IRON DEFICIENCY ANEMIA IN ACUTE STROKE AND TRANSIENT ISCHEMIC ATTACK PATIENTS

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ABSTRACT

Iron deficiency anemia (IDA) has been implicated as a possible risk factor for stroke and transient ischemic attack (TIA) on the basis of epidemiological findings and numerous case reports. The study objectives were to document the prevalence of IDA in a series of patients ≥ 65 years of age admitted with TIA or stroke, and to investigate whether previous dietary intake was a predictor of iron status. Blood samples were collected within 24 hours of admission for 94 patients, and IDA was identified using an algorithm containing values for hemoglobin, ferritin, total iron binding capacity, transferrin saturation, and serum transferrin receptor (sTfR). The Clue II food frequency questionnaire was administered to obtain information about usual dietary intake. The prevalence of IDA in this study was 6.4%. Two methods were used to compare this prevalence figure with findings from the Third National Health and Nutrition Examination Survey (NHANES III). The first comparison was with published prevalence figures for males and females ≥ 70 years of age, and revealed a significantly higher IDA prevalence in the current study for both genders. The second analysis, a case-control comparison, compared prevalence figures with data for 94 age and gender-matched, non-Hispanic white controls with no previous history of stroke from the NHANES III database; this difference approached statistical significance. These findings suggest that IDA should be further investigated as an independent risk factor using a prospective case-control design, using age and gender-matched controls. An unexpected finding from this study was the high percentage of subjects (30%) with sTfR values below the reference range; problematic reference ranges for the elderly or a stroke response might be implicated. Multivariate regression analysis was used to identify significant predictors of iron status, but few significant associations were apparent. Gender emerged as a significant predictor of hemoglobin, and supplemental iron and heme iron were found to be significant predictors of total iron binding capacity. Limited study power or underreporting of intake may have contributed to the lack of association between iron status and previous dietary intake.

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LIST OF ABBREVIATIONS

Acetysalicylic acid	ASA
Anemia of chronic disease	ACD
Body mass index	BMI
Estimated average requirement	EAR
First National Health and Nutrition Examination Survey	NHANES I
Food frequency questionnaire	FFQ
Gastrointestinal	GI
Iron deficiency anemia	IDA
Iron regulatory protein	IRP
Iron responsive element	IRE
Non-steroidal anti-inflammatory drug	NSAID
Population attributable risk	PAR
Recommended dietary allowance	RDA
Recommended nutrient intake	RNI
Serum transferrin receptor	sTfR
Third National Health and Nutrition Examination Survey	NHANES III
Transferrin receptor	TfR
Transient ischemic attack	TIA
Total iron binding capacity	TIBC

1. Introduction

1.1 Background

Stroke, a reduction in blood flow to an area of the brain, is a major cause of death and disability in Canada (Juurlink and Sweeney, 1997). The elderly comprise the group at highest risk for stroke, and are also the most severely disabled following stroke injury (Pohjasvaara et al., 1996). Since the population of elderly individuals is quickly rising in Canada, efforts to reduce the burden of disease in this age group are essential.

There are many well-known risk factors for stroke such as hypertension, smoking, atrial fibrillation, and increasing age (Sacco, 1998). However, only about 60% of all strokes can be attributed to known causes (Whisnant, 1997). Thus, there is a need to examine other possible risk factors for stroke injury. To date, little research has been done in the area of nutrition and stroke.

Many elderly individuals are at risk for nutritional deficiencies due to problems such as impaired functional and cognitive status, multiple medical conditions and medications, decreased education, poor oral health, poverty, changes in taste and smell, and dysphagia (ADA Reports, 2000). Estimates of malnutrition range from 4 to 31% in this age group (Seiler, 1999). Iron is one particular nutrient that may be compromised (Johnson et al., 1994).

1.2 Rationale

Iron status may be implicated in the etiology of stroke. Many studies have examined the association between excess iron status and stroke, but findings remain inconclusive (Gillum et al., 1996). Conversely, there is some evidence to suggest that there may be an association between iron deficiency anemia and stroke. Numerous case reports have described stroke events in individuals with iron deficiency anemia (Hartfield et al., 1997; Akins et al., 1996; Belman et al., 1990; Bruggers et al., 1990). In a follow-up study to the First National Health and Nutrition Examination Survey (NHANES I), Gillum et al. (1996) found a U-shaped relationship between transferrin

saturation and stroke in white women 45-74 years of age; this suggests increased stroke risk at both **low** and **high** levels of circulating iron. The association between iron deficiency anemia and stroke warrants further investigation.

Iron deficiency anemia may be problematic in the elderly. Prevalence estimates range considerably from 1% (Looker et al., 1997) to 23% (Health and Welfare Canada, 1973) using different markers of iron status. Gastrointestinal blood loss, non-steroidal anti-inflammatory drugs, anticoagulants, genitourinary disease, or frequent blood drawings may contribute to iron deficiency in the elderly (Johnson et al., 1994). Dietary factors may also be implicated in iron deficiency as previous authors have demonstrated relationships between iron, vitamin C, and alcohol intake with various markers of iron status (Fleming et al., 1998; Beard et al., 1996; Payette and Gray-Donald, 1991).

With the increasing burden of stroke injury in the elderly in Canada, there is an urgent need for research into both preventative and treatment strategies. There is intriguing evidence to suggest that iron deficiency anemia, a common problem in elderly individuals, may be associated with stroke injury. At present, there are no available reports that describe the prevalence of iron deficiency anemia in elderly stroke patients. Thus the relationship between inadequate iron status, stroke, and dietary factors deserves further investigation.

1.3 Hypothesis

The prevalence of iron deficiency anemia in a group of adults ≥ 65 years of age presenting with acute stroke or transient ischemic attack will be higher than that previously published for free-living individuals in a similar age group who have not had a stroke. Dietary intake will be a predictor of iron status in this group of stroke/transient ischemic attack patients.

1.4 Objectives

1. To determine the prevalence of iron deficiency anemia in a series of 100 consecutive patients (≥65 years) admitted with stroke or transient ischemic attack.
2. To compare the prevalence data for iron deficiency anemia with existing published figures for free-living elderly.
3. To determine whether previous dietary intake is a predictor of iron status in individuals presenting with stroke or transient ischemic attack.

2. Literature Review

2.1 Stroke and Transient Ischemic Attack (TIA)

2.11 Implications of Stroke Injury

The effects of stroke injury are devastating. In 1997, cerebrovascular disease (consisting mainly of stroke) accounted for 7% (n=16,048) of all deaths in Canada (Heart and Stroke Foundation of Canada, 1999). In Saskatchewan alone, approximately 2000 adults suffer a stroke, 400 die, and 500 to 700 are permanently disabled every year (Reeder et al., 1994). Of long term survivors (>6 months), 48% have hemiparesis, 22% cannot walk, 24 to 53% are completely or partially dependent for functional activities, 12-18% are aphasic, and 32% are clinically depressed (Sacco et al., 1997). One third of all stroke survivors exhibit dementia (Zhu et al., 1998). Clearly stroke is a major cause of death and disability.

Stroke is of particular concern in the elderly. The risk of stroke doubles for each decade after 55 years of age (American Heart Association, 1999). Elderly individuals are also more severely disabled following stroke injury (Pohjasvaara et al., 1996). In 1994, Petrasovits and Nair reported that Canada had experienced a significant decline in age-adjusted stroke mortality since 1961 that may have resulted from overall improvements in health status, access to health care, and lifestyle changes. However since 1994, both the number and age-adjusted mortality for stroke have been rising again (Wolf & D'Agostino, 1998). The total number of people affected by stroke will also continue to increase due to changing population demographics. In 1996, 12.23 % of the Canadian population (n=3,527,000) was $\geq$ 65 years of age; this figure is projected to increase to 15.88% (n=5,894,300) by 2016 (Statistics Canada, 2000). Unfortunately these demographic changes translate into an increased burden of disease that includes stroke.

2.12 Stroke Definitions

Stroke may be defined as " rapidly developed clinical signs of focal or global disturbance of cerebral function, lasting more than 24 hours or until death" (WHO Monica Project Principal Investigators, 1988). Stroke results from cerebral ischemia; cerebral ischemia occurs when blood flow decreases to the point that metabolic substrate delivery fails to meet the metabolic tissue demand (Sharp et al., 1998). Focal ischemia may be "complete" or "incomplete". Complete ischemia results from occlusion of a cerebral artery in regions with no collateral flow; incomplete ischemia occurs in the surrounding penumbra region with collateral blood supply. Situations such as cardiac arrest or severe hypotension may result in global ischemia (i.e. ischemia to the entire brain) (Sharp et al., 1998).

Transient ischemic attacks (TIAs) are defined as temporary, focal neurologic deficits that are presumably related to ischemia, that last less than 24 hours (Warlow et al., 1996). The 24-hour guideline that is used to distinguish between TIAs and mild ischemic stroke is an arbitrary cut-point (Warlow et al., 1996)

2.13 Classification of Stroke Injury

The term "stroke" may be classified further. Information collected from the Stroke Data Bank in the United States from 1983-1986 (n=1,805) suggests that 70% of all strokes are ischemic infarcts; 27% are hemorrhagic, and 3% are other types (Sacco et al., 1998). Hemorrhagic infarcts result from bleeding from an arterial source directly into the brain substance; hypertension is the main causative factor (Kase et al., 1998). However, since ischemic stroke comprises the largest group and is of particular interest in this research study, it will be the focus of this review. Ischemic stroke may be classified into four different groups, based on the origin and type of predisposing injury (Sacco et al., 1998).

a) An infarction or TIA may be due to **large artery thrombosis.** A site of severe large vessel occlusion or stenosis ultimately results in distal perfusion failure. The vascular occlusion may result from a local atherosclerotic lesion, or the migration of an embolism from another artery.

b) An infarction may result from **cardiac embolism**. Conditions such as atrial fibrillation with valvular disease may result in the migration of thrombi or platelet aggregates to smaller vasculature of the brain.

c) **Lacunar infarction** refers to arterial disease of the vessels penetrating the brain to supply the capsule, basal ganglia, thalamus, and paramedian regions of the brain stem. The zone of ischemia is usually limited to the smaller territory of a single vessel, and the cause of occlusion is often unknown.

d) Infarcts may also be classified as being of **undetermined cause**. These patients typically do not have risk factors or a medical history that suggests a cardiac embolus or large artery thrombosis.

2.14 Mechanism of Injury

An interruption in cerebral blood flow and oxygen supply for 4-5 minutes can lead to a core area of irreversible brain damage, surrounded by a penumbra area of potentially reversible ischemia (Juurlink & Sweeney, 1997). In the ischemic core region, an interruption in blood flow is promptly followed by decreased protein synthesis, depolarization of neurons, and a failure of membrane ion transport systems (Sharp et al., 1998; Kasner & Grotta, 1997). Subsequently, increasing intracellular Na^+ result in ATP depletion, and a marked rise in intracellular Ca^{2+} and extracellular K^+ lead to the release of glutamate and other neurotransmitters (Juurlink & Sweeney, 1997). Excess glutamate release in turn leads to a further rise in intracellular Ca^{2+}, ATP depletion, and depolarization of other neurons (Juurlink & Sweeney, 1997). Sustained rises in Ca^{2+} can activate proteases, lipases, and endonucleases, leading to the destruction of cellular homeostatic mechanisms, cytoskeleton, mitochondria, and cell membranes; the generation of free radicals and other reactive oxygen species further increase neurological damage (Sharp et al., 1998; Kasner & Grotta, 1997). Waves of depolarization known as "spreading depression" also endanger the penumbra region (Juurlink & Sweeney, 1997).

2.15 Risk factors

There are many well-documented risk factors for stroke injury. A "risk factor" may be defined as "an aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic, which on the basis of epidemiologic evidence is known to be associated with health-related condition(s) considered important to prevent" (Last, 1995).

The well-known non-modifiable risk factors for first stroke include increasing age, gender, race or ethnicity, and hereditary factors (i.e. birth parent) (Sacco, 1998). A recent review by Rubattu et al. (2000) suggests that genetics may have a major role in the etiology of stroke. The numerous modifiable risk factors include hypertension, atrial fibrillation, cigarette smoking, heavy alcohol use, transient ischemic attack, asymptomatic carotid stenosis, and cardiac disease (Sacco, 1997; Sacco, 1998; Aronow, 1999). Population-attributable risk (PAR)—i.e. "the proportion of ischemic strokes in the population that can be attributed to a particular risk factor" (Goldstein et al., 2001)--is ranked as **high** for hypertension, **medium** for coronary heart disease and blood lipids, **low-medium** for asymptomatic carotid artery stenosis (60-99% occlusion), and **low** for atrial fibrillation, diabetes mellitus, cigarette smoking, and heavy alcohol consumption (Gorelick et al., 1999). A "high" PAR is defined as 40% or greater, medium ranges from 15 to 40%, and low is less than 15%. Although PAR is ranked as medium for blood lipids, estimates are uncertain and the role of hypercholesterolemia in the etiology of stroke remains unclear (Gorelick et al. 1999; Sacco, 1999). Elevated homocyst(e)ine levels appear to be associated with increased risk of stroke (Giles et al., 1998; Coull et al., 1990; Yoo et al., 1998), but the exact mechanism has not yet been defined (Rubatta et al. 2000). Risk for stroke will increase significantly when a combination of these predisposing factors occurs (Rubatta et al., 2000). Since only about 60% of all strokes can be linked to known risk factors (Whisnant, 1997), there is a need to identify other possible causes for stroke injury.

Other variables may be implicated in the etiology of stoke, but there may be insufficient evidence at this time to firmly establish causation. These following criteria are commonly used when attempting to describe an observed association as causation (Hill, 1965): a) strength of association; b) consistency of an observed association;

c) specificity (i.e. an association is limited to particular circumstances); d) temporality of relationship between cause and effect; e) biological gradient (i.e. dose response); f) biologically plausible explanation; g) coherence with current knowledge of history and biology of disease; h) experimental evidence; and i) analogy (i.e. previous known relationships may provide support). Thus, many factors must be considered before any given variable can be classified as a "risk factor".

2.16 Novel Nutritional Risk Factors

The relationship between diet and stroke remains controversial. An ecological analysis of the World Health Organization stroke mortality rates from 1986 to 1988, and dietary surveys from 17 countries (Sasaki et al., 1995) provides interesting insight into the relationship between diet and stroke. Overall, these authors found a significant positive correlation between urinary sodium excretion, saturated fatty acid intake, and alcohol with log-stroke mortality, and a negative correlation with urinary potassium (urinary sodium and potassium excretion are indicators of dietary intake). The effect of high dietary sodium is mediated through its effect on hypertension (Gorelick et al., 1999). Thus, as a result of this analysis, dietary sodium, saturated fatty acids, and alcohol were positively associated with stroke mortality, and dietary potassium appeared to be protective.

Longitudinal studies have suggested that the consumption of fruits and vegetables is protective against stroke development in both men and women. In the Nurses' Health Study and the Health Professionals' Follow-Up Study, a 31% lower relative risk of ischemic stroke was evident for men and women in the highest quintile of fruit and vegetable intake (Joshipura et al., 1999); each one serving increment of fruit or vegetable per day was associated with a 6% decrease in relative risk. Similarly, Gillman et al. (1995) reported a 22% reduction in relative risk for all strokes (and a 24% reduction for ischemic stroke specifically) per 3 serving increment of fruit and/or vegetables for men in the Framingham study. The specific protective components of fruits and vegetables remain unknown, but may include dietary fibre, potassium, and/or folate (Joshipura et al., 1999). Two other cohort studies (Khaw and Barrett-Connor, 1987; Ascherio et al., 1998) corroborate the protective effect of potassium in stroke risk.

Ascherio et al. (1998) also found that dietary magnesium and cereal fibre were protective against stroke, particularly in hypertensive men.

There is conflicting evidence about the role of dietary fat and protein in stroke. Sasaki et al. (1995) had noted a positive association between saturated fatty acid intake and stroke mortality in their ecological analysis, but other authors have described negative associations. In the Framingham Heart Study, Gillman et al. (1997) noted a significant inverse association between ischemic stroke risk and total fat, saturated fat, and monounsaturated fat. An analysis of The Nurses Health Study participants revealed a higher risk for intraparenchymal hemorrhage with lower intakes of both saturated fat intake and protein (Iso et al., 2001); this trend was not apparent with other stroke subtypes, or with other types of fat. In a comparison between Japanese men living in Japan and Hawaii, Takeya et al. (1984) also found an inverse relationship between animal protein and fat (including saturated fat) and risk of both hemorrhagic and thrombo-embolic strokes.

In a longtitudinal study of Hawaiian Japanese men, Kagan et el. (1985) found that low levels of protein intake were associated with a higher risk of total stroke in univariate analysis; however this trend was not significant in multivariate analysis. A diet low in food from animal sources (including protein) is suspected to be involved in the paradox of high stroke occurrence in populations at low risk for coronary heart disease (Reed et al., 1990). Although there are no available published reports that investigate a potential association between dietary iron and stroke risk, many animal sources of protein are also rich in iron. Therefore, reduced dietary iron could also be implicated in stroke, or function as a confounding variable in the relationship between dietary protein and stroke. The premise that compromised iron status resulting from poor diet or medical factors may increase risk for ischemic stroke forms the basis for this thesis.

Overall, the role of diet in the etiology of stroke is unclear. Interpretation of findings is confounded by different dietary assessment methods, and varying study endpoints. The protective effect of fruits and vegetables (including a high potassium diet) appears to be well established. The role of other nutrients such as dietary fat, protein and iron remains to be established.

2.2 Iron Status and Stroke

Preliminary attempts have been made to explore the relationship between iron and stroke. The effect of excess iron status in mediating stroke injury and altering outcome has been investigated in vitro and in animal and human subjects. The association between both excess and inadequate iron status and future risk of stroke has also been addressed in several reports.

2.21 Iron Excess and Stroke

At a cellular level, it is known that excess iron stores can exacerbate free radical damage following stroke injury, and thus potentially increase the amount of neural damage. Normally transferrin and ferritin bind ferric iron tightly, preventing reduction to ferrous iron, and serving as a protective mechanism against redox participation (McCord, 1998). However, the reoxygenation and reperfusion that follow cerebral ischemia leads to an increased production of reactive oxygen species, including the superoxide anion (Juurlink & Sweeney, 1997). One of the most destructive properties of superoxide is its ability to liberate iron from ferritin stores; superoxide enters the ferritin core and reduces Fe^{3+} to Fe^{2+}, thereby enabling its release (McCord, 1998). Ferrous iron then serves as a mediator in the formation of the hydroxyl radical in the Fenton reaction. The hydroxyl radical, an extremely potent oxidizing species, can cause DNA strand breakage, inactivate enzymes, and initiate lipid peroxidation which in turn creates further cycles of free radical formation (McCord, 1996). The ability of iron-chelating agents (e.g. deferoxamine) to reduce damage during and following ischemic insult in vitro and in animal models is supportive of the role of cellular iron in reactive oxygen species mediated damage (McCord, 1996). Excess iron stores has been associated with adverse outcomes in stroke patients. In a prospective study of acute ischemic stroke patients, Dávalos et al. (1994) found that a high serum ferritin level ($\geq$190 µg/dL) within the first 24 hours of stroke injury was an independent predictor of poor outcome.

The subject of this thesis however focuses on the role of iron status, and particularly iron deficiency anemia (IDA), as a risk factor for the development of

stroke. At an epidemiologic level, several studies have examined the relationship between excess iron status and stroke risk with conflicting results. The Framingham Study, a prospective cohort design, reported a modest but insignificant association between elevated hemoglobin levels and stroke risk after adjustment for other risk factors (Kannel et al., 1972). Tohgi et al. (1978) reported a significantly higher incidence of cerebral infarction in younger elderly patients (60 – 77 years) with hematocrit values >46%, but this trend was not significant in older adults. Two other studies, a multivariate analysis of stroke predictors in Hawaiian Japanese men (Kagan et al., 1980), and a Chinese case-control analysis (Woo et al., 1990), failed to identify hematocrit as a risk factor. The fact that hemoglobin and hematocrit are not specific markers for iron status is a major limitation of these studies; the use of better markers might have elicited different findings. There are no recent reports of excess iron status and stroke. Increased blood viscosity with reduced blood flow, compounded by the presence of atherosclerosis, has been proposed as a biological basis for an association between iron excess and stroke (Tohgi et al., 1978). To date, this relationship remains unclear and warrants further investigation.

2.22 Iron Deficiency and Stroke

Conversely, there is also evidence to suggest an association between iron deficiency anemia and incidence of stroke and / or transient ischemic attack (TIA). The follow-up study to the First National Health and Nutrition Examination survey (NHANES I) (Gillum et al., 1996) found a U-shaped relationship between transferrin saturation and stroke incidence in white women 45-74 years of age (Figure 2.1). There was a significantly increased relative risk of stroke (adjusted for baseline age and other risk factors) in both the highest and lowest quintiles of transferrin saturation levels. Transferrin saturation levels >44% and <20% were associated with a relative risk of 1.96 and 1.80 respectively when compared with the 30-36% quintile. This trend remained when hemorrhagic stroke cases were excluded. These findings were also present in white women in the 65-74 year age group, but not in white men. This study suggests an increased risk of stroke with both high and low levels of circulating iron.

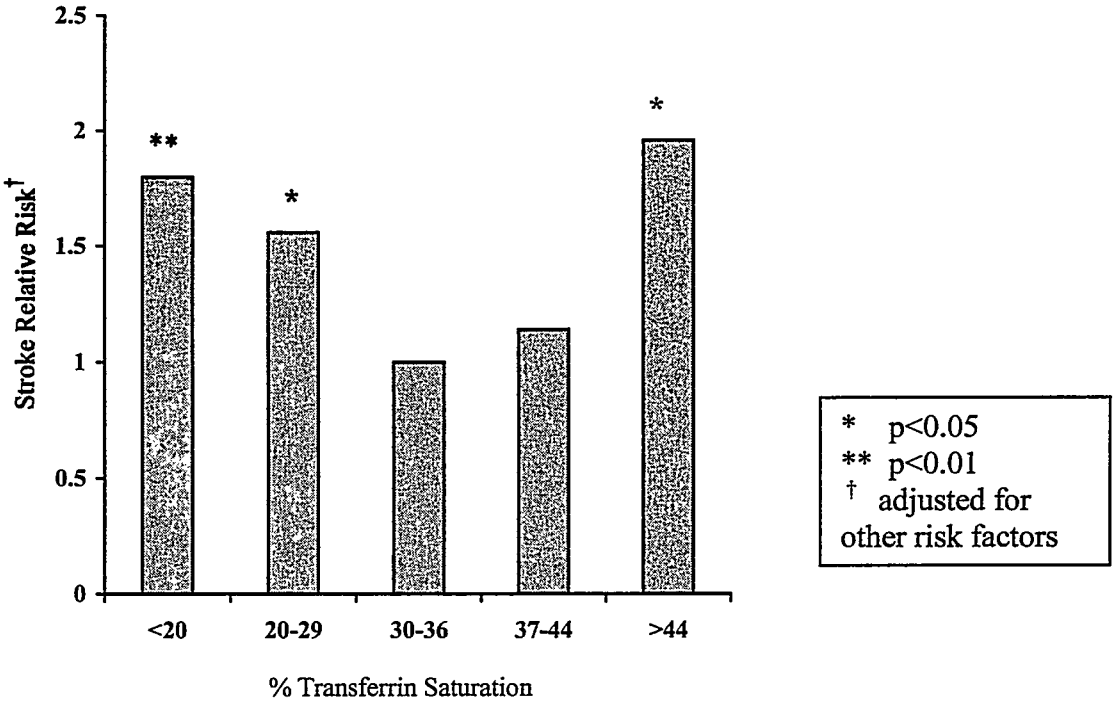

Figure 2.1: Relative risk† for stroke incidence for white women (45-74 years) from NHANES I follow-up study (Gillum et al., 1996).

There are also many published case reports that suggest that iron deficiency and/or iron deficiency anemia or its sequelae (i.e. thrombocytosis) may be associated with cerebrovascular events. Stroke, venous thrombosis, carotid artery stenosis, retinal stroke, papilledema, and transient ischemic attacks have all been cited as possible outcomes of impaired iron status.

Numerous pediatric case reports have linked cerebrovascular events with iron deficiency anemia. In a retrospective review of 53 children, Hartfield et al. (1996) described 6 unexplained cases of stroke or venous thrombosis associated with iron deficiency after a viral prodrome. Five of these six children had hypochromic, microcytic anemia. Swann and Kendra (2000), and Ready and Lowry (1989) describe single cases of cerebral infarction associated with IDA. Belman et al. (1990) report a

case of hemorrhagic infarction associated with venous thrombosis and IDA. An earlier paper by Huttenlocher & Smith (1968) details 2 cases of acute infantile hemiplegia associated with thrombocytosis; iron status was not reported. A recent review of risk factors for arterial ischemic stroke in children reports that one fifth of patients in the Great Ormond Street Hospital stroke database were anemic; the number of cases and diagnostic criteria for IDA were not described (Kirkham et al., 2000).

Iron deficiency anemia resulting from menorrhagia may also be problematic. Both Alexander (1983) and Scoditti et al. (1990) described single cases of ischemic stroke associated with IDA and thrombocytosis. IDA and thrombocytosis were also evident in 3 women who presented with carotid artery thrombi; two of these cases manifested as TIAs, one individual had a parietal infarct (Akins et al., 1996).

IDA associated with other types of severe bleeding may be implicated in cerebrovascular events. Noninfectious thrombosis of the superior sagittal sinus was found in an individual with rectal prolapse (Stehle et al., 1991). Shahar & Sadeh (1991) describe 2 cases of TIA with carotid stenosis associated with fecal blood loss. TIAs were also problematic in an adolescent female (Bruggers et al., 1990) and six other adults (Knizley & Noyes, 1972; Siekert et al., 1960); etiology of IDA included telangiectasis, gastrointestinal bleeding, and hematuria. A recent case control analysis (Kim & Kang, 2000) provides further support for a significant association between anemia from blood loss (hemoglobin ≤ 9.0 mg/dL) and ischemic stroke.

IDA has also been associated with cerebrovascular events in individuals with cyanotic congenital heart disease. The use of therapeutic phlebotomy to reduce hyperviscosity in these patients may lead to decompensation of erythropoiesis and IDA (Perloff et al., 1988). In a retrospective review of 162 adult patients with cyanotic congenital heart disease, Ammash and Warnes (1996) found that microcytosis (mean corpuscular volume <82) was the risk factor most strongly associated with a cerebrovascular event such as transient ischemic attack, reversible ischemic deficit, or completed infarct; they concluded that IDA might be an independent risk factor for cerebrovascular events in these patients. Previously, Phornphutkil et al. (1973) and Cottril and Kaplan (1973) had identified iron deficiency as a risk factor for stroke in children with congenital heart disease.

In summary, there is intriguing evidence to support a possible association between IDA and risk for stroke. The NHANES I follow-up study data (a prospective cohort study) provides sound epidemiological data to suggest an increased risk of stroke at both high and low levels of circulating iron, but falls short of establishing causation. Shortcomings of case reports include findings may be highly susceptible to bias, may not be generalizable, and may be due to chance alone (Fletcher et al., 1988). The findings also do not establish whether risk is associated specifically with iron deficiency anemia; anemia of other causes might also be equally correlated. Nevertheless, a unique pattern of iron deficiency anemia has emerged amongst a subset of stroke and TIA patients. These previous reports form a solid foundation for more formalized research endeavours.

2.23 Mechanisms of Association Between IDA and Stroke

Several theories exist to explain an association between IDA and stroke. IDA might be involved in the pathophysiology of arterial thrombosis, a predisposing condition for stroke, through adverse effects on the blood, blood vessels, and blood flow. Increased stroke risk might also result from insufficient iron for enzymes, or a combination of predisposing factors.

Thrombus formation may result from blood abnormalities such as thrombocytosis (increased platelet count) (Rodgers, 1999). Since iron appears to be a regulator of thrombopoiesis, IDA may be implicated in thrombocytosis and thrombosis (Hartfield et al., 1997; Akins et al., 1996). It is thought that iron is necessary for both adequate platelet production and inhibition of excess platelet production. Although thrombocytopenia has been reported with severe IDA (Karpatkin et al., 1974), IDA is more commonly associated with thrombocytosis in both humans (Gross et al., 1964; Schloesser et al., 1965) and animals (Karpatkin et al., 1974; Schloesser et al., 1965). Platelet counts may increase to twice normal values, and normalize with iron therapy (Karpatkin et al., 1974; Gross et al., 1964; Schloesser et al., 1965). Although the precise mechanism for this relationship between IDA and platelets is not known, the hypothesis that megakaryocytic and erythrocytic cell lines share a common precursor cell supports an inverse relationship between red blood cell and platelet production

(McDonald and Sullivan, 1993). Beguin (1999) suggests that increased endogenous erythropoietin stimulation in response to IDA may be involved; moderate iron deficiency would cause a modest increase in serum erythropoietin and stem cell shunting that would lead to thrombocytosis. Alterations in activity of iron-dependent enzymes involved in platelet synthesis may also be implicated (Levine, 1999; Beguin, 1999). Thrombosis is common in patients with essential thrombocythemia, a myeloproliferative disorder, but is less frequent in the setting of the reactive thrombocytosis associated with IDA (Levine, 1999). Nevertheless, there are several case reports that describe stroke events in patients with thrombocytosis and IDA (Knizley & Noeyes, 1972; Alexander, 1983; Scoditti et al., 1990; Huttenlocher & Smith, 1968). Some authors (Heller et al., 1988; Scoditti et al., 1990) have suggested that even mild elevations in platelet count may be problematic in thrombus formation. However it is also possible that abnormal platelet activation and function may be more important in the etiology of thrombus formation than absolute platelet count (Akins et al., 1996). The relationship between IDA, thrombocytosis, and thrombosis is unclear and deserves further investigation.

Alterations in blood flow due to hyperviscosity may lead to thrombosis (Rodgers, 1999). IDA may be involved in this mechanism, since decreased red blood cell plasticity (Card & Weintraub, 1971) and deformability (Yip et al., 1983) has been demonstrated in both iron deficient humans and animals. This may result in increased blood viscosity (Foerster, 1999). In contrast, other authors (Metivier et al., 2000) have suggested that blood viscosity might decrease with IDA as a result of a decrease in erythrocyte count and haematocrit. The clinical significance of these IDA-associated changes in blood viscosity changes is unclear.

Iron deficiency anemia may induce a hyperkinetic circulatory state that predisposes to stroke injury through effects that include damaged blood vessel endothelium (Kim & Kang, 2000). Iron deficiency anemia may lead to increases in left ventricular stroke volume, cardiac output, and heart rate. Left ventricular hypertrophy, a predisposing factor for stroke, may develop (Metivier et al., 2000; Georgieva & Georgieva, 1997). Arterial bruits -- a physical sign of turbulent blood flow -- are also more common in patients with severe anemia (Wales & Martin, 1963; Akins et al.,

1996). This turbulence may damage vessel endothelium and lead to platelet aggregation and subsequent stroke (Akins et al., 1996). Increased blood flow and turbulence may also lead to migration of existing thrombi (Kim & Kang, 2000).

Insufficient iron for key metabolic enzymes may be problematic with IDA. Iron is an essential component of cytochrome enzymes and other metalloenzymes involved in oxygen metabolism, DNA synthesis, and catecholamine and steroid metabolism (Bruggers et al., 1990). Decreased levels of iron-requiring enzymes may lead to inefficient oxygen utilization (Bruggers et al., 1990). Concomittant increases in oxygen requirements (i.e. illness or surgery) may lead to metabolic demands that cannot be met, and neurological sequelae may result (Hartfield et al., 1990; Ready & Lowry, 1989; Heller et al., 1988; Huttenlocher & Smith, 1968).

Two or more of the above mechanisms may act synergistically to promote stroke injury. For example, turbulence of blood flow combined with thrombocytosis may promote thrombus formation (Akins et al., 1996). Normally, cerebral blood flow changes in response to arterial oxygen content, and decreases in the oxygen carrying capacity of blood with IDA should lead to compensatory increases in cerebral blood flow in order to maintain constant oxygen transport to the brain (Brown et al., 1985). However, in the setting of ischemic cerebrovascular disease coexisting with IDA, the relationship between cerebral blood flow, oxygenation, and hematocrit may be impaired (Kusonoki et al., 1981); this could reduce available oxygen below brain tissue requirements, and predispose to stroke injury (Shahar & Sadeh, 1991).

In summary, there are several ways by which IDA might increase risk for stroke. Further research is needed to clarify the precise mechanisms and clinical significance of these changes. It is also possible that the broader category of anemia, rather than IDA specifically, has a key role in stroke injury.

2.3 Iron Metabolism

In order to lay the foundation for an understanding of IDA, a brief review of iron metabolism follows.

2.31 Intestinal Iron Absorption

There are three major factors that influence intestinal iron absorption: the iron content of the diet, the bioavailability of the diet, and the ability of the mucosal cells to absorb iron (Baynes and Stipanuk, 2000). The latter of the three will be addressed here. Absorption of iron from the intestinal mucus layer and into the mucosa occurs primarily in the duodenum and upper jejunum. In the setting of iron deficiency, these areas are able to increase iron absorption as an adaptive response (Schumann et al., 1990). Iron absorption is dependent upon the adequacy of dietary supply, the bioavailability of iron sources, and sufficient exposure to the intestinal absorptive surface (Conrad et al., 1999).

Gastrointestinal secretions interact to influence the absorption of non-heme (inorganic) dietary iron. In the stomach, hydrochloric acid enables protein denaturation with removal of protein-bound iron, and may reduce insoluble ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) (Beard et al., 1996). Ferric iron is also chelated with substances such as mucin, ascorbate, histidine, and fructose which keep iron soluble in the higher pH of the small intestine (Conrad et al., 1999; Powell et al., 1999). Bile contains ascorbic acid and other reducing substances that enhance iron absorption (Conrad et al., 1999). Pancreatic and intestinal enzymes produce amino acids and sugars that also enhance iron absorption; pancreatic bicarbonate diminishes iron absorption (Conrad et al., 1999). In contrast, heme iron (from myoglobin and hemoglobin) is soluble in the alkaline environment of the small intestine (Beard et al., 1996).

Iron absorption can be divided into 3 stages: iron uptake, intraenterocyte transport and storage, and extraenterocyte transfer (Beard et al., 1996).

a) Iron uptake

The precise mechanisms of iron uptake through the luminal (apical) cell membrane of the enterocyte remain unclear. In fact, three separate mechanisms for iron uptake may exist (Conrad et al., 1999): 1) The integrin complex - paraferritin - containing integrin , mobilferrin, and flavin monooxygenase is believed to be involved in intestinal uptake of soluble ferric chelates and the subsequent reduction to ferrous iron (Conrad et al., 1999; Powell et al., 1999; Wolf, 1994). 2) A divalent cation transporter 1 (DCT-1 or Nramp 2) appears to facilitate the entry of ferrous

iron or soluble chelates into enterocytes (Conrad et al., 1999; Rolfs & Hediger, 1999). 3) Heme is digested from hemoglobin in the intestinal lumen, and then enters the enterocyte as an intact metalloprotein likely via an endosome (Conrad et al., 1999). Heme is released from protoporphyrin by heme oxygenase and degraded to inorganic iron, carbon monoxide, and bilirubin IXa (Beard et al., 1996.).

b) Intraenterocyte transport and storage

Iron derived from both heme and non-heme sources is processed in the same manner once it enters the common intracellular (enterocyte) pool of iron (Beard et al., 1996). Although the methods of intracellular iron transport are poorly defined, mobilferrin (a transferrin-like protein) may play a role in iron transport (Powell et al., 1999; Wolf, 1994). Ferrous iron may be utilized by mitochondria for the formation of heme proteins and other end products (Conrad et al., 1999), transported to the basolateral membrane of enterocytes (Powell et al., 1999; Wolf, 1994), or stored in ferritin (Powell et al., 1999; Wolf, 1994). Ferritin acts as an "iron sink" for intestinal mucosal cells by sequestering excessive levels of iron; this may in turn prevent free radical related damage to the cell (Powell et al., 1999; Beard et al., 1996). This storage iron is subsequently lost when the enterocyte dies and is shed (Beard et al., 1996).

c) Extraenterocyte transfer

Non-storage iron is delivered and bound to a transferrin-like basolateral transporter, and then shuttled through the basolateral membrane into the circulation; again, the specific mechanisms have yet to be defined (Beard et al., 1996; Powell et al., 1999). Two recently identified proteins – Ferroportin 1/Ireg1/MTP1 and hephaestin (a ceruplasmin homologue) -- appear to be involved in iron export (Roy & Enns, 2000). Apotransferrin receptor (Conrad et al., 1999), and lactoferrin (Wessling-Resnick, 2000) may also play a role. The basolateral membrane also contains holotransferrin receptors that allow the influx of iron-transferrin (Conrad et al., 1999; Powell et al., 1999).

2.32 Regulation of Iron Absorption

It is well established that the enterocyte is able to alter iron absorption according to iron status (Beard et al., 1996; Schumann et al., 1990; Gibson, 1990). Iron deficiency is the most potent inducer of both heme and non-heme iron absorption (Beard et al., 1996). Iron excretion in humans is limited to fecal and insensible losses (skin, sweat and bile), but the absorption of dietary iron is sufficient (1 to 2 mg/day) to balance these losses in iron replete individuals (Conrad et al., 1999). Recently, it has been hypothesized that several humoral "regulators" may function to maintain iron homeostasis (Roy & Enns, 2000): a) a "stores regulator" may increase non-heme iron uptake when iron levels in liver, skeletal muscle, and blood drop below a critical level, and decrease iron absorption in the setting of high iron stores; b) an "erythropoietic regulator" may increase iron uptake when erythropoietic demand exceeds iron supply; and c) hypoxia may also serve as an independent regulator. The soluble components involved in signalling between body tissues have not yet been identified (Roy & Enns, 2000).

Other factors are involved in maintaining iron balance at a cellular level. Iron responsive elements (IREs) work in conjunction with iron regulatory proteins (IRPs) to sense and respond to changes in the "labile iron pool" of enterocyte precursor cells (Roy & Enns, 2000). The iron regulatory proteins are directly involved in the modulation of enterocyte-specific ferritin and transport proteins, thereby altering the absorptive capacity of the mature enterocyte (Roy & Enns, 2000). A heredity hemochromatosis protein (HFE) at the basolateral membrane is believed to interact with both transferrin and transferrin receptor to inhibit iron uptake from the circulation, and influence the absorptive capacity of the enterocyte in normal individuals (Roy & Enns, 2000).

2.33 Iron Transport and Storage

As free iron is an extremely toxic substance, iron circulates in a protein-bound form. Transferrin is the most significant iron transport protein in the plasma. Circulating levels of transferrin reflect the body's iron status; serum levels increase with iron deficiency (Wessling-Resnick, 2000). Although transferrin-bound iron comprises

less than 0.1% of total body iron, it is the iron pool with the highest rate of turnover (Ponka & Lok, 1999). The primary site of transferrin synthesis is the liver; the brain, kidney, testes, and fetal muscle are involved to a lesser degree (Beard et al., 1996; Lee, 1995). Transferrin contains two half-sites for binding of ferric iron, and is normally 25-50% saturated (Beard et al., 1996). Transferrin acquires iron from both dietary absorption and heme from senescent erythrocytes, and functions to deliver iron to peripheral tissues, redistribute iron to various body compartments, and protect iron from glomerular filtration (Beard et al., 1996). Other proteins are involved to a lesser degree in the plasma transport of iron. Ferritin is found in small amounts in plasma, relative to iron stores (Baynes & Stipanuk, 2000). The exact role of lactoferritin – found in human milk, plasma, and mucous secretions such as tears – remains uncertain (Beard et al., 1996), but it may be bacteriostatic and fungistatic in nature (Baynes et al., 1986). Free heme is detected in the serum only in hemolytic conditions, and normally circulates bound to albumin (Beard & Dawson, 1997).

Transferrin receptor (TfR) is the primary mediator involved in iron uptake from the circulation into body cells (Cook, 1999). Transferrin receptor (TfR) is a membrane associated glycoprotein that is comprised of two identical transmembrane subunits (Ponka & Lok, 1999). The extracellular domain of the receptor contains the transferrin binding site and each domain binds one transferrin molecule (Ponka & Lok, 1999). The affinity of TfR is highest for diferric transferrin, lower for monomeric transferrin, and negligible for apotransferrin (Cook, 1999). The number of transferrin receptors is proportional to the requirements of the cell; consequently 70 to 80% of total body TfR is found in erythroid precursor cells.

Ponka and Lok (1999) describe the following steps for cellular iron uptake: 1) transferrin attaches to its receptor by a physiochemical interaction and bound transferrin-receptor complexes cluster in clathrin-coated vesicles; 2) the transferrin-complex is internalized via endocytosis; 3) the endosome is acidified, iron is released from transferrin and likely reduced to ferrous iron; 4) iron is transported through the endosomal membrane via the "natural resistance associated macrophage protein 2" (Nramp2) and used for metabolic functions or stored as ferritin; 5) apo-transferrin returns to the cell surface and is released. Vesicles fuse with the plasma membrane, and

exosomes containing transferrin receptor are released. Subsequent proteolysis leads to the appearance of a truncated form of the transfer-receptor complex in the serum (Shih et al., 1990).

Total body storage iron varies from 0-15mg/kg body, depending on gender and iron status (Beard, 2001). The majority of iron in the body is stored as ferritin. 60% of ferritin is contained in the liver, and 40% is found in muscle tissues and cells of the reticulo-endothelial system (Beard, 2001). 95% of storage iron in liver tissue is contained within hepatocytes as ferritin; the remaining five percent is contained within Kuppfer cell lysosomal remnants as hemosiderin (Beard, 2001).

The ferritin protein is comprised of 24 polypeptide subunits with an iron core (Beard & Dawson 1997). Although ferritin contains 4500 binding sites for ferric iron, normally it is 20% saturated (Beard & Dawson, 1997). Ascorbic acid or reduced flavin mononucleotide may be involved in facilitating the rapid release of iron from the ferritin core (Beard & Dawson, 1997). Ceruloplasmin may be necessary for the oxidation of ferritin-derived iron and attachment to transferrin (Beard & Dawson, 1997).

Hemosiderin, the less available storage protein, is formed from ferritin with increasing tissue iron stores (Beard et al., 1996). Lysosomal proteases degrade ferritin to hemosiderin when ferritin contains about 4000 atoms of iron per molecule (Weir et al., 1984). The iron contained within hemosiderin is also less chemically reactive than ferritin (Beard et al., 1996).

In order to maintain intracellular iron homeostasis, iron is directly involved in regulating the synthesis and action of proteins involved in iron acquisition, utilization and storage (Beard and Dawson, 1997). When intracellular iron levels are suboptimal, iron acquisition will increase by either mobilizing storage iron or increasing uptake of plasma iron, and the cell will reprioritize its iron distribution (Beard and Dawson, 1997). These processes are regulated at a genetic level in part by the iron regulatory proteins 1 and 2 (IRP1 and IRP2) and iron-responsive elements (IREs) that are located within the messenger RNA of TfR and ferritin (Lee, 1999). IRP1 is an iron-sulfur protein that becomes cytosolic aconitase when saturated with iron, a Krebs cycle enzyme that catalyzes the conversion of citrate to isocitrate (Lee, 1999). When

intracellular iron is sufficient, the affinity of IRP1 for messenger RNA IREs is low and this protein has aconitase activity; conversely when intracellular iron is low, this enzyme activity is lost and affinity for IREs increases (Lee, 1999). IRP2 does not have a known enzyme function and is less abundant than IRP, but since it is rapidly degraded in iron replete cells it is only available to bind IREs in iron-depleted cells (Lee, 1999; Ponka and Lok, 1999). Although the precise mechanisms are undefined, this system appears to be instrumental in the down-regulation of ferritin synthesis, and up-regulation of TfR synthesis with low intracellular iron (Baynes and Stipanuk, 2000). Heme is also directly involved in regulating hemoglobin synthesis and several heme containing gene products (Beard and Dawson, 1997). Factors other than cellular iron such as nitric oxide also appear to induce changes in the activity of the IRPs (Ponka and Lok, 1999).

2.34 Functions of Iron

Iron is both an essential nutrient and potential toxicant to cells, and can exist in several oxidation states in biological systems. Interconversion between the ferrous (+2), ferric (+3) and ferryl (+4) states in biological systems enables iron to participate in electron transfer and reversibly bind ligands such as oxygen, nitrogen and sulfur atoms (Beard, 2001). The electronic spin state of iron and biological redox potential of some heme proteins can change according to the ligand to which it is bound (Beard et al., 1996). These characteristics can enable iron to participate in a large number of biochemical reactions. Electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell growth and differentiation are dependent on adequate iron supply (Beard, 2001).

There are many iron containing proteins that are involved in these essential functions. These proteins can be classified as heme and non-heme proteins. Heme proteins contain a heme coenzyme that is comprised of one atom of ferrous iron atom bound to the complex organic ring structure of protoporphyrin (Brody, 1994). The heme proteins hemoglobin and myoglobin are involved in oxygen transport and storage. Hemoglobin, the major protein of red blood cells, is synthesized in immature erythrocytes, and contains four subunits that can each bind one molecule of dioxygen

(Brody, 1994). The monomeric protein myoglobin is contained in muscle tissue in small amounts, and is used for short-term storage of oxygen (Brody, 1994). Cytochromes are also heme proteins that are involved in numerous activities such as the respiratory chain, lipid metabolism, and conversion of cholesterol to steroid hormones. Many non-heme, iron containing enzymes are also found in the human body.

The numerous functions of these iron-containing proteins may be classified into three main areas (Beard & Dawson, 1997):

a) Oxygen transport and storage

Hemoglobin and myoglobin are instrumental in oxygen transport and storage. Hemoglobin functions as an oxygen carrier, and its affinity for oxygen varies depending on the concentration of oxygen in the bloodstream. In areas of high oxygen concentration (i.e. lung capillaries), oxygen binding is strong. As the red blood cell reaches areas with lower oxygen levels, hemoglobin's affinity for oxygen diminishes, and oxygen dissociates and enters the tissues (Brody, 1994). Oxygen unloading is also increased in conditions of decreasing pH (i.e. exercising muscle) via the Bohr effect, and in anemia (Beard & Dawson, 1997). Myoglobin increases the rate of diffusion of dioxygen from capillary red blood cells to cytoplasm and mitochondria (Beard & Dawson, 1997). Eighty-five percent of body iron is contained in hemoglobin and myoglobin (Wessling-Resnick, 2000).

b) Electron transport

The electron-transport chain functions to transfer electrons from NADH (or other intermediate electron donors) to molecular oxygen with water formation; several heme and non-heme proteins are involved (Beard & Dawson, 1997). The iron-porphyrin ring structure of heme-containing cytochromes reduces ferrous iron to ferric iron with the acceptance of electrons; iron-sulfur proteins also function as electron carriers via iron bound to two or four sulfur atoms and cysteine side chains (Beard & Dawson, 1997).

c) Substrate oxidation and reduction

Numerous iron-containing proteins are involved in metabolic processes of substrate oxidation and reduction. The catalytic **oxidoreductase** enzymes include xanthine dehydrogenase which is involved in uric acid formation, and

ribonucleotide reductase which functions in DNA synthesis (Brody, 1994). The **monooxygenases** include amino acid monooxygenases that catalyze the formation of serotonin and dopamine precursors, the cytochrome P450 family of enzymes (involved in numerous activities including steroid hormone synthesis and metabolism of xenobiotics), and fatty acid desaturases (Beard & Dawson, 1997). **Dioxygenases** include amino acid or amine dioxygenases, lipoxygenases, peroxidases (all contain iron except glutathione peroxidase), and nitric oxide synthases.

2.4 Anemia in the Elderly

Anemia is found in elderly individuals for a variety of reasons. IDA and anemia of chronic disease (ACD) are particularly problematic in this age group, and distinguishing between these two conditions may be difficult. Blood loss is frequently implicated in IDA in the elderly, and may be attributed to specific medical conditions or medications; dietary factors may also be involved in IDA.

2.41 Assessment of Iron Status

Traditionally, the prevalence of IDA has been determined on the basis of either hemoglobin or hematocrit concentrations (Fleming et al., 2001). However, since anemia may result from other causes, the use of hemoglobin values alone will overestimate prevalence of IDA. There is also a large overlap in hemoglobin between healthy and anemic subjects. The use of several indices of iron status simultaneously provides a more accurate measure of iron status than any single index, and the presence of two or more abnormal values for iron status indices is considered indicative of impaired iron status. Previous models have utilized parameters that cover the stages of IDA (Gibson, 1990). Overall, the use of a combination of different laboratory tests is likely to be more powerful tool for correctly classifying individuals as having/not having a particular disease (Wians et al., 2001).

Iron deficiency is often classified into three stages, each with specific laboratory findings (Gibson, 1990). Anemia is accompanied by alterations in the levels of the

three key iron-related proteins: transferrin, ferritin, and transferrin receptor (Cook, 1999).

a) **Iron depletion** is associated with a gradual reduction in the amount of iron stored in the liver, spleen, and bone marrow (Beard & Dawson, 1997). Clinical findings include declining serum ferritin levels, a marker for reduced iron stores, with normal levels of transport iron and hemoglobin.

b) **Iron deficient erythropoiesis** is associated with complete exhaustion of iron stores. Decreased supply of plasma iron to erythropoietic cells results, and is commonly identified by depressed transferrin saturation and serum iron levels. Hemoglobin levels usually remain within the normal range.

c) **Iron deficiency anemia** (tissue iron depletion) ultimately results when iron stores are completely depleted, and levels of circulating iron remain depressed. Insufficient iron for erythropoiesis results in microcytic, hypochromic anemia (i.e. small, pale red blood cells). Reduced levels of hemoglobin, hematocrit and red cell indices are also present.

Serum ferritin is the single best measure to assess iron stores in a non-invasive manner, and provides a quantitative measure of iron stores in the absence of inflammation. In normal individuals, every 1μg/L of serum ferritin is equivalent to approximately 10 mg of storage iron (Beard & Dawson, 1997). Values below the reference range indicate iron deficiency, and values above the reference range may reflect excess iron stores. However, serum ferritin values increase irrespective of iron status in disease states such as chronic infection or inflammation, liver disease and malignancies, and thus is not a useful indicator of iron stores in these conditions (Borch-Iohnson, 1995). In contrast, serum ferritin concentrations do not rise with the acute phase response of stroke injury, and values are stable up to 48 hours following a stroke event (Armengou et al., 1998). In cases where iron deficiency coexists with infection or inflammation, ferritin values may remain in the normal range (Gibson, 1990). Ferritin values increase with age, an effect that may be attributed to a higher preponderance toward infections, inflammation, or regular alcohol use with advancing age (Hallberg, 2001). Some authors (Holyoake et al., 1993; Guyatt et al., 1990;

Patterson et al., 1985) have suggested higher cut-off points for identifying iron deficiency in the elderly.

Serum iron, total iron binding capacity (TIBC), and iron saturation of transferrin provide measurements of transport iron (Baynes and Stipanuk, 2000). Serum iron and TIBC reflect the amount of iron in transit from the reticuloendothelial system to the bone marrow. Serum iron is a measure of the number of atoms of iron bound to transferrin and iron deficiency results in low values; in contrast, TIBC reflects the amount of iron that transferrin will bind and rises with iron deficiency (Lee, 1999). Transferrin saturation[1], the percentage result of TIBC divided into serum iron, reflects iron supply to the erythroid bone marrow and low values are present with iron deficiency. Serum iron is considered to be an unstable parameter for several reasons: a) non-fasting values are significantly higher, b) diurnal variation can lead to morning values that are 30% higher than evening values, c) there is significant short term variability, and d) values decrease with infections and inflammation (Borch-Iohnson, 1995). TIBC is less affected by biological variations, but is more subject to analytical errors (Gibson, 1990). Transferrin saturation is also influenced by variations in serum iron.

Serum transferrin receptor (sTfR) is a recently developed useful and highly sensitive indicator of iron deficiency. The number of transferrin receptors (TfR) is highest on rapidly dividing cells, tissues synthesizing hemoglobin, and the placenta (Borch-Iohnson, 1995), and sTfR levels correlate with total body mass of cellular transferrin receptor (Cook, 1999). In the setting of uncomplicated IDA, sTfR is a highly sensitive marker of iron deficiency, and serum elevations usually exceed reference range values (Cook, 1999). sTfR is also useful for detecting iron deficient erythropoiesis, as sTfR values start to increase when ferritin stores are depleted (Cook, 1999). sTfR does not differ between men and women and is normally distributed in healthy subjects (Cook, 1999). sTfR values are reported to remain unchanged with age (Cook, 1999). However little research has been conducted with elderly individuals, and Ahluwalia (1998) has suggested that sTfR values may in fact be lower with this age group. Individuals living at high altitudes and those of black race have sTfR values that

[1] Transferrin saturation (%) = Serum iron (μmol/L) / TIBC (μmol/L) x 100%

are 9% higher than white subjects and those living at sea level (Allen et al., 1998). Since sTfR values are also higher with medical conditions such as thalassemia, hemolytic anemia, and polycythemia, it has been recommended that sTfR be used in conjunction with other measures of iron status such as serum ferritin for assessing iron status (Cook, 1999). sTfR values are generally lower in individuals with aplastic anemia, anemia post-transplant, and chronic renal failure (Ponka and Lok, 1999). Since there is currently a variety of commercial kits available for determining TfR values, each with different reference ranges, there is an urgent need to standardize the different assays to obtain uniform and comparable results (Yeung et al., 1998).

2.42 Anemia of Chronic Disease

Anemia of chronic disease (ACD) is a mild to moderate, often microcytic, hypochromic anemia that is associated with iron deficient erythropoiesis (Fleming et al., 2001; Cook, 1999). ACD may result from infection, malignancy, inflammation, and liver disease. The release of iron from reticuloendothelial cells, and transport of iron from these stores to transferrin is defective. This defect (i.e. 'mucosal block') results in reduced iron supply to the bone marrow. Since iron stores remain adequate, neither dietary iron absorption nor transferrin synthesis is increased. Typical laboratory values include low serum iron and total iron binding capacity (TIBC) levels, and low to normal transferrin saturation values. As ferritin is an acute phase protein, increased ferritin synthesis in the reticuloendothelial system leads to elevated serum ferritin concentrations, thus providing a false indicator of iron stores with ACD (Fleming et al., 200; Gibson, 1990). Bone marrow examination to determine the presence or absence of stainable iron in reticuloendothelial cells is the "gold standard" for distinguishing between IDA and ACD, since the presence of stainable iron excludes IDA as the cause of anemia (Cook, 1999).

Much research has been conducted into the use of sTfR for distinguishing between IDA and ACD, since this would ideally negate the need for invasive bone marrow examinations. Many studies have reported sTfR to be very useful for this purpose since sTfR increases with IDA, but values remain within the normal range in the setting of ACD (Ferguson et al., 1992; Petterson et al., 1994; Chua et al., 1999; Suominen, 2000).

Wians et al. (2001) countered that although sTfR offers good discriminatory power for distinguishing between IDA and ACD, there was no apparent advantage over the use of TIBC. The use of the sTfR/log ferritin ratio or "TfR-F index" has also been used to discriminate between IDA and ACD (Suominen et al., 2000). This tool, which captures the relationship between increased sTfR and decreased serum ferritin in the setting of IDA, may in fact be a more sensitive and specific indicator than sTfR alone (Punnomen et al., 1997).

sTfR also appears to be useful for identifying IDA coexisting with ACD. Several studies suggest that sTfR is a good indicator of iron status in this setting; sTfR values are higher with IDA coexisting with ACD than with ACD alone (Bultink et al., 2001, Punnonen et al., 1997; Petterson et al., 1994. It is possible that there may be limitations to the use of sTfR as a single indicator, and that the TfR-F Index might also be a superior tool in this setting (Punnonen et al., 1997; Bultink et al., 2001). Overall, comparison of these reports is complicated by differing subject characteristics and techniques for the determination of sTfR. It is also difficult to extrapolate these research findings to the elderly, as none of these studies reported findings specific to this age group.

2.43 IDA in the Elderly

Elderly individuals are at risk for IDA for a variety of medical reasons. Blood loss is a frequent cause of IDA, and may result from: (a) gastrointestinal conditions such as polyps, stomach or colon cancer, peptic ulcer disease, hiatus hernia, diverticular disease, or hemorrhoids, (b) the use of non-steroidal anti-inflammatory or anticoagulant drugs, or (c) frequent blood drawings (Johnson et al., 1994). Clinical manifestations of IDA may include: glossitis, angular stomatitis, koilonchyia (spoon nails), blue sclera, esophageal webbing, or microcytic hypochromic anemia and behavioural disturbances (geophagia, pacophagia, and restless legs syndrome) (Beard, 2001; Gibson, 1990). Less specific symptoms may include decreased energy, tiredness, anorexia, susceptibility to infection, and reduced work capacity (Beard, 2001; Gibson, 1990). These functional consequences are more strongly related to anemia rather than tissue iron deficits (Beard et al., 1996).

Generally poor nutritional status has also been documented in subgroups of elderly and this is a contributor to IDA. Malnutrition is problematic with many elderly individuals. A recent review by Seiler (1999) cites prevalence figures for malnutrition in the elderly ranging from 4 to 31% in "healthy", free-living elderly. Decreased muscle mass and sedentary lifestyle contribute to declining energy needs with aging (ADA Reports, 2000). Despite these changing needs, many older individuals fail to consume sufficient intakes of energy and other nutrients. Factors that may adversely impact nutritional status in the elderly include impaired functional and cognitive status, medical comorbidities, multiple medications, decreased education, poor oral health, poverty, taste and smell alterations, and dysphagia (ADA Reports, 2000; Ranieri et al., 1996; Ritchie et al., 1997). Aging-related changes in neurotransmitter and hormone levels, and heightened cytokine release in response to injury may further impair appetite and nutritional status in many elderly individuals (Baez-Franceschi & Morley, 1999).

A significant number of stroke patients are also malnourished at the time of admission to hospital. Davalos et al. (1996) reported a 16.3% prevalence of protein-energy malnutrition in 104 patients admitted with first stroke; protein-energy malnutrition was defined as below normal values for serum albumin, triceps skinfold thickness, or midarm muscle circumference. Other authors (Unosson et al., 1994; Axelsson et al., 1988) reported prevalence figures of 8% and 16% respectively using different classification schemes. During hospitalization following stroke injury, the nutritional status of stroke patients has been shown to deteriorate further (Unosson et al., 1994, Gariballa et al., 1998, Davalos et al., 1996). Impaired nutritional status at time of stroke injury has also been associated with adverse outcomes such as increased stress response, frequent infections and bedsores (Davalos et al., 1996, Axelsson et al., 1988). Furthermore, impaired baseline nutritional status has been shown to be worse amongst those stroke patients who later die or remained in hospital (Garbiballa et al., 1988).

There are various reports of the prevalence of IDA in the elderly. Interpretation of these findings is complicated by differing methods of assessment, and difficulty in distinguishing between IDA and ACD in this age group. Furthermore, some studies conducted separate analyses for "well elderly" versus those with disease; one would

expect a higher prevalence of IDA in a sample that includes individuals with variety of diagnoses. A recently published, prospective analysis of 1016 free-living, elderly members of the original Framingham Heart Study cohort sought to evaluate the iron status of this non-institutionalized US population (Fleming et al., 2001). The overall prevalence of IDA was 1.2% in this group, but when subjects with chronic disease were excluded from the analysis, the prevalence of IDA decreased to 0.8%. In this study, IDA was defined as having two out of three abnormal values for ferritin, transferrin saturation, or mean corpuscular volume, plus low hemoglobin values. Interestingly, a much higher prevalence of excess iron stores, 12.9% overall, was found in this population, but the disease effect on this prevalence estimate was considered insignificant (excess iron stores was defined as serum ferritin >200 μg/L for women, and > 300 μg/L for men) (Fleming et al., 2001). The Third National Health and Nutrition Examination Survey (NHANES III) aimed to determine the prevalence of IDA and iron deficiency in the US population. Looker et al. (1997) reported a 2% prevalence of IDA in non-black elderly males and 1% in elderly females using a combination of low hemoglobin with altered values in two of the following: transferrin saturation, serum ferritin, or erythrocyte protoporphyrin. A stratified, multistage probability design was used to select the sample for NHANES III, and elderly subjects included free-living individuals with a variety of medical conditions. The Nutrition Canada Survey (Health and Welfare Canada, 1973) is the most comprehensive Canadian data and is now almost 30 years old. This study used a stratified design with random sampling of subjects. Using single markers of hemoglobin and mean corpuscular hemoglobin concentration to identify prevalence of IDA in individuals over 65 years of age, values of 16.5% and 23.2 % respectively were cited for men, and figures of 4% and 22.1% for women. These data appear to apply to free-living, elderly individuals, and did not exclude individuals with disease. There are no available reports that document the prevalence of iron deficiency anemia in acute stroke patients.

2.5 Diet as a Contributor to IDA in Elderly

Given the numerous medical factors that influence iron status in the elderly, the issue of whether diet is a major contributor to compromised iron status is controversial,

and thus represents a secondary objective of the thesis research. Several authors have examined the relationship between diet and iron stores, with conflicting findings. Garry et al. (2000) recently examined the association between iron intake and serum ferritin (a marker of storage iron) in elderly men and women by both longitudinal (n=125) and cross-sectional (n=226) study design. No association was apparent between heme iron intake and iron stores in either study. However supplemental iron intake had a significant and positive association with increased iron stores in women in the cross-sectional study, and in both genders in the longitudinal study. In a cross-sectional analysis of 634 free-living elderly participants of the Framingham Heart Study, Fleming et al. (1998) reported heme iron, supplemental iron, dietary vitamin C, and alcohol as significant positive predictors, and coffee as a negative predictor of serum ferritin. Dietary protein, but not dietary iron, was positive correlated with serum ferritin in a Canadian study of 82 healthy elderly subjects (Payette and Gray-Donald, 1991). In 56 homebound elderly persons, Beard et al. (1996) noted a positive association between iron supplement use and serum ferritin. The relationship between diet and other markers of iron status in the elderly has also been examined: Payette and Gray-Donald (1991) noted a positive correlation between dietary vitamin C and serum iron, and Yearick et al. (1980) found a correlation between dietary iron and hemoglobin in 100 non-institutionalized elderly. Study designs and dietary assessment methods vary, further complicating the relationship between diet and iron status. Factors such as alcohol, vitamin C, and heme iron, dietary iron, or supplemental iron may indeed play a role, but further research is warranted.

2.51 Iron Requirements of the Elderly

The Dietary Reference Intakes for iron, the newest recommendations for dietary iron intake, were released in 2001 (Institute of Medicine, 2001). The methods used to determine iron requirements are also contained within this report. The process of factorial modeling was used whereby iron requirements are based on estimated iron losses (feces, exfoliation and urine) in non-menstruating adults. As estimated basal iron loss is related to body weight, data from NHANES III was used to determine the median and variability for body weight. An upper limit of dietary iron absorption of

18% was used to adjust requirements. The Estimated Average requirement (EAR) or the "median requirement of a distribution of requirements for a life stage or gender group" was set at 6 mg/day for adult men and 5 mg/day for post-menopausal women. The Recommended Dietary Allowance (RDA), or the estimated requirement that would theoretically meet iron needs for 97.5% of a population, was set at 8 mg/day. The Tolerable Upper Intake Level, or "the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals" (i.e. gastrointestinal side effects) was set at 45 mg/day for all adults ≥ 19 years of age. It is notable that the methods used to determine all of the above requirements were not unique to the elderly; the requirements of all adult men >19 years were determined by the same method, as were the requirements of all post-menopausal women. These calculations did not consider that iron absorption diminishes with increasing iron stores, or that men have higher iron stores than women. Additionally, these requirements may not be sufficient for individuals consuming a diet with lower iron bioavailability. The previous Recommended Nutrient Intake (RNI) for iron was set at 9 and 8 mg/day for elderly men and women respectively (Health and Welfare Canada, 1990), and was based simply on known estimates of average requirements using a dietary absorption factor of 12.5%. Despite the methodological limitations, the Dietary Reference Intakes are the most current published recommendations available for iron intake.

The Dietary Reference Intakes may be utilized in several different ways. In the assessment of an individual, comparison of dietary intake to the EAR (with consideration of the within-subject variability of intake) may be used to estimate risk of inadequacy. At a group level, the EAR may be used to estimate prevalence of nutrient inadequacy, and to plan intakes. The RDA is useful as a target in planning the dietary intake of an individual. The Tolerable Upper Intake Level is used to determine the possibility of overconsumption of a nutrient for an individual or a group (Institute of Medicine, 2001).

2.52 Bioavailability of Dietary Iron

The bioavailability of dietary iron and iron content of the diet are the two main factors that affect iron absorption, other than the absorptive capacity of the intestine as

previously discussed. Since iron status does not appear to be directly affected by iron intake, many research efforts have sought to identify how specific foods affect the bioavailability of dietary iron. Numerous dietary factors have been shown to either enhance or inhibit the absorption of dietary iron via effects that modify iron digestion and uptake. Enhancing factors may reduce iron to its ferrous form, or form soluble and stable complexes that easily release iron for intestinal uptake; in contrast, inhibiting factors may form insoluble complexes or complexes that do not release easily for intestinal uptake (Carpenter, 1992). There are two types of dietary iron: **heme iron** is found mainly in meat, and comprises up to 15% of all iron in a mixed diet; **nonheme** iron is found in cereals, fruits and root vegetables, and comprises the remaining part of dietary iron (Hallberg, 2001). The role of enhancing and inhibiting factors is more pronounced with nonheme sources of iron.

a) Nonheme Iron

The concurrent intake of any food is almost always inhibitory to the bioavailability of nonheme iron, as the bioavailability of iron administered alone is higher (Carpenter, 1992). However specific food components have been identified that impede nonheme iron absorption. Fibre, the dietary component resistant to digestion by the endogenous secretions of the upper digestive tract, has demonstrated an inhibitory effect on nonheme iron absorption (Morris, 1998; Carpenter, 1992); a specific component of fibre such as tannins or phytates may be responsible for this effect (Hallberg et al., 1987). Phytates, found in whole grains, bran and soy products, are problematic since they form insoluble complexes with nonheme iron (Morris, 1998). Polyphenols (i.e. tannic acid, gallic acid, catechin), found in coffee, tea, red wines, and certain spices and fruits and vegetables, also demonstrate inhibitory effects on nonheme iron absorption (Gillooