations between serum fatty acids and ischemic stroke may not be consistent for all ischemic stroke subtypes. In the Japan study described previously, the results were driven by lacunar stroke, this subtype comprising 95 of the 122 ischemic strokes in that study (Iso et al., 2002). Importantly, this study did not have sufficient power to examine associations for

atherothrombotic stroke, the subtype of ischemic stroke directly related to blood cholesterol (Ohira et al., 2006). Combining atherothrombotic stroke with lacunar and cardioembolic strokes would dilute expected associations. However, to our knowledge, only one study has examined serum fatty acids in relation to atherothrombotic stroke and other ischemic stroke subtypes.

Second, the inconsistent findings could be due to individual fatty acids having different effects on ischemic stroke. In the Japan study described previously, despite finding that serum myristic and palmitic, but not stearic, saturated fatty acids were positively associated with total ischemic stroke in unadjusted analysis, investigators combined these three fatty acids in multivariable analysis (Iso et al., 2002). Depending on the relative contribution of each fatty acid, it is possible to find inconsistent associations for total serum saturated fatty acids. However, few studies have examined serum fatty acids individually while controlling for potential confounders.

To address these possibilities, we conducted a prospective case-control study nested in a large cohort of postmenopausal US women to examine the associations between 25 individual serum fatty acid concentrations from stored serum samples collected at baseline and the incidence of ischemic stroke and ischemic stroke subtypes. We hypothesize that individual serum trans and saturated fatty acids would be positively associated with atherothrombotic stroke, whereas individual serum monounsaturated and polyunsaturated fatty acids would be inversely associated.

C. METHODS

1. Study Population

We conducted a case-control study of 964 incident ischemic stroke cases and 964 matched control subjects nested within the Women's Health Initiative Observational Study (WHI-OS) (Wassertheil-Smoller et al., 2008). Briefly, the WHI was conducted in 40 clinical centers across the US to examine the impact of a number of factors on the major causes of morbidity and mortality in postmenopausal women (Prentice et al., 1998). Eligible women were 50 to 79

years of age at baseline, postmenopausal, had no medical conditions associated with a predicted survival of <3 years, and provided informed consent to be a part of the study as approved by the institutional review boards. The WHI-OS enrolled 93,676 women between October 1993 and December 1998. There were 972 confirmed incident ischemic stroke cases between 1993 and 2003. Control subjects were individually matched 1:1 to the cases on age and race at the time of ischemic stroke. Blood quantity was not sufficient to analyze fatty acid content for 9 participants, leaving a total of 964 case-control pairs.

2. Case identification and classification

Incident ischemic strokes during the follow-up period were identified through self-report during annual medical history updates (annual response rate 94%) (Curb et al., 2003). Using additional details from medical charts, brain imaging, or death certificates, the potential outcomes were subject to local adjudication by physicians, then central adjudication by trained neurologists, according to standard criteria. Over 95% of participant-reported stroke cases were classified based on CT or MRI findings (Chen et al., 2008).

Central adjudicators further classified ischemic strokes by subtypes according to the Trial of ORG 10172 Acute Stroke Trial (TOAST) Classification (Goldstein et al., 2001). The TOAST classification focuses on the presumed underlying stroke mechanism; requires detailed investigations (such as brain computed tomography, magnetic resonance imaging, angiography, carotid ultrasound, and echocardiography); and distinguishes 5 categories of stroke, which include large artery atherothrombotic stroke, small vessel lacunar stroke, cardioembolic stroke, other, and undetermined mechanism (Wassertheil-Smoller et al., 2003). Probable and possible subtypes determinations were combined in this report. Transient ischemic attack, hemorrhagic stroke, strokes not requiring hospitalization, and strokes not confirmed by central adjudication were not included as a stroke outcome.

3. Control selection

Controls were sampled from women at risk at the time ischemic stroke events occurred during the follow-up period and matched 1:1 to case subjects on age at baseline (±2 years), race/ethnicity (white, black, Hispanic, Asian, American Indian, or other/unspecified), date of study enrollment (±3 months), and follow-up time.

4. Data collection

Fasting blood samples were collected from all WHI-OS participants by clinic staff members following a standardized protocol for ventipuncture at baseline (Anderson et al., 2003). Samples were centrifuged, separated by layers, frozen onsite at -70℃, and shipped to the central WHI repository for long-term storage.

Fatty acids were extracted according to the general technique of Bligh and Dyer using 1, 2-dinonadecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL) (50 µg of 19:0) as an internal standard (Bligh & Dyer, 1959). The lipid extracts were resuspended in 1.5 ml 14% boron trifluoride methanol and derivatized according to the procedure of Morrison and Smith (Morrison & Smith, 1964). The extracts were analyzed by capillary GC:column (SP-2380, 105 m x 53 mm ID, 0.20 um film thickness). The gas chromatograph was a Perkin Elmer Clarus 500 equipped with a flame ionization detector. Operating conditions: the oven temperature was 140℃ for 35 min, then ramped at 8℃/min to 220℃, and held for 12 min. Injector and detector temperatures were both at 260℃ and helium, the carri er gas, was at 15 psi. Identification of components was by comparison of retention times with those of authentic standards (Sigma Chemical Co., St. Louis, MO). Duplicate samples and control pools were analyzed with each batch of samples. The coefficients of variation of fatty acids ranged from 3.1% to 13.2%. The Spearman correlation coefficients between dietary fatty acid intake (% of total fat intake assessed by a FFQ) and serum fatty acid concentrations among non-cases (both measured at baseline) were 0.53 for DHA, 0.42 for EPA, 0.18 for DPA, 0.16 for palmitic acid, 0.15 for linelaidic acid, 0.07 for arachidonic acid, and 0.03 for oleic acid.

Participants completed sociodemographic and lifestyle questionnaires during baseline visits to a WHI clinical center. (Anderson et al., 2003). Physical activity was assessed based on participant report about the frequency, intensity, and duration of walking, exercise, or recreational activity and summarized into total metabolic equivalent task (MET)-hours per week. Certified clinic staff measured participant height, weight, and blood pressure. Height and weight were measured without shoes or heavy clothing and with pocket contents removed. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Following standardized procedures, blood pressure was measured twice after a 5-minute seated rest with a 30-second rest in between. The average of two blood pressure measurements was used in analysis. Hypertension was defined as self-report of antihypertensive medication use or systolic blood pressure ≥ 140 mm Hg or a diastolic blood pressure ≥ 90 mm Hg. Diabetes was defined as being on treatment for diabetes by self-report or having a fasting glucose level ≥ 126 mg/dL. Participants reported their history of smoking, diabetes, atrial fibrillation, angina, or revascularization (coronary bypass surgery or angioplasty of the coronary arteries), as well as current use of medications.

Lipoprotein profiles were assayed at Liposcience using nuclear magnetic resonance. Low-density lipoprotein (LDL) cholesterol was calculated from high-density lipoprotein (HDL) cholesterol, total cholesterol, and triglyceride concentrations among women who had a triglyceride value <400 mg/dL using the Friedewald equation (National Cholesterol Education Program Expert Panel on Detection, 2002). LDL cholesterol values were set to missing for those women whose triglyceride value was >400 mg/dL (n=35) or who were missing HDL cholesterol, total cholesterol, or triglyceride values (n=7).

5. Statistical analysis

The McNemar's chi-square test was used to compare covariate proportions between ischemic stroke cases and controls. The paired Student's *t* test was used to compare serum fatty acid concentration and covariate means between ischemic stroke cases and controls. The

distributions of the serum fatty acids were highly skewed to the right. Therefore, we used the median as the measure of central tendency for descriptive statistics.

Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for ischemic stroke and its subtypes, conditioning on matching factors in all models. We divided women into quintiles based on distribution of serum fatty acid concentrations in the case-control study population. We estimated multivariable ORs and 95% CIs comparing the odds of ischemic stroke among women in the higher quintiles of serum fatty acids with the odds of ischemic stroke in the first quintile. Linear trend tests were performed by assigning the median value of the quintile of the fatty acid to each category and entering this variable into the model as a continuous variable. Since trends were generally linear, multivariable ORs were calculated per 1-standard deviation (SD) increment in each fatty acid. Covariates were determined using previous knowledge, existing literature, and statistical tests. Our primary model was adjusted for BMI, smoking status, diabetes, and aspirin use. Subsequent models were adjusted for blood pressure and blood cholesterol variables, since these factors are the likely mediators of the association between serum fatty acids and ischemic stroke. Two-sided $P \leq 0.001$ was considered significant to account for multiple comparisons and to decrease the risk of detecting a false positive association (Type I error). All analyses were performed using SAS statistical software (Version 9.2, SAS Institute Inc., Cary, NC, USA).

D. RESULTS

1. Study Population

Between 1993 and 2003, we identified 964 cases of incident ischemic stroke. Of these, 96 were atherothrombotic strokes, 250 were lacunar strokes, 209 were cardioembolic strokes, 42 were ischemic strokes of other determined etiology, 366 were ischemic strokes of undetermined etiology, and 1 was missing subtype information. The mean age at baseline of women with ischemic stroke was 68.7 (SD 6.4) years (**Table 12**). Women with incident ischemic stroke were

more likely to be overweight or obese, be a current smoker, have diabetes, and to use aspirin. We accounted for these differences by adjusting for these variables in multivariable analysis.

2. Distribution of serum fatty acids

Twenty-five individual serum fatty acids were examined (**Table 13**). The major contributors (median >5%) to the composition of serum fatty acids were linoleic n6 polyunsaturated (27%), palmitic saturated (24%), oleic monounsaturated (18%), stearic saturated (9%), and arachidonic n6 polyunsaturated (8%) fatty acids. The serum concentrations of 18:1t and linelaidic trans fatty acids, myristic and palmitic saturated fatty acids, and 7-hexadecenoic and oleic monounsaturated fatty acids were higher among ischemic stroke cases than controls. The concentrations of alpha-linolenic, eicosatetraenoic, EPA, DPA, DHA n3 polyunsaturated fatty acids and linoleic, arachidonic, and 22:5n6 n6 polyunsaturated fatty acids were lower among ischemic stroke cases than controls.

3. Total ischemic stroke

With adjustment for BMI status, smoking status, diabetes, and aspirin use and accounting for the matching factors, serum linelaidic acid (18:2tt trans, SD=0.04%, OR 1.38, 95%CI 1.17 to 1.63), palmitic acid (16:0 saturated, SD=2.74%, OR 1.27, 95%CI 1.14 to 1.41), and oleic acid (18:1n9 monounsaturated, SD=2.32%, OR 1.20, 95%CI 1.08 to 1.33) were associated with increased incidence of ischemic stroke. Serum EPA (20:5n3 n3 polyunsaturated, SD=0.54%, OR 0.84, 95%CI 0.75 to 0.93), DPA (22:5n3 n3 polyunsaturated, SD=0.18%, OR 0.72, 95%CI 0.64 to 0.80), DHA (22:6n3 n3 polyunsaturated, SD=0.91%, OR 0.72, 95%CI 0.64 to 0.80) n3 polyunsaturated fatty acids and arachidonic (20:4n6 n6 polyunsaturated, SD=2.02%, OR 0.81, 95%CI 0.73 to 0.91) acid were associated with decreased incidence (**Figure 7, Table 14**). Further adjustment for blood pressure and blood cholesterol did not change estimates by more than 10%. Neither did further adjustment for physical activity, angina, atrial fibrillation, or revascularization change estimates by more than 10% (data not shown).

To examine the possibility that the positive association for serum oleic acid was due to its correlations with serum arachidonic acid or serum DHA (Spearman rank correlation coefficients -0.43 and -0.31, respectively), we additionally adjusted for these fatty acids using the residual method (Willett, 1998). The multivariable OR (95%CI) for total ischemic stroke associated with a 1-SD increment in oleic acid was attenuated by 10% (OR 1.09, 95% CI 1.00, 1.18) after adjustment for serum arachidonic acid, by 12% (OR 1.06, 95% CI 0.99, 1.14) after adjustment for serum DHA, and completely attenuated after adjustment for serum total polyunsaturated fatty acids (OR 1.00, 95% CI 0.91, 1.11).

4. Ischemic stroke subtypes

We further examined the fatty acids that were significantly associated with total ischemic stroke (serum linelaidic acid, palmitic acid, oleic acid, EPA, DPA, DHA, and arachidonic acid) in relation to ischemic stroke subtypes. The associations between these serum fatty acids and total ischemic stroke were generally consistent for atherothrombotic stroke, lacunar stroke, and ischemic stroke of undetermined etiology (**Figure 8, Table 15**). There were a few exceptions to this generalization. We did not observe a positive association between palmitic acid and atherothrombotic stroke. Neither did we observe an inverse association between EPA and ischemic strokes of undetermined etiology. However, we did observe an inverse association between serum DHA and cardioembolic stroke, making DHA the only serum fatty acid to be consistently inversely associated with all subtypes of ischemic stroke. We did not have sufficient statistical power to examine associations for ischemic strokes of other determined etiology (42 matched pairs).

Further adjustment for blood pressure attenuated associations for lacunar stroke (by 11% for EPA and 12% for DHA), but not atherothrombotic stroke. Further adjustment for blood cholesterol attenuated associations for lacunar stroke (by 13% for linelaidic fatty acid) and atherothrombotic stroke (by 48% for linelaidic fatty acid, 28% for palmitic fatty acid, and 60% for oleic fatty acid).

E. DISCUSSION

In the largest case-control study of ischemic strokes among postmenopausal US women, serum linelaidic (trans) acid, palmitic (saturated) acid, and oleic (monounsaturated) acid were associated with increased incidence of ischemic stroke. Serum EPA, DPA, and DHA (n3 polyunsaturated) acids and arachidonic (n6 polyunsaturated) acid were associated with decreased incidence. These associations were generally consistent for atherothrombotic and lacunar stroke, but not cardioembolic stroke.

Our results suggest that individual serum fatty acids have different associations with ischemic stroke. However, large prospective cohort studies were not able to examine fatty acids individually. These studies assessed fatty acid intake through participant report (e.g. FFQ) and focused on major types of fat (e.g., saturated fat). Two prospective studies reported that saturated fat intake and animal fat intake (mostly saturated fat) were associated with decreased risk of ischemic stroke and cerebral infarction death, respectively (Gillman et al., 1997; Sauvaget et al., 2004). However, four prospective studies failed to find associations between saturated fat intake and ischemic stroke (Seino et al., 1997; Iso, Stampfer, et al., 2001; He et al., 2003; Yaemsiri et al., 2012 [in press]). One possible explanation for these inconsistent results is that individual fatty acids, even those of the same type, may have different effects on cardiovascular disease risk. For example, myristic (14:0) and palmitic (16:0) acids tend to increase plasma total and LDL-cholesterol concentrations compared with oleic acid, whereas stearic acid (18:0) does not (Kris-Etherton & Yu, 1997). It is possible that examining types of fat intake masked the associations of individual fatty acids and ischemic stroke. However, individual fatty acids were highly correlated in prospective studies due to participant reported intake assessment methods. These high correlations make distinguishing their individual relations with ischemic stroke risk difficult (Hu et al., 2001).

In contrast to the prospective studies using participant reported fatty acids intakes, case-control studies using biomarkers of fatty acid intake – serum, erythrocytes, and adipose tissue –

are able to examine fatty acids individually (e.g., palmitic acid). In the largest of these studies, Iso and colleagues found that serum myristic and palmitic acids, but not stearic acid, were associated with increased risk of ischemic stroke, even though all three are saturated fatty acids. (Iso et al., 2002). In addition, serum linoleic acid, but not other polyunsaturated fatty acids, was associated with decreased risk of ischemic stroke. Smaller case-control studies also suggest that individual fatty acids, even those of the same type, may have different effects on ischemic stroke risk (Bucalossi & Mori, 1972; Miettinen et al., 1986; Ricci et al., 1987; Ricci et al., 1997). These studies highlight the importance of examining individual fatty acids in relation to ischemic stroke risk.

Our results also suggest that the associations between serum fatty acids and ischemic stroke may not be consistent across all ischemic stroke subtypes. To our knowledge, only two studies have reported associations for ischemic stroke subtypes. In these studies, the associations between individual fatty acids and total ischemic stroke were consistent for lacunar stroke, but not atherothrombotic or cardioembolic stroke. Iso and colleagues reported that the associations between serum saturated and linoleic fatty acids and ischemic stroke were driven by lacunar stroke, this subtype comprising 95 of the 122 ischemic strokes in that study (Iso et al., 2002). More recently, Yaemsiri and colleagues reported that the positive association between trans fat intake and ischemic stroke was consistent for lacunar stroke using data from the full WHI-OS cohort in a longitudinal study (Yaemsiri et al., 2012 [in press]). These studies highlight the importance of examining ischemic stroke subtypes individually.

The associations between fatty acids and ischemic stroke may not be consistent across all subtypes because ischemic stroke is a heterogeneous condition. There is evidence that blood cholesterol may play a role in the development of some ischemic stroke subtypes, but not others (Ohira et al., 2006). Kuller and Reisler examined the incidence of ischemic stroke subtypes in populations with varying blood cholesterol levels (Kuller & Reisler, 1971). Based on this analysis, they hypothesized that blood cholesterol may play a role in atherothrombotic

stroke, the ischemic stroke subtype with an atherogenic basis. Fatty acid intakes have a powerful effect on blood cholesterol concentrations, and they were hypothesized to play a role in the development of atherothrombotic stroke, as well. One of the challenges in studying atherothrombotic stroke, the least prevalent ischemic stroke subtype, is low statistical power. However, we had sufficient power to detect general trends in atherothrombotic stroke incidence according to serum fatty acid concentration. Consistent with Kuller and Reisler's hypothesis, linelaidic, oleic, EPA, DPA, DHA, and arachidonic acids were associated with atherothrombotic stroke.

While the effect of fatty acid intake on blood cholesterol likely explains associations for atherothrombotic stroke, the explanation for the apparent associations for lacunar stroke is not clear. However, a few possibilities can be proposed. First, intake of EPA and DHA through fish oil supplementation may lower blood pressure. A meta-analysis of 36 randomized trials reported that fish oil supplementation lowered systolic and diastolic blood pressures by 3.5 and 2.4 mm Hg, respectively (Geleijnse et al., 2002). Importantly, serum DHA, which may have a stronger blood pressure lowering effect than EPA, (Mori et al., 1999) was the only serum fatty acid that was consistently associated with all subtypes of ischemic stroke in the present study. Second, intake of EPA and DHA inhibit thrombosis through their anti-platelet effects (Agren et al., 1997). In the present study, serum EPA and DHA were both inversely correlated with d-dimer concentration, a measure of thrombotic disease, such as lacunar stroke. Third, intake of trans fat intake and n3 polyunsaturated fatty acids have pro- and anti-inflammatory effects, respectively (James et al., 2000; Mozaffarian et al., 2004). High concentrations of inflammatory markers have been associated with greater neurological deterioration in lacunar strokes (Castellanos et al., 2002). Finally, there is limited evidence suggesting that trans fat intake may adversely affect glucose tolerance, which may lead to development of diabetes. Diabetes is a major risk factor for lacunar stroke. Most observational studies report a positive

association between trans fat intake and diabetes, however these results have not been supported by the results of controlled feeding studies (Thompson et al., 2011).

Our study has some limitations. One concern is the stability of serum fatty acids during storage and freeze/thaw procedures. However, serum storage for up to 12 years at -80℃ protected polyunsaturated fatty acids, the most susceptible fatty acids to storage and freeze-thaw, from oxidation (Zeleniuch-Jacquotte et al., 2000). We conducted an analysis of 15 samples analyzed originally in 2005, one year later in 2006, and then after an additional freeze/thaw procedure. No statistically significant differences were detected between the mean values of the fatty acids from the original and one-year or freeze/thaw samples.

When these ischemic strokes were classified, TOAST was the best currently available with moderate to good inter-observer reliability with training (Wassertheil-Smoller et al., 2003). The field of ischemic stroke classification has been evolving to address the large number of cases that are classified as having “undetermined etiology.” In the newer ASCO Phenotypic System, cases can be characterized by all 4 phenotypes based on the level of diagnostic certainty (Shang & Liu, 2012). Using the ASCO classification could reduce the proportion of ischemic stroke cases with underdetermined etiology and increase statistical power.

Another concern is that the TOAST classification uses a history of diabetes mellitus and hypertension in its lacunar stroke definition (Adams et al., 1993). This classification bias could, in part, explain the association between serum fatty acid and lacunar stroke, assuming that serum fatty acids act through hypertension to increase risk of ischemic stroke. More recent studies have advocated the use of a “risk factor-free classification” to avoid classification bias (Ohira et al., 2006). However, the prevalence of hypertension among the ischemic stroke subtypes was relatively uniform (72% of atherothrombotic, 73% of lacunar, and 68% of cardioembolic stroke cases) suggesting that this misclassification was not a major source of bias in this study.

Finally, there is evidence that associations between fatty acid biomarkers and ischemic stroke may vary by the age of the study population and type of biomarker used (Tilvis et al., 1987). The results derived from this study population of predominately white postmenopausal women may not be generalizable to other populations.

In conclusion, we report that serum linolaidic trans fatty acid, palmitic saturated fatty acid, and oleic monounsaturated fatty acid were positively associated with incidence of ischemic stroke, while serum EPA, DPA, DHA n3 polyunsaturated fatty acids and arachidonic n6 polyunsaturated fatty acid were inversely associated with incidence of ischemic stroke. These associations were generally consistent for atherothrombotic and lacunar strokes. These findings highlight the importance of individual fatty acids in the development of particular subtypes of ischemic stroke.

VIII. CONCLUSIONS

The goal of this dissertation was to answer the questions: Why were there discrepancies in previous studies relating fatty acid biomarkers versus fatty acid intakes to ischemic stroke and why were there discrepancies in previous studies relating dietary fat intake to ischemic stroke versus CHD? We examined the correlations between serum fatty acids and fatty acid intakes to answer the first question. We examined the associations between individual serum fatty acids and ischemic stroke subtypes to answer the second question.

A. Aim 1 conclusions

1. Individual fatty acid correlations

In this study, we found moderate correlations between serum fatty acid concentration and fatty acid intakes for DHA, EPA, and 18:1t fatty acids. Thus, we expected to find consistent associations between these serum fatty acids and fatty acid intakes and ischemic stroke. However, studies relating intake to ischemic stroke focused on fatty acid groups, rather than individual fatty acids. They did not examine fatty acids individually because they measured fat intake through participant report (e.g. FFQ and 24-hour recall). These methods may result in high correlations among individual fatty acids within the same group, making distinguishing their individual relations with disease risk difficult (Hu et al., 2001). Therefore, we investigated the correlations between individual serum fatty acids and fatty acid intake groups.

2. Fat group correlations

The correlations between serum fatty acids and fat intake groups help explain previous inconsistent results. With respect to fat intake groups, we found a moderate correlation

between serum 18:1t fatty acid and trans fat intake and between serum DHA and EPA and n3 polyunsaturated fat intake. Thus, we expected to find consistent associations between these fatty acids measured in serum and diet and ischemic stroke. Indeed, the positive associations between serum 18:1t fatty acid and ischemic stroke in this dissertation were consistent with the positive associations found between trans fat intake and ischemic stroke (Yaemsiri et al., 2012 [in press]). In addition, the inverse associations between serum DHA and EPA and ischemic stroke in this dissertation were consistent with the inverse associations found between n3 polyunsaturated fat intake and ischemic stroke (Iso, Rexrode, et al., 2001; He et al., 2002).

We found low correlations between serum DHA and EPA and total polyunsaturated fat intake. Given these low correlations, consistent results between DHA and EPA versus total polyunsaturated fat intake and ischemic stroke should not be expected. Indeed, the inverse associations between serum DHA and EPA and ischemic stroke in this dissertation were consistent with studies that examined DHA and EPA (specifically) and ischemic stroke (Iso, Rexrode, et al., 2001; He et al., 2002), but not consistent with the studies that examined total polyunsaturated fatty acids and ischemic stroke (Gillman et al., 1997; Seino et al., 1997; He et al., 2003; Sauvaget et al., 2004).

In summary, studies that examined total polyunsaturated fat intake reported null associations with ischemic stroke (Gillman et al., 1997; Seino et al., 1997; He et al., 2003; Sauvaget et al., 2004), while studies that examined n3 polyunsaturated fatty acids specifically reported significant inverse associations (Iso, Rexrode, et al., 2001; He et al., 2002). The implication for future studies is that studies should examine the associations between individual fatty acid intakes and disease, not fat intake groups. Combining fatty acids into groups, in particular combining n3 and n6 polyunsaturated fatty acids, could mask any potential association of individual fatty acids, depending on their relative contribution.

3. Serum biomarker of fatty acid intakes

Adipose tissue and erythrocyte fatty acids are often thought to be better biomarkers of long-term fatty acid intake than serum based on the longer half-lives of fatty acids in these tissues (Sun et al., 2007). However, based on our results and that of others,' serum DHA, EPA, and 18:1t fatty acids have correlations with long-term intake that are similar to or better than those reported for adipose tissue (Hunter et al., 1992; Tjonneland et al., 1993; Garland et al., 1998; Lemaitre et al., 1998; Andersen et al., 1999; Baylin et al., 2002) and erythrocytes (Andersen et al., 1999; Hodge et al., 2007; Sun et al., 2007). Since adipose tissue fatty acids are more difficult to collect and erythrocyte fatty acids are prone to degradation during storage (Glatz et al., 1989; Romon et al., 1995), serum may be a good alternative to rank participants according to intake of DHA, EPA and 18:1t fatty acids.

4. Implications for future research

The moderate correlations found between serum DHA, EPA, and 18:1t fatty acids and their corresponding fatty acid intake, fat intake groups, and food groups have implications for future studies. Those that find associations between serum concentration of DHA, EPA, or 18:1t fatty acids and disease may make cautious dietary recommendations based on these moderate correlations. Those that find associations between dietary intake of DHA, EPA, 18:1t fatty acid, fish, and added fats and disease are strengthened by the moderate correlations with the objective serum fatty acid measurements reported in this study.

We found that serum DHA, EPA, and 18:1t fatty acids were suitable biomarkers of their corresponding fatty acid intake. However, the correlations between serum and intake of thirteen other fatty acids were low. This work highlights the need for further research to find suitable biomarkers of intake for several fatty acids, especially specific saturated fatty acids, which are associated with increased risk of ischemic stroke.

B. Aim 2 conclusions

1. Total ischemic stroke

In this study, serum linelaidic, palmitic, and oleic acids were positively associated, and serum EPA, DPA, DHA, and arachidonic acids were inversely associated, with ischemic stroke. Based on studies of dietary fat groups and coronary heart disease, we expected that trans and saturated fatty acids would be positively associated with ischemic stroke and monounsaturated and polyunsaturated fatty acids inversely associated with ischemic stroke. However, not all individual serum fatty acids within a fatty acid group followed these associations. For example, serum 18:1t fatty and linelaidic acids were positively associated with ischemic stroke, while palmitelaidic acid showed no association. Similarly, myristic and palmitic acids were positively associated with ischemic stroke, while pentanoic, margaric, and stearic acids showed no association. These results further support our recommendation that future studies examine associations between individual fatty acid intakes and disease, not fatty acid groups.

Few studies have examined the associations between trans fatty acids and ischemic stroke; none used a biomarker of trans fatty acid intake. Two prospective studies of trans fat intake among male and female health care professionals did not find significant associations with ischemic stroke after adjustment for potential confounders (Iso, Stampfer, et al., 2001; He et al., 2002). However, a longitudinal prospective study of ischemic stroke in the WHI-OS observed that women in the highest quartile of trans fat intake were 39% more likely to experience ischemic stroke compared with women in the lowest. Our results support the available evidence that 18:1t fatty acid and linelaidic acid, the trans fatty acids found in partially hydrogenated vegetable oils, have stronger cardiovascular disease effects than palmitelaidic acid (Mozaffarian et al., 2009).

One unexpected result was the positive association between serum oleic acid and total ischemic stroke, given oleic acid's neutral effect on serum cholesterol (Mensink & Katan, 1992) and beneficial effect on blood pressure (Teres et al., 2008). We suggest one possibility that could explain this unexpected association. Serum fatty acids are expressed as a percent of total fatty acids, not absolute amounts. Therefore, increases of a specific fatty acid can drive down the relative percentage of another fatty acid even though its absolute amount is unaltered. Serum oleic acid was inversely correlated with arachidonic acid and DHA, suggesting that increased concentration of serum oleic acid drove down concentrations of DHA and arachidonic fatty acids. Both DHA and arachidonic fatty acids were inversely associated with ischemic stroke. When we statistically remove these correlations, the association between oleic acid and ischemic stroke was attenuated. The oleic acid finding may be a result of examining serum fatty acid concentrations rather than absolute amounts. Still, with the opposing effects of individual fatty acids, the concentration of fatty acids, rather than the absolute amount appears to be important to cardiovascular disease risk (Hu et al., 1997).

2. Ischemic stroke subtypes

Our results also suggest that the associations between serum fatty acids and ischemic stroke may not be consistent across all ischemic stroke subtypes. To our knowledge, only two studies have reported associations for ischemic stroke subtypes. In these studies, the associations were consistent for lacunar stroke, but not atherothrombotic or cardioembolic stroke. Iso and colleagues reported that the associations between serum saturated and linoleic fatty acids and ischemic stroke were driven by lacunar stroke, this subtype comprising 95 of the 122 ischemic strokes (Iso et al., 2002). More recently, Yaemsiri and colleagues reported that the positive association between trans fat intake and ischemic stroke was consistent for lacunar stroke using data from the full WHI-OS cohort in a

longitudinal study (Yaemsiri et al., 2012 [in press]). These studies highlight the importance of examining ischemic stroke subtypes individually.

The associations between fatty acids and ischemic stroke may not be consistent across all subtypes because ischemic stroke is a heterogeneous condition. There is evidence that serum cholesterol may play a role in the development of some ischemic stroke subtypes, but not others (Ohira et al., 2006). Kuller and Reisler examined the incidence of ischemic stroke subtypes in populations with varying serum cholesterol levels (Turan et al., 2010). Based on this analysis, they hypothesized that serum cholesterol may play a role in atherothrombotic stroke, the ischemic stroke subtype with an atherogenic basis. Fatty acid intakes have a powerful effect on serum cholesterol concentrations, and they were hypothesized to play a role in the development of atherothrombotic stroke, as well. One of the challenges in studying atherothrombotic stroke, the least prevalent ischemic stroke subtype, is low statistical power. However, we had sufficient power to detect general trends in atherothrombotic stroke incidence according to serum fatty acid concentration. Consistent with Kuller and Reisler's hypothesis, linelaidic, oleic, EPA, DPA, DHA, and arachidonic acids were associated with atherothrombotic stroke.

While the effect of fatty acid intake on serum cholesterol likely explains associations for atherothrombotic stroke, the mechanisms for the apparent associations for lacunar stroke are not clear. However, a few possibilities can be proposed. First, intake of EPA and DHA through fish oil supplementation may lower blood pressure. A meta-analysis of 36 randomized trials reported that fish oil supplementation lowered systolic and diastolic blood pressures by 3.5 and 2.4 mm Hg, respectively (Geleijnse et al., 2002). Importantly, serum DHA, which may have a stronger blood pressure lowering effect than EPA (Mori et al., 1999), was the only serum fatty acid that was consistently associated with all subtypes of ischemic stroke in the present study. Second, intake of EPA and DHA inhibit thrombosis through their anti-platelet effects (Agren et al., 1997). In the present study, serum EPA and

DHA were both inversely correlated with d-dimer concentration, a measure of thrombotic disease, such as lacunar stroke. Third, intake of trans fat intake and EPA and DHA have pro- and anti-inflammatory effects, respectively (James et al., 2000; Mozaffarian et al., 2004). High concentrations of inflammatory markers have been associated with greater neurological deterioration in lacunar strokes (Castellanos et al., 2002). Finally, there is limited evidence suggesting that trans fat intake may adversely affect glucose tolerance, diabetes being a key risk factor for lacunar stroke. Most observational studies report a positive association between trans fat intake and diabetes, however these results have not been supported by the results of controlled feeding studies (Thompson et al., 2011).

With the exception of serum DHA, serum fatty acids were not associated with cardioembolic stroke. It could be that hypertension and serum cholesterol are more distal causes of cardioembolic stroke than lacunar and atherothrombotic stroke. More proximate causes include atrial fibrillation, cardiac valve disease, or recent myocardial infarction. Indeed, serum DHA, which has been shown to reduce risk of atrial fibrillation in some studies, was inversely associated with cardioembolic stroke. These other more proximate causes of cardioembolic stroke may be unrelated to serum fatty acids.

Two fatty acids stand out for being associated with a subtype of ischemic stroke, but having no detectable association with total ischemic stroke. The first was serum stearic acid, which was inversely associated with lacunar stroke, but not total ischemic stroke or other subtypes. Serum stearic acid has been linked with lower systolic (Leng et al., 1994) and diastolic blood pressures (Simon et al., 1996), which is the primary cause of lacunar strokes. The second was serum alpha-linolenic acid, which was inversely associated with cardioembolic stroke, but not total ischemic stroke or other subtypes. The alpha-linolenic acid-rich Mediterranean diet has been linked with a reduction in recurrent myocardial infarction (de Lorgeril et al., 1994), which is a primary cause of cardioembolic strokes. These

results show that combining ischemic strokes with different etiologies may mask associations between individual fatty acids and ischemic stroke subtypes.

There is some controversy about whether lacunar strokes should be investigated separately from other ischemic strokes in epidemiologic studies based on their association with risk factors. In an investigation of risk factors of ischemic stroke subtypes, Ohira and colleagues reported that the population attributable fractions (PAF) of diabetes and current smoking was twice as great for lacunar versus non-lacunar stroke. However, the PAF of hypertension was around 35% for all ischemic stroke subtypes (Ohira et al., 2006). In contrast, another study using risk factor-free definitions of ischemic stroke subtypes found only a marginal excess of hypertension and no difference for diabetes with lacunar vs. non-lacunar strokes (Jackson & Sudlow, 2005). Our results suggest something different entirely, that cardioembolic stroke should be investigated separately from atherothrombotic and lacunar strokes.

3. Implications for future research

We found that not all serum fatty acids in the same group have the same association with ischemic stroke. These results suggest that fatty acids should be examined individually with respect to ischemic stroke, and likely other cardiovascular diseases. The effect of combining individual fatty acids into groups is to dilute the association of fatty acids that are associated with disease. Studies using participant report of intake through an FFQ will find examining individual fatty acids difficult because of the correlations between fatty acids of the same type. These methods may be improved to better distinguish between individual fatty acids or other measures of fatty acid intake may be used, such as a tissue biomarker of fatty acid intake.

In addition, we found that the associations between serum fatty acids were not consistent across ischemic stroke subtypes. These results suggest that ischemic strokes should be examined by subtype, especially for exposures related to serum cholesterol. This

work highlights the need for studies with a large number of ischemic strokes. The barriers to examining ischemic stroke subtypes are having the brain imaging data to classify ischemic strokes and having a sufficient number of ischemic strokes so that examination of subtypes will have sufficient statistical power. It may be useful to conduct meta-analyses or pooling studies in order to combine data from several studies to examine ischemic stroke subtypes.

C. Dietary recommendations

In this dissertation, we report inverse associations between serum DHA and EPA and ischemic stroke. In addition, we report that serum DHA and EPA are correlated with their corresponding dietary fat intakes, as well as fish intake. While these associations are not causal and should be investigated in an experimental setting, these results suggest that intake of DHA and EPA or fish may reduce ischemic stroke risk.

Intake of DHA and EPA through fish oil supplementation has been shown to beneficially influence cardiovascular risk factors. Fish oil supplementation lowers serum triglyceride concentrations by 25% to 30% and raises HDL cholesterol concentrations by 3% (Harris, 1997). Fish oil supplementation also lowers blood pressure, a major risk factor of ischemic stroke (Geleijnse et al., 2002). In addition to being a major source of dietary DHA and EPA, fish is an excellent source of protein, vitamins, and trace elements.

The results of this dissertation add to the body of literature suggesting that we should avoid 18:1t intake. A trans fatty acid, 18:1t has no nutritional benefit and potential to harm (Mozaffarian et al., 2006). The FDA suggests that you keep trans fat intake as low as possible. Since 2003, the FDA has required the labeling of trans fats in foods. The problem is if a food has <0.5g trans fat then food label can read 0g of fat. However, we found that even small amounts of trans fat may associated with increased odds of stroke. So it is best to avoid the commercially prepared baked and fried goods that use trans fats. Since many foods don't have labels, this points to a need for big changes in the food and restaurant

industry. Many manufacturers and restaurants have been voluntarily removing trans fats. Some states and cities have passed trans fat bans.

Getting enough dietary EPA and DHA is important too, but should the fatty acids be from fish or fish oil? The FDA recommends that you eat 2 servings of fish per week. Fish is an excellent source of protein, vitamins, and trace elements (He, 2009). It is a great substitute for red meat, which has more saturated fats. However, contaminants in fish are also an important issue. For most people, the benefits of eating fish outweigh the possible risks. But children and women who are trying to become pregnant, are pregnant, or are nursing will want to avoid fish that have high contaminants, but should make sure that they have enough EPA and DHA from other fish or from fish oil.

D. Strengths and Limitations

This dissertation has several strengths. First, we used serum fatty acids, an objective measure that does not rely on participant report of intake, to characterize the exposure. Compared with measurement of serum fatty acids, measurement of fatty acid intake is subject to considerable random error, as well as systematic error. The effect of systematic error – individuals with high fat intake and high risk of ischemic stroke being more likely to underreport fat intake – is to bias associations toward the null. Measurement of fatty acid concentrations in tissue may have less systematic error, which may increase our capability to detect associations with ischemic stroke. In addition, our use of serum fatty acids allowed us to examine individual fatty acids in relation to ischemic stroke. We added to the literature on serum trans fatty acids, which have not been well characterized in previous correlations studies and ischemic stroke studies. Second, ischemic strokes were classified by etiology. Few studies have the brain imaging data needed to classify ischemic strokes subtypes or central adjudication by trained neurologist. This classification allowed us to hypothesize about the mechanism linking serum fatty acids and ischemic stroke. Third, our study has over 7 times the number of ischemic strokes in previously the largest case-control studies

and twice the number of ischemic strokes in previous cohort studies. This substantial increase in statistical power may have further increased our ability to detect associations with serum fatty acids. Our use of the matched case-control study design also increased statistical efficiency. In addition, this dissertation included the largest number of participants in a study examining the correlations between serum fatty acids and fatty acid intakes, allowing us to examine correlations in specific subgroups of postmenopausal women. Finally, this dissertation was conducted among a cohort of postmenopausal women who have been understudied in previous ischemic stroke studies.

We also acknowledge the limitations of this dissertation. First, serum fatty acids are not free of measurement error. The stability of serum fatty acids during storage and freeze/thaw procedures was of concern. However, serum storage for up to 12 years at -80℃ preserved polyunsaturated fatty acids from oxidation very well (Zeleniuch-Jacquotte et al., 2000). We conducted an analysis of 15 samples that demonstrated that there were no substantial changes in the composition of the serum fatty acids analyzed originally in 2005, one year later in 2006, and then after an additional freeze/thaw procedure. No statistically significant differences were detected between the mean values of the fatty acids from the original and one-year or freeze/thaw samples. Polyunsaturated fatty acids, the most susceptible fatty acids to storage and freeze/thaw, gave the best results according to this analysis.

The second concern involves the TOAST criteria for categorizing ischemic strokes by etiology. Ohira and colleagues noted that more than half of previous studies examining ischemic stroke subtypes included hypertension or diabetes in their definition of lacunar stroke, obviously making these risk factors more common among patients with lacunar stroke (Ohira et al., 2006). This bias could, in part, explain the association between serum fatty acid and lacunar stroke, assuming that serum fatty acids act through hypertension to increase risk of ischemic stroke. Ohira and colleagues have advocated the use of a “risk factor-free classification” to avoid classification bias. However, the prevalence of

hypertension among the ischemic stroke subtypes was relatively uniform (72% of atherothrombotic, 73% of lacunar, and 68% of cardioembolic stroke cases) suggesting that this misclassification was not a major source of bias in this study. In addition, the strict TOAST guidelines improve the specificity of three main ischemic stroke subtypes, but they also limit statistical power to examine those categories. We had the lowest power to examine atherothrombotic stroke. However, the number of atherothrombotic strokes in this dissertation exceeded the total number of ischemic strokes in several previous studies.

We are also concerned about not controlling for important confounders. In a matched case-control study where the inclusion of a case-control pair depends on both having complete covariate data, we were careful to add only variables that were justified and necessary to maximize statistical power. In any observational study, the possibility of residual confounding by some unknown factors cannot be excluded.

Another concern is that our study population of mostly non-Hispanic Caucasian, postmenopausal US women is relatively homogenous, limiting generalizability to other populations. Correlations between biomarkers of serum fatty acids and intake may vary by population, likely due to the distribution of serum fatty acids or fatty acid intakes in those populations. These results emphasize the importance of examining the correlations of fatty acids biomarkers and intake in various populations. Despite the variation in correlations across studies, trans and marine n3 polyunsaturated fatty acids consistently have the highest correlations between intake and biomarker measurements. There is limited evidence that the association between fatty acids and ischemic stroke may be modified by participant age (Tilvis et al., 1987) and type of biomarker (He et al., 2007). However, these results were obtained in studies with a small number of ischemic stroke and the apparent modification may disappear in larger studies with more stable estimates of the association between fatty acid biomarkers and ischemic stroke.

E. Conclusion

In aim 1, we found moderate correlations between serum DHA, EPA and 18:1t fatty acid and their corresponding fatty acid intakes. These correlations suggest that studies examining the associations between serum DHA, EPA, and 18:1t fatty acid and disease should be consistent with studies examining the association between intake of these fatty acids and disease. In addition, these correlations suggest that studies that find associations between serum DHA, EPA, and 18:1 fatty acid and disease may make cautious dietary recommendations based on their results.

In aim 2, we found that serum linelaidic, palmitic, and oleic acids were positively associated with total ischemic stroke, while serum EPA, DPA, DHA, and arachidonic acids were inversely associated. These associations were generally consistent for atherothrombotic and lacunar stroke, but not cardioembolic stroke. The associations between these fatty acids and atherothrombotic and lacunar strokes were generally consistent with fatty acid intake and CHD studies. These findings highlight the importance of individual fatty acids in the development of particular subtypes of ischemic stroke.

APPENDIX I: TABLES

Table 1. Trial of ORG 10172 in Acute Stroke Trial (TOAST) classification of subtypes of acute ischemic stroke (Adams et al., 1993).

Atherothrombosis
Cardioembolism
Lacunae
Other Determined Etiology
Undetermined Etiology
Two or more causes identified
Negative evaluation
Incomplete evaluation

Table 2. Characteristics of Women's Health Initiative Observational Study (WHI-OS) women (1993-1998) and US women age 50-79 (1999-2000).

	WHI-OS Women	US women age 50-79
Total N	93,676	1,003
Age group, %		
50 to 59	31.7	42.2
60 to 69	44.0	33.6
70 to 79	24.3	24.2
Race/ethnicity, %		
White	83.3	74.1
Black	8.2	9.1
Hispanic	3.9	12.4
Other	16.6	4.4
Education, %		
Did not complete high school	5.2	32.9
High school/GED	16.3	30.3
Some training after high school	36.5	23.8
College graduate	42.0	13.4
Family income, %		
<$20,000	16.1	37.4
$20,000 to <$35,000	23.3	22.4
$35,000 to <$75,000	40.3	28.1
≥$75,000	20.3	12.1
Body mass index category, kg/m^2, %		
<18.5	1.2	1.1
18.5 to <25	39.6	29.2
25 to <30	34.0	30.8
≥30	25.2	39.0
Current smoker, %	6.3	36.7
Hormone therapy use ever, %	59.5	48.8
Hypertension, %	33.5	60.6

*NHANES (Nutritional Health and Nutrition Examination Survey) 1999-2000 results weighted to obtain estimates for US women ages 50-79.

Table 3. Women's Health Initiative (WHI) baseline data collection procedures.

Self-administered forms	Interviews	Clinical measurement
Personal information	Hormone use	Blood pressure
Medical history	Current medications	Height and weight
Personal habits		Blood specimen
Food questionnaire		

Table 4. Serum fatty acids and their common names.

Fatty acids	Common name
TFA	
16:1*t*	Palmitelaidic Acid
18:1*t*	(none)
18:2*tt*	Linelaidic Acid
SFA	
14:0	Myristic Acid
15:0	Pentanoic Acid
16:0	Palmitic Acid
17:0	Margaric Acid
18:0	Stearic Acid
MUFA	
16:1n7	7-Hexadecenoic Acid
18:1n7	Vaccenic Acid
18:1n9	Oleic Acid
20:1n9	Eicosenoic Acid
24:1	Nervonic Acid
PUFA	
18:3n3	Alpha-linolenic Acid
20:4n3	Eicosatetraenoic Acid
20:5n3	Eicosapentaenoic Acid (EPA)
22:5n3	Docosapentaenoic Acid (DPA)
22:6n3	Docosahexaenoic Acid (DHA)
18:2n6	Linoleic Acid
18:3n6	Gamma-linolenic Acid
20:2n6	Eicosadienoic Acid
20:3n6	Dihomo-gamma-linolenic Acid
20:4n6	Arachidonic Acid
22:4n6	Adrenic Acid
22:5n6	Osbond Acid

Table 5. Fatty acid intakes and selected food groups rich in fat derived from the Women's Health Initiative Food Frequency Questionnaire (WHI FFQ).

Nutrients (per day)	Food groups (servings/day)
Trans fatty acid 18:1, g	Red meat
Trans fatty acid 18:2, g	Ground meat
	Beef, pork, or lamb
Saturated fatty acid 14:0, g	
Saturated fatty acid 16:0, g	Fish
Saturated fatty acid 17:0, g	Dark fish
Saturated fatty acid 18:0, g	White fish
	Tuna
Monounsaturated fatty acid 16:1, g	Shellfish
Monounsaturated fatty acid 18:1, g	Fried fish
Monounsaturated fatty acid 20:1, g	
	Poultry
Polyunsaturated fatty acid 18:2, g	Eggs
Polyunsaturated fatty acid 18:3, g	Dairy
Polyunsaturated fatty acid 20:4, g	Milk
Polyunsaturated fatty acid 20:5, g	Nuts
Polyunsaturated fatty acid 22:5, g	Chips
Polyunsaturated fatty acid 22:6, g	Added fat
	Butter
	Pastries

Table 6. Conditional logistic regression models of the odds of ischemic stroke according to a 1-standard deviation increment in serum fatty acid concentration.

Model 1[a]	Ischemic stroke = 1-SD Fatty acid
Model 2[b]	Ischemic stroke = 1-SD Fatty acid + Confounders
Model 3[c]	Ischemic stroke = 1-SD Fatty acid + Confounders + Blood pressure
Model 4[d]	Ischemic stroke = 1-SD Fatty acid + Confounders + Blood pressure + Serum cholesterol

[a] Unadjusted models accounted only for the matching factors: age, race, and time on follow-up.
[b] Confounders: BMI status (<25.0, 25.0 to 29.9, ≥30.0 kg/m^2), smoking status (never, past, current), diabetes (yes, no), and aspirin use (yes, no)
Blood pressure variables: systolic blood pressure (continuous) and antihypertensive medication use (yes, no)
Serum cholesterol variables: total cholesterol to HDL-C ratio (continuous) and log normalized-triglycerides (continuous)

Table 7. Characteristics of the correlations paper study population.[a]

Characteristic	N (%)
N	925
Age, y	
Mean (SD)	69 (6)
50-59	91 (10)
60-69	369 (40)
70-79	465 (50)
Race/ethnicity	
White (not of Hispanic origin)	803 (87)
Black or African-American	69 (7)
Hispanic/Latino	16 (2)
Asian or Pacific Islander	21 (2)
American Indian or Alaskan Native	4 (<1)
Other	10 (1)
Education, y	
≤12	211 (23)
13-15	343 (37)
≥16	368 (40)
Household income, $	
<35,000	408 (44)
35,000 – 74,999	327 (35)
≥75,000	119 (13)
Body mass index, kg/m^2	
Mean (SD)	27 (5)
<30	375 (41)
30 to <35	328 (35)
≥35	209 (23)
Current smoker	32 (3)
Diabetes[b]	69 (7)
Hypertension[c]	432 (47)
Mean baseline daily intake (SD)	
Energy, kcal	1550 (587)
Fat, g	53 (28)
Fat, % energy	30 (8)
Carbohydrates, g	202 (78)
Carbohydrates, % energy	53 (9)
Protein, g	65 (27)
Protein, % energy	17 (3)

[a]Data are N (%), unless specified otherwise. Percentages may not add to 100% because of rounding and missing values.
[b]Diabetes was defined as having a fasting plasma glucose level ≥ 126 mg/dL or report of a diagnosis of sugar diabetes.
[c]Hypertension was defined as having a systolic or diastolic blood pressure equal to or greater than 140 and 90 mm Hg, respectively, or report of use of antihypertensive medication.

Table 8. Correlations between serum DHA, EPA, and 18:1*t* and their corresponding fatty acid intakes by subgroups.

	DHA	EPA	18:1*t*
Overall	0.53	0.42	0.39
Age group, y			
50 to 59	0.44	0.38	0.52
60 to 69	0.53	0.40	0.34
70 to 79	0.54	0.45	0.40
P for difference[a]	0.52	0.60	0.16
Race			
White	0.56	0.42	0.41
Black	0.22	0.32	0.09
P for difference	0.001	0.37	0.007
Education, y			
<12	0.41	0.36	0.28
13-15	0.52	0.41	0.36
≥16	0.55	0.43	0.39
P for difference	0.10	0.63	0.36
Household income, $			
<35,000	0.47	0.32	0.33
35,000 to <75,000	0.53	0.41	0.33
≥75,000	0.44	0.39	0.39
P for difference	0.43	0.36	0.79
BMI, kg/m^2			
<25	0.59	0.45	0.45
25 to <30	0.49	0.40	0.34
≥30	0.41	0.38	0.30
P for difference	0.02	0.46	0.08

[a]*P* for difference calculated using Fisher's *z* transformation.

Table 9. Agreement between quintiles of serum fatty acids and fatty acid intakes.

	Percent Agreement[a]	Weighted Kappa[b]
TFA		
16:1*t*	19	-0.04
18:1*t*	30	0.25
18:2*tt*	21	0.07
SFA		
14:0	24	0.10
16:0	24	0.10
17:0	21	0.05
18:0	21	0.04
MUFA		
16:1n7	24	0.11
18:1n9	21	0.02
20:1n9	19	-0.05
N3 PUFA		
18:3n3	25	0.13
20:5n3	28	0.26
22:5n3	22	0.11
22:6n3	34	0.35
N6 PUFA		
18:2n6	20	0.04
20:4n6	21	0.04

[a]Proportion of participants who were ranked in the same quintile for serum fatty acids and fatty acid intakes.
[b] The weighted kappa represents agreement when agreement due to chance is discounted. It is used for ordered categories and assigns less weight to agreement as categories are further apart. Kappa<0.00, 0.00 to 0.20, and 0.21 to 0.40 represents less than chance agreement, slight agreement, and fair agreement, respectively (Viera & Garrett, 2005).

Table 10. Spearman rank correlation coefficients between serum fatty acids and fatty acid intake groups.

Serum	Intake					
	TFA	**SFA**	**MUFA**	**PUFA**	**N3 PUFA**	**N6 PUFA**
TFA						
16:1*t*	-0.04	-0.10	-0.03	0.11	0.09	0.10
18:1*t*	0.39	0.02	0.15	-0.06	-0.27	0.00
18:2*tt*	0.14	0.08	0.04	-0.10	-0.19	-0.05
SFA						
14:0	-0.06	0.14	-0.15	-0.03	-0.02	-0.03
15:0	-0.05	0.24	-0.12	-0.16	-0.12	-0.15
16:0	-0.08	0.12	-0.18	-0.03	0.02	-0.04
17:0	0.02	0.07	-0.02	-0.06	-0.02	-0.07
18:0	0.00	0.02	-0.03	-0.02	0.00	-0.02
MUFA						
16:1n7	-0.10	0.14	-0.18	-0.08	0.02	-0.09
18:1n7	-0.08	0.01	-0.07	-0.03	0.12	-0.07
18:1n9	0.03	0.04	0.04	-0.07	-0.06	-0.06
20:1n9	0.09	0.08	0.06	-0.11	-0.20	-0.07
24:1n9	0.02	0.01	0.02	-0.01	0.04	-0.03
N3 PUFA						
18:3n3	-0.06	-0.19	0.04	0.21	0.20	0.19
20:4n3	-0.17	0.02	-0.08	0.01	0.13	-0.03
20:5n3	-0.24	-0.03	-0.10	0.07	0.29	-0.01
22:5n3	-0.12	0.03	-0.05	-0.01	0.11	-0.05
22:6n3	-0.20	-0.12	-0.06	0.13	0.33	0.06
N6 PUFA						
18:2n6	0.09	-0.13	0.16	0.08	-0.02	0.11
18:3n6	0.01	-0.03	-0.03	0.05	-0.01	0.06
20:2n6	-0.02	-0.09	0.02	0.09	0.04	0.09
20:3n6	-0.02	0.10	-0.06	-0.09	-0.10	-0.08
20:4n6	0.04	0.03	0.04	-0.08	-0.10	-0.07
22:4n6	0.03	0.03	-0.01	-0.04	-0.09	-0.01
22:5n6	0.11	0.10	0.03	-0.12	-0.21	-0.08

Table 11. Spearman rank correlation coefficients between serum fatty acids and selected food groups rich in fat.

	Red meat			Fish					
	Overall	**Ground meat**	**Pork, beef, or lamb**	**Overall**	**Dark fish**	**White fish**	**Tuna**	**Shellfish**	**Fried fish**
TFA									
16:1*t*	-0.10	-0.06	-0.11	0.05	0.07	0.05	0.07	-0.04	-0.12
18:1*t*	0.15	0.13	0.06	-0.21	-0.26	-0.18	-0.09	-0.18	0.14
18:2*tt*	0.09	0.12	0.06	-0.14	-0.18	-0.09	-0.05	-0.04	0.04
SFA									
14:0	0.05	0.02	0.08	0.04	-0.01	0.03	0.04	0.05	-0.06
15:0	0.03	0.02	0.03	-0.04	-0.01	-0.02	0.02	-0.01	-0.05
16:0	0.02	-0.01	0.04	0.12	0.07	0.04	0.07	0.09	-0.05
17:0	-0.03	0.01	-0.02	0.02	0.04	0.01	0.03	-0.05	0.00
18:0	0.01	0.00	0.04	0.03	-0.04	0.02	-0.01	0.02	0.06
MUFA									
16:1n7	0.02	0.00	0.06	0.10	0.07	0.10	0.06	0.06	-0.13
18:1n7	-0.08	-0.07	-0.07	0.10	0.15	0.12	0.03	0.02	-0.12
18:1n9	0.06	0.01	0.04	-0.05	-0.02	-0.04	-0.07	-0.02	-0.01
20:1n9	0.11	0.10	0.09	-0.13	-0.17	-0.07	-0.05	0.01	0.02
24:1n9	0.00	0.01	-0.01	0.07	0.04	0.08	0.07	-0.01	0.01
N3 PUFA									
18:3n3	-0.11	-0.05	-0.11	0.06	0.11	0.08	0.09	0.00	-0.02
20:4n3	-0.09	-0.08	-0.02	0.12	0.13	0.12	0.08	0.08	-0.14
20:5n3	-0.17	-0.14	-0.07	0.35	0.40	0.29	0.16	0.20	-0.13
22:5n3	-0.10	-0.06	-0.07	0.10	0.17	0.11	0.05	0.05	-0.10
22:6n3	-0.27	-0.22	-0.18	0.39	0.47	0.31	0.21	0.17	-0.11
N6 PUFA									
18:2n6	0.00	0.02	-0.03	-0.12	-0.12	-0.08	-0.05	-0.06	0.10
18:3n6	0.03	0.00	0.05	-0.01	-0.03	-0.02	0.02	-0.05	-0.04
20:2n6	-0.15	-0.08	-0.09	-0.05	-0.01	-0.02	0.00	-0.05	-0.08
20:3n6	0.06	0.06	0.08	-0.08	-0.09	-0.05	-0.02	-0.05	-0.07
20:4n6	0.08	0.08	0.05	-0.07	-0.08	-0.04	-0.04	-0.04	0.05
22:4n6	0.06	0.08	0.04	-0.13	-0.14	-0.11	-0.07	-0.04	0.00
22:5n6	0.11	0.08	0.08	-0. 26	-0.23	-0.19	-0.13	-0.15	0.01

Table 11 (continued). Spearman rank correlation coefficients between serum fatty acids and selected food groups rich in fat.

	Poultry	Eggs	Dairy	Milk	Added fat[a]	Butter	Pastries	Chips	Nuts
TFA									
16:1*t*	0.05	-0.11	0.11	0.04	-0.15	-0.10	-0.09	-0.13	0.00
18:1*t*	-0.06	0.03	-0.01	0.08	0.22	0.26	0.30	0.19	0.00
18:2*tt*	-0.02	0.04	0.04	0.06	0.10	0.05	0.12	0.07	-0.01
SFA									
14:0	0.04	0.03	0.20	0.05	0.05	0.09	0.00	0.04	0.03
15:0	0.01	0.04	0.16	0.10	0.04	0.09	0.09	-0.02	0.03
16:0	0.10	0.04	0.17	0.01	-0.02	-0.01	-0.09	-0.02	0.00
17:0	-0.01	-0.01	0.08	0.04	-0.04	-0.02	0.03	-0.05	-0.01
18:0	-0.02	0.03	-0.09	-0.06	0.03	-0.04	0.03	0.07	-0.06
MUFA									
16:1n7	0.07	-0.01	0.18	0.06	-0.07	-0.05	-0.11	-0.11	-0.06
18:1n7	0.06	-0.08	0.09	0.01	-0.17	-0.17	-0.17	-0.21	-0.15
18:1n9	0.00	-0.03	0.03	0.01	0.00	-0.01	-0.03	-0.04	-0.02
20:1n9	-0.05	0.02	0.03	0.01	0.10	0.06	0.09	0.10	0.02
24:1n9	0.08	-0.02	0.04	0.04	-0.02	-0.03	-0.01	-0.07	-0.04
N3 PUFA									
18:3n3	0.01	-0.03	0.01	-0.03	-0.09	0.01	-0.01	-0.05	0.02
20:4n3	0.05	-0.06	0.10	0.03	-0.12	-0.10	-0.07	-0.14	-0.02
20:5n3	0.10	-0.08	0.06	-0.01	-0.20	-0.15	-0.16	-0.21	-0.06
22:5n3	0.06	-0.11	0.06	0.00	-0.13	-0.11	-0.08	-0.15	-0.08
22:6n3	0.11	-0.08	-0.01	-0.04	-0.24	-0.22	-0.17	-0.26	-0.05
N6 PUFA									
18:2n6	-0.13	0.03	-0.16	-0.04	0.09	0.11	0.12	0.11	0.08
18:3n6	0.00	-0.05	0.04	0.08	0.00	0.02	0.06	0.04	0.01
20:2n6	-0.07	-0.11	0.02	-0.04	-0.09	-0.06	-0.03	-0.07	0.07
20:3n6	0.01	-0.04	0.12	0.06	-0.03	-0.01	0.00	0.01	0.00
20:4n6	0.07	-0.01	-0.05	0.02	-0.01	-0.06	0.03	-0.02	-0.05
22:4n6	-0.03	-0.02	0.04	0.04	0.08	0.02	-0.02	0.07	0.04
22:5n6	-0.02	0.04	0.03	0.06	0.03	-0.02	0.05	0.03	-0.02

[a]Fat (butter, margarine, sour cream, oils, or other fat) added to vegetables, beans, rice, and potatoes after cooking.

Table 12. Baseline characteristics of the ischemic stroke paper study population by ischemic stroke case and control status.

	Non-case	Case
N	964	964
Age, yr	68.7 (6.4)	68.7 (6.4)
Race		
African American	79 (8)	79 (8)
Native American	5 (1)	5 (1)
Asian	21 (2)	21 (2)
Hispanic	20 (2)	20 (2)
White	826 (86)	826 (86)
Other	10 (1)	10 (1)
Education		
≤ High school/ GED	226 (24)	235 (25)
Some college/ training	358 (37)	391 (41)
College graduate	377 (39)	330 (35)
Family income		
<$35,000	427 (48)	479 (54)
$35,000 to $74,999	332 (38)	302 (34)
≥$75,000	126 (14)	114 (13)
Moderate/strenuous activities ≥20 minutes*		
None	121 (13)	149 (16)
Some	356 (37)	419 (44)
2-4 episodes/wk	198 (21)	160 (17)
>4 episodes/wk	282 (29)	225 (24)
Alcohol intake		
Non-drinker	111 (12)	117 (12)
Past drinker	177 (18)	211 (22)
<7 drinks/wk	559 (58)	511 (53)
≥7 drinks/wk	116 (12)	122 (13)
Blood pressure, mm Hg		
Systolic*	130 (18)	137 (20)
Diastolic*	74 (10)	75 (10)
Hypertension*	450 (47)	647 (67)
Serum lipids, mg/dL		
Total cholesterol	231 (38)	233 (39)
HDL-C*	60 (16)	57 (16)
LDL-C	139 (37)	141 (37)

Triglycerides*	161 (80)	180 (90)
Total cholesterol to HDL-C ratio*	4.2 (1.4)	4.4 (1.5)
BMI, kg/m^2*		
Mean	27.0 (5.3)	27.7 (5.9)
< 25.0	388 (41)	330 (35)
25.0 to 29.9	342 (36)	365 (38)
≥30.0	221 (23)	260 (27)
Smoking status*		
Never	523 (55)	501 (53)
Past	397 (42)	374 (39)
Current	36 (4)	78 (8)
Comorbidities		
Diabetes*	72 (7)	153 (16)
Atrial fibrillation*	55 (6)	92 (10)
Angina*	53 (6)	90 (9)
Revascularization*	11 (1)	38 (4)
Medications		
Aspirin*	238 (25)	295 (31)
Antihypertensive*	319 (33)	449 (47)
Lipid-lowering	157 (16)	182 (19)
Statins	83 (9)	92 (10)
HRT	363 (38)	381 (40)

GED, General Equivalency Diploma; BMI, body mass index; HRT, hormone replacement therapy
*McNemar's chi-square or paired Student's *t* test $P \leq 0.05$, as appropriate.

Table 13. Composition of serum fatty acids.[a]

	Non-case			Case			
	Q1	**Median**	**Q3**	**Q1**	**Median**	**Q3**	**Diff[b]**
18:2n6	24.22	27.12	30.40	23.78	26.73	29.70	*-0.55
16:0	22.07	23.77	25.77	22.56	24.37	26.44	* 0.65
18:1n9	16.12	17.59	19.07	16.66	18.17	19.68	* 0.46
18:0	7.83	8.54	9.18	7.67	8.42	9.20	-0.06
20:4n6	7.02	8.27	9.67	6.63	8.01	9.43	*-0.31
16:1n7	1.65	2.23	2.96	1.79	2.34	3.09	* 0.15
20:3n6	1.71	2.05	2.40	1.75	2.03	2.34	-0.01
22:6n3	1.50	1.97	2.65	1.34	1.75	2.32	*-0.26
18:1n7	1.29	1.47	1.69	1.31	1.49	1.68	0.01
14:0	0.64	0.84	1.09	0.66	0.89	1.14	* 0.06
18:1*t*	0.56	0.75	1.03	0.60	0.80	1.05	* 0.05
20:5n3	0.44	0.60	0.85	0.40	0.56	0.76	*-0.09
22:5n3	0.45	0.56	0.68	0.41	0.51	0.63	*-0.05
18:3n3	0.44	0.55	0.68	0.42	0.53	0.66	*-0.02
18:3n6	0.32	0.41	0.53	0.31	0.41	0.52	-0.01
16:1*t*	0.27	0.33	0.38	0.28	0.33	0.39	0.001
22:4n6	0.15	0.30	0.43	0.15	0.29	0.42	-0.005
17:0	0.25	0.28	0.32	0.24	0.28	0.32	-0.003
20:2n6	0.22	0.26	0.30	0.22	0.25	0.30	-0.004
22:5n6	0.19	0.25	0.32	0.19	0.24	0.31	*-0.012
15:0	0.15	0.19	0.23	0.15	0.19	0.23	0.003
20:1n9	0.09	0.14	0.24	0.10	0.14	0.26	0.004
20:4n3	0.05	0.08	0.11	0.05	0.07	0.10	*-0.004
18:2*tt*	0.02	0.03	0.06	0.02	0.04	0.07	* 0.005
24:1n9	0.00	0.00	0.04	0.00	0.00	0.05	-0.004

Q, quartile; Diff, difference; t, trans.
[a]Fatty acids are shown in order of contribution to total serum fatty acids.
[b]The average of the differences between case and control serum fatty acid concentration.
*Paired *t* test $P \leq 0.05$.

Table 14. Odds ratios and 95% confidence intervals for total ischemic stroke associated with a 1-standard deviation increase in individual serum fatty acids.

Fatty Acid	SD	Model 1	Model 2	Model 3	Model 4
TFA					
16:1 t	0.10	1.02 (0.93, 1.11)	0.996 (0.91, 1.09)	1.02 (0.92, 1.13)	0.94 (0.83, 1.07)
18:1 t	0.40	1.15 (1.05, 1.27)	1.13 (1.02, 1.25)	1.13 (1.02, 1.25)	1.12 (1.01, 1.24)
18:2 t	0.04	1.39 (1.19, 1.63)	1.38 (1.17, 1.63)	1.34 (1.13, 1.59)	1.27 (1.06, 1.51)
SFA					
14:0	0.36	1.21 (1.10, 1.34)	1.18 (1.06, 1.31)	1.16 (1.04, 1.29)	1.09 (0.94, 1.25)
15:0	0.06	1.09 (0.97, 1.21)	1.10 (0.98, 1.24)	1.11 (0.98, 1.25)	1.07 (0.94, 1.21)
16:0	2.74	1.31 (1.19, 1.45)	1.27 (1.14, 1.41)	1.23 (1.10, 1.37)	1.23 (1.07, 1.41)
17:0	0.06	0.94 (0.86, 1.04)	0.97 (0.88, 1.07)	1.001 (0.90, 1.11)	1.01 (0.91, 1.12)
18:0	1.11	0.93 (0.84, 1.03)	0.92 (0.83, 1.03)	0.93 (0.83, 1.03)	0.99 (0.87, 1.11)
MUFA					
16:1 n7	1.01	1.17 (1.06, 1.28)	1.15 (1.05, 1.27)	1.13 (1.02, 1.25)	1.09 (0.97, 1.22)
18:1 n7	0.32	1.03 (0.94, 1.12)	1.03 (0.94, 1.14)	1.05 (0.95, 1.15)	1.03 (0.93, 1.14)
18:1 n9	2.32	1.26 (1.14, 1.38)	1.20 (1.08, 1.33)	1.18 (1.06, 1.31)	1.13 (0.995, 1.27)
20:1 n9	0.12	1.16 (0.97, 1.38)	1.16 (0.96, 1.40)	1.12 (0.93, 1.36)	1.06 (0.87, 1.29)
24:1n9	0.08	0.86 (0.73, 1.02)	0.87 (0.74, 1.03)	0.88 (0.74, 1.05)	0.92 (0.78, 1.09)
N3 PUFA					
18:3 n3	0.22	0.87 (0.78, 0.97)	0.90 (0.80, 1.003)	0.92 (0.82, 1.03)	0.87 (0.77, 0.99)
20:4 n3	0.05	0.89 (0.80, 0.99)	0.92 (0.83, 1.03)	0.94 (0.84, 1.05)	0.92 (0.82, 1.03)
20:5 n3	0.54	0.82 (0.74, 0.91)	0.84 (0.75, 0.93)	0.88 (0.79, 0.99)	0.89 (0.80, 1.001)
22:5 n3	0.18	0.69 (0.62, 0.77)	0.72 (0.64, 0.80)	0.75 (0.67, 0.84)	0.75 (0.66, 0.84)
22:6 n3	0.91	0.70 (0.63, 0.78)	0.72 (0.64, 0.80)	0.75 (0.67, 0.84)	0.76 (0.67, 0.85)
N6 PUFA					
18:2 n6	4.41	0.89 (0.81, 0.97)	0.92 (0.84, 1.02)	0.94 (0.85, 1.03)	0.998 (0.89, 1.12)
18:3 n6	0.16	0.93 (0.85, 1.02)	0.91 (0.83, 1.01)	0.91 (0.82, 1.01)	0.89 (0.80, 0.99)
20:2 n6	0.06	0.94 (0.86, 1.03)	1.01 (0.91, 1.11)	1.02 (0.92, 1.13)	1.02 (0.92, 1.13)
20:3 n6	0.50	0.97 (0.88, 1.07)	0.97 (0.88, 1.08)	0.94 (0.85, 1.05)	0.91 (0.82, 1.02)
20:4 n6	2.02	0.81 (0.73, 0.90)	0.81 (0.73, 0.91)	0.82 (0.73, 0.92)	0.85 (0.75, 0.96)
22:4 n6	0.18	0.91 (0.77, 1.07)	0.93 (0.78, 1.10)	0.91 (0.77, 1.08)	0.92 (0.77, 1.09)

22:5 n6	0.11	0.88 (0.80, 0.98)	0.88 (0.80, 0.98)	0.86 (0.77, 0.97)	0.85 (0.76, 0.96)

Model 1: Unadjusted models accounted only for the matching factors: age, race, and time on follow-up.
Model 2: Adjusted for BMI status (<25.0, 25.0 to 29.9, ≥30.0 kg/m^2), smoking status (never, past, current), diabetes, and aspirin use.
Model 3: Adjusted for the previous model variables plus systolic blood pressure and antihypertensive medication use.
Model 4: Adjusted for the previous model variables plus total cholesterol to HDL-C ratio and normalized-triglycerides.

Table 15. Odds ratios and 95% confidence intervals for ischemic stroke subtypes associated with a 1-standard deviation increase in individual serum fatty acids.

Fatty Acid	Model 1	Model 2	Model 3	Model 4
Atherothombotic stroke (n=96)				
18:2*t*	1.83 (1.05, 3.17)	1.77 (0.91, 3.45)	1.74 (0.88, 3.43)	1.13 (0.53, 2.45)
16:0	1.42 (1.01, 2.00)	1.12 (0.74, 1.69)	1.10 (0.72, 1.68)	0.86 (0.48, 1.52)
18:1n9	1.43 (1.01, 2.04)	1.40 (0.93, 2.10)	1.41 (0.92, 2.16)	0.89 (0.50, 1.56)
20:5n3	0.66 (0.48, 0.92)	0.51 (0.31, 0.82)	0.47 (0.28, 0.79)	0.38 (0.20, 0.71)
22:5n3	0.82 (0.61, 1.10)	0.83 (0.59, 1.17)	0.86 (0.60, 1.23)	0.81 (0.55, 1.21)
22:6n3	0.63 (0.44, 0.88)	0.70 (0.47, 1.04)	0.65 (0.42, 1.01)	0.66 (0.40, 1.08)
20:4n6	0.77 (0.55, 1.07)	0.70 (0.48, 1.04)	0.66 (0.44, 0.99)	0.74 (0.48, 1.14)
Lacunar stroke (n=250)				
18:2*t*	1.41 (1.05, 1.90)	1.39 (1.01, 1.90)	1.38 (0.98, 1.94)	1.24 (0.89, 1.72)
16:0	1.22 (1.01, 1.47)	1.20 (0.98, 1.47)	1.17 (0.94, 1.46)	1.14 (0.86, 1.52)
18:1n9	1.26 (1.05, 1.51)	1.23 (1.01, 1.49)	1.30 (1.04, 1.63)	1.17 (0.90, 1.52)
20:5n3	0.56 (0.43, 0.74)	0.58 (0.44, 0.78)	0.65 (0.48, 0.87)	0.65 (0.48, 0.89)
22:5n3	0.61 (0.49, 0.77)	0.64 (0.51, 0.80)	0.70 (0.55, 0.89)	0.64 (0.49, 0.83)
22:6n3	0.60 (0.47, 0.75)	0.61 (0.48, 0.79)	0.68 (0.52, 0.88)	0.69 (0.53, 0.91)
20:4n6	0.76 (0.61, 0.93)	0.75 (0.60, 0.94)	0.73 (0.57, 0.92)	0.81 (0.62, 1.05)
Cardioembolic stroke (n=209)				
18:2*t*	0.94 (0.67, 1.33)	0.96 (0.66, 1.39)	0.85 (0.57, 1.25)	0.94 (0.62, 1.42)
16:0	1.19 (0.98, 1.44)	1.12 (0.91, 1.38)	1.05 (0.84, 1.31)	1.14 (0.86, 1.51)
18:1n9	1.30 (1.04, 1.64)	1.22 (0.96, 1.56)	1.15 (0.90, 1.49)	1.43 (1.05, 1.94)
20:5n3	0.85 (0.66, 1.09)	0.84 (0.65, 1.09)	0.89 (0.68, 1.15)	0.88 (0.67, 1.14)
22:5n3	0.80 (0.63, 1.02)	0.82 (0.64, 1.05)	0.87 (0.68, 1.12)	0.87 (0.67, 1.13)
22:6n3	0.67 (0.53, 0.86)	0.66 (0.51, 0.85)	0.70 (0.54, 0.91)	0.70 (0.54, 0.90)
20:4n6	0.85 (0.67, 1.08)	0.86 (0.67, 1.11)	0.92 (0.70, 1.20)	0.82 (0.61, 1.10)
Ischemic strokes of undetermined etiology (n=366)				
18:2*t*	1.51 (1.14, 1.98)	1.51 (1.13, 2.02)	1.46 (1.09, 1.97)	1.35 (1.003, 1.82)
16:0	1.45 (1.22, 1.73)	1.42 (1.18, 1.70)	1.39 (1.16, 1.68)	1.39 (1.10, 1.74)
18:1n9	1.19 (1.02, 1.38)	1.14 (0.97, 1.35)	1.13 (0.96, 1.33)	1.06 (0.88, 1.28)
20:5n3	0.98 (0.86, 1.11)	0.99 (0.87, 1.13)	1.02 (0.89, 1.17)	1.03 (0.90, 1.18)
22:5n3	0.63 (0.53, 0.77)	0.65 (0.54, 0.79)	0.67 (0.55, 0.81)	0.67 (0.55, 0.81)
22:6n3	0.81 (0.69, 0.96)	0.83 (0.70, 0.99)	0.85 (0.71, 1.02)	0.86 (0.72, 1.03)
20:4n6	0.85 (0.72, 1.01)	0.86 (0.72, 1.03)	0.88 (0.73, 1.05)	0.92 (0.76, 1.11)

Model 1: Unadjusted models accounted only for the matching factors: age, race, and time on follow-up.
Model 2: Adjusted for BMI status (<25.0, 25.0 to 29.9, ≥30.0 kg/m^2), smoking status (never, past, current), diabetes, and aspirin use.
Model 3: Adjusted for the previous model variables plus systolic blood pressure and antihypertensive medication use.
Model 4: Adjusted for the previous model variables plus total cholesterol to HDL-C ratio and normalized-triglycerides.

APPENDIX II: FIGURES

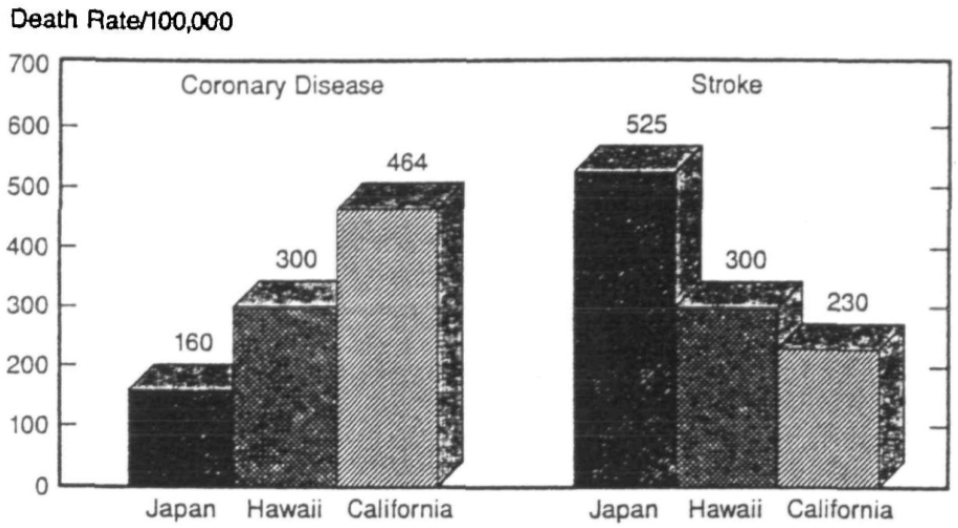

Figure 1. Mortality rates for Japanese men aged 55-64 years in California, Hawaii, and Japan, 1950 (Gordon, 1957; D. M. Reed, 1990).

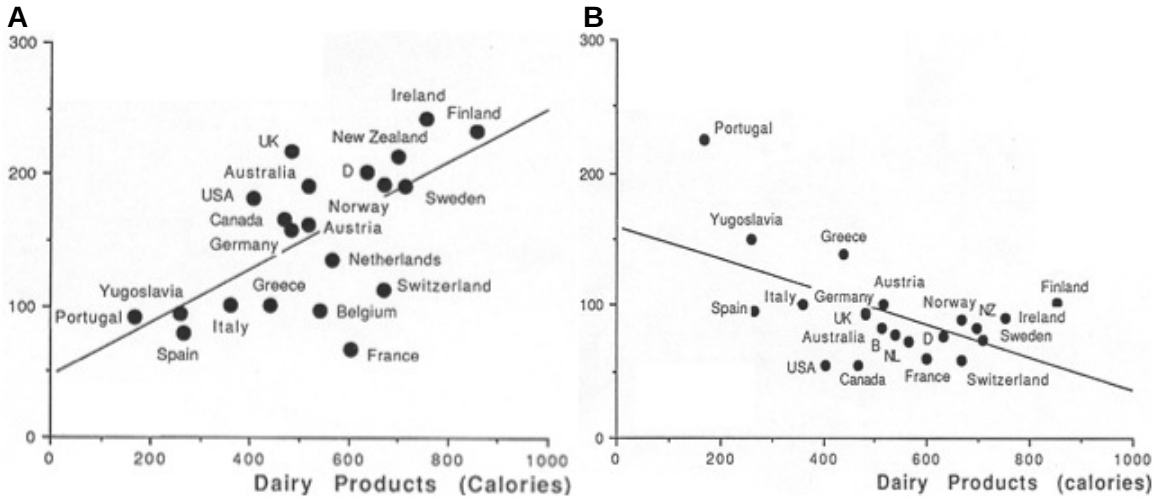

Figure 2. Coronary heart disease (CHD mortality (A, r=0.66, p<0.001) and cerebrovascular mortality (B, r=-0.56, p<0.01) per 100,000 men and women from 21 countries, 1990 (Renaud, 2001).

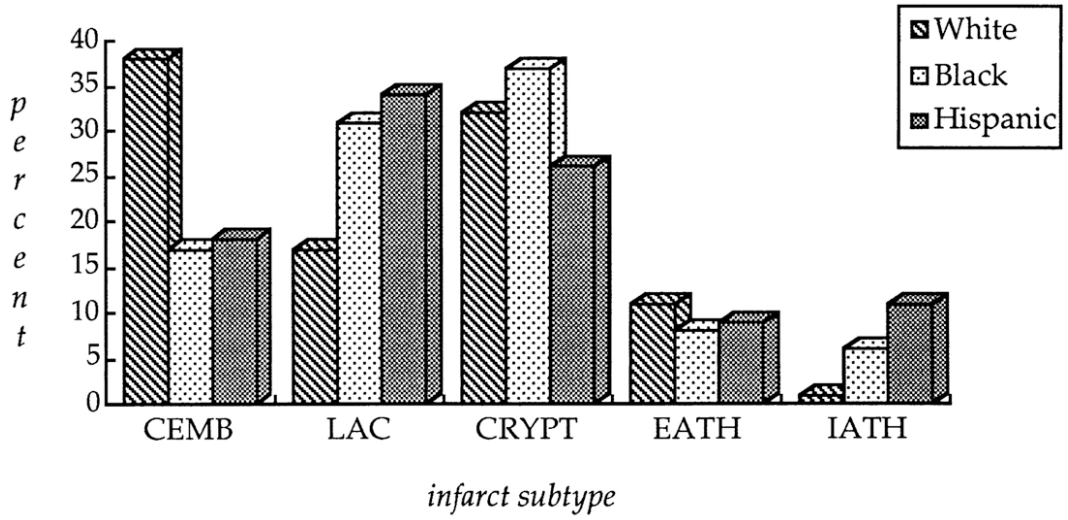

Figure 3. Distribution of ischemic stroke subtypes among 438 patients enrolled in the Northern Manhattan Stroke Study, 1993-1997. CEMB=coronary embolism, LAC=lacunar, CRYPT=cryptogenic stroke, EATH=extracranial atherosclerotic stroke, IATH=intracranial atherosclerotic stroke, (R. L. Sacco et al., 1995)

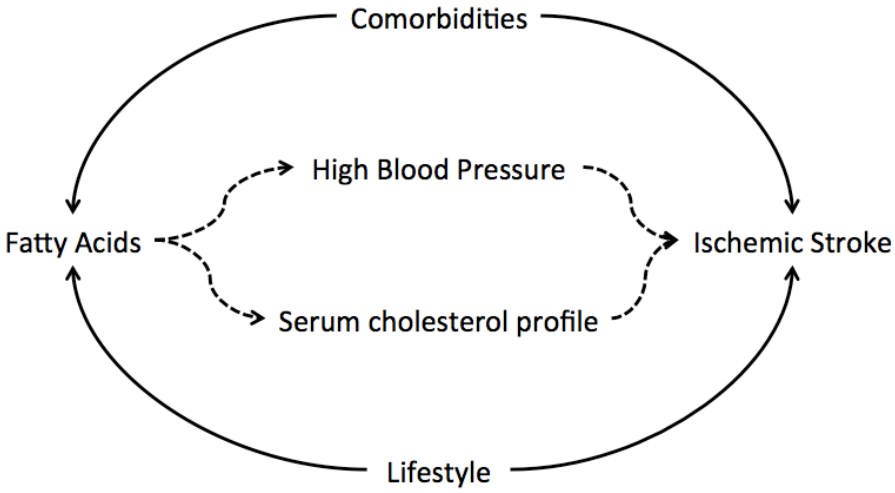

Figure 4. Conceptual model of the association between serum fatty acids and ischemic stroke.

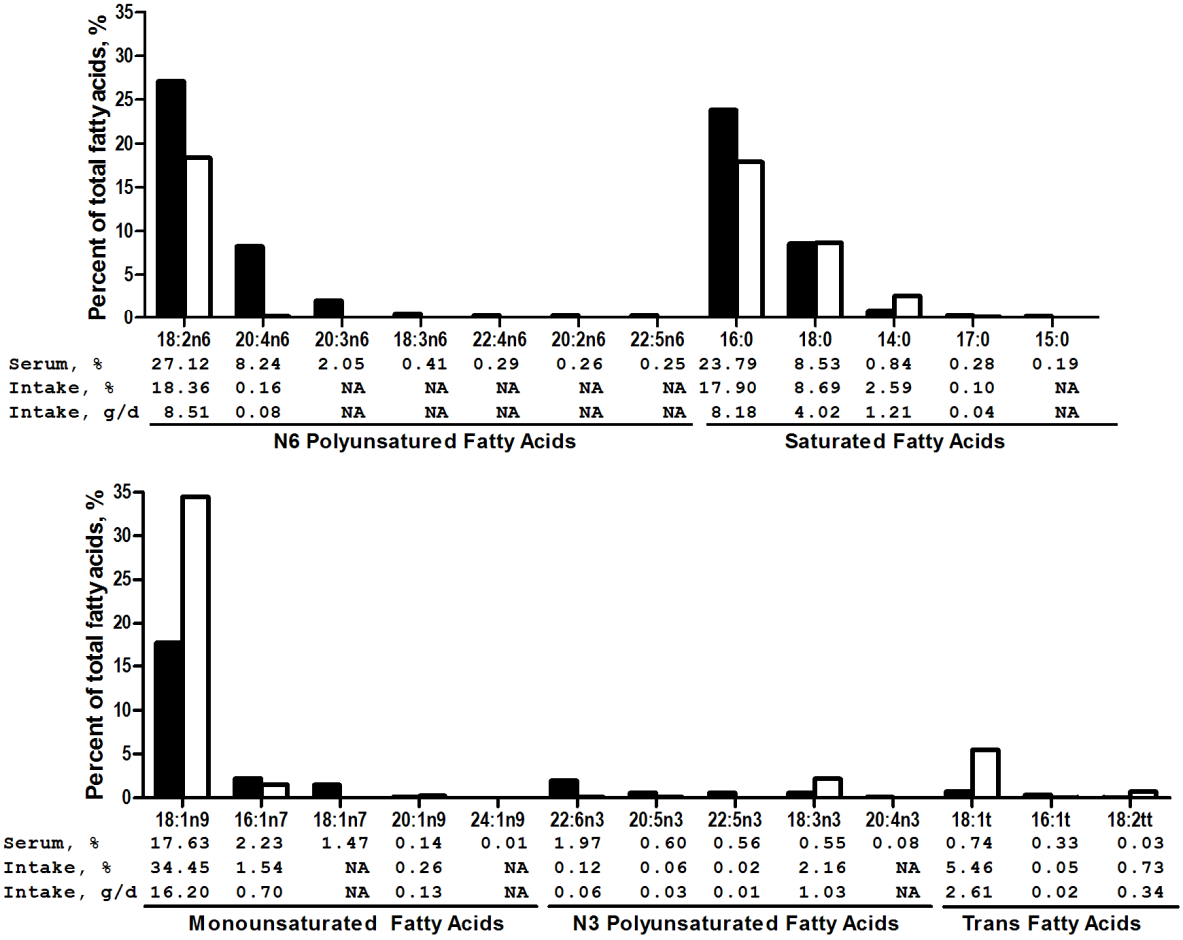

Figure 5. Composition of serum fatty acids (■) and fatty acid intakes (□). Fatty acids are grouped by type and shown in order of contribution to total serum fatty acids. Data are medians.

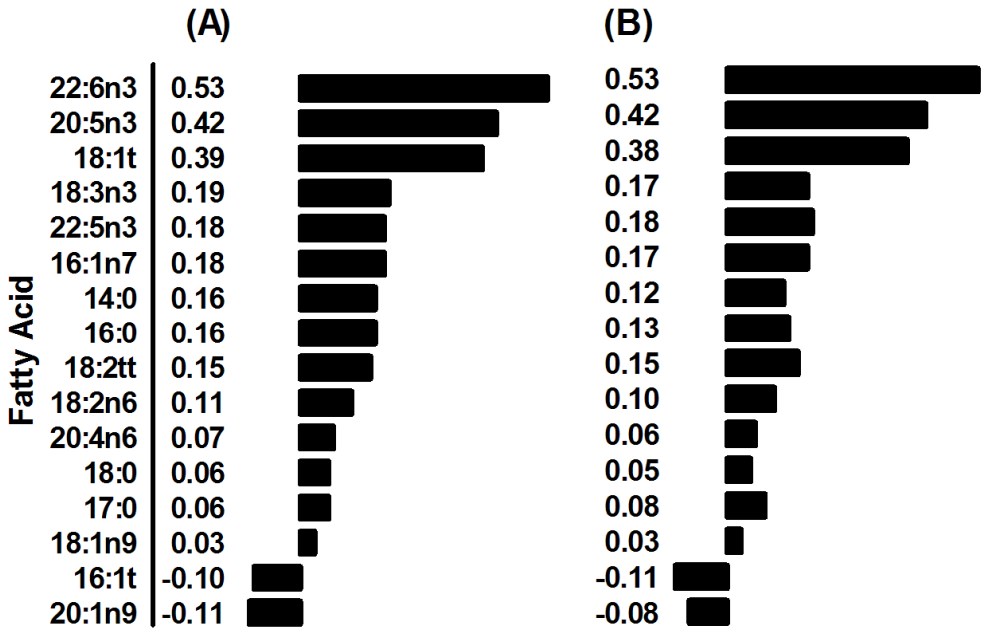

Figure 6. (A) Unadjusted and (B) partial Spearman rank correlation coefficients between serum fatty acid concentrations and fatty acid intakes. Partial correlations coefficients were adjusted for age (continuous), race/ethnicity (white, non-white), body mass index (<25, 25 to <30, ≥30 kg/m^2), and current smoking status (no, yes). Correlations coefficients r ≥ 0.20 were statistically significant ($P \leq 0.003$)

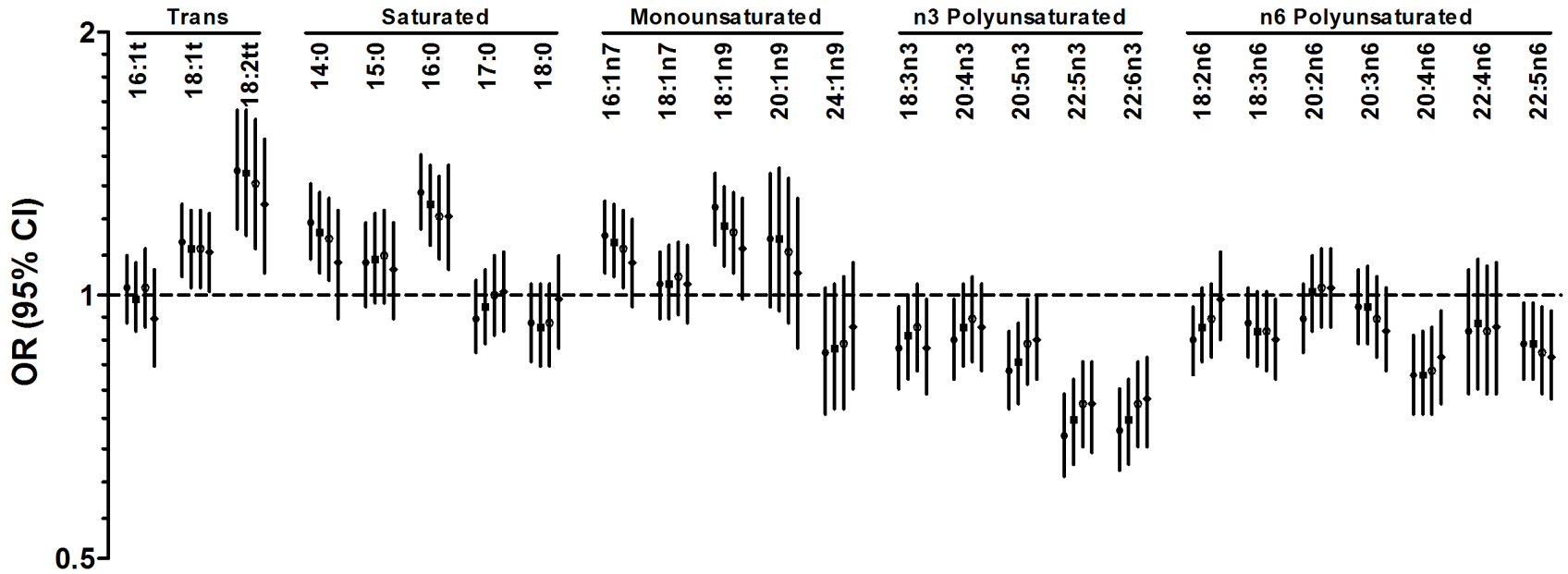

Figure 7. Odds ratios and 95% confidence intervals for total ischemic stroke associated with a 1-standard deviation increment in individual serum fatty acids.

●: Unadjusted models accounted only for the matching factors: age, race, and time on follow-up.
■: Adjusted for BMI status (<25.0, 25.0 to 29.9, ≥30.0 kg/m^2), smoking status (never, past, current), diabetes, and aspirin use.
□: Adjusted for the previous model variables plus systoic blood pressure and antihypertensive medication use.
◆: Adjusted for the previous model variables plus total cholesterol to HDL-C ratio and normalized-triglycerides.

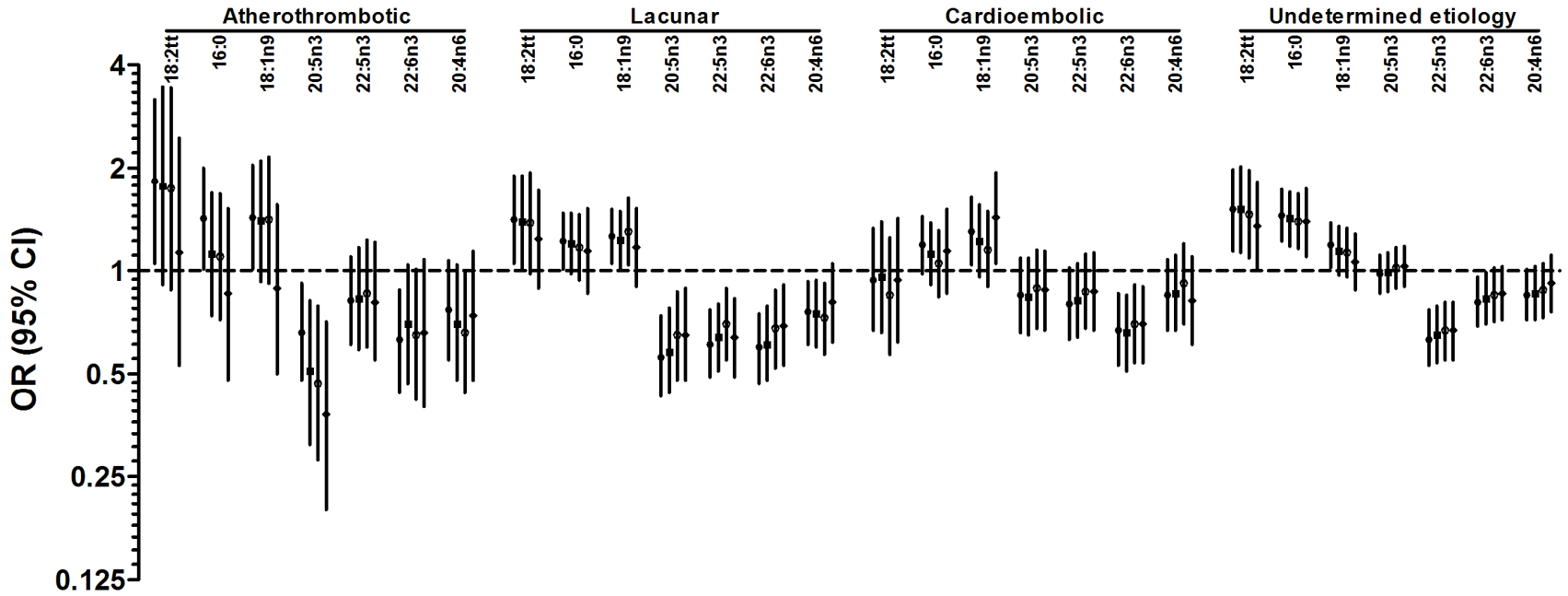

Figure 8. Odds ratios and 95% confidence intervals for ischemic stroke subtypes associated with a 1-standard deviation increment in individual serum fatty acids.

●: Unadjusted models accounted only for the matching factors: age, race, and time on follow-up.
■: Adjusted for BMI status (<25.0, 25.0 to 29.9, ≥30.0 kg/m^2), smoking status (never, past, current), diabetes, and aspirin use.
□: Adjusted for the previous model variables plus systolic blood pressure and antihypertensive medication use.
◆: Adjusted for the previous model variables plus total cholesterol to HDL-C ratio and normalized-triglycerides.

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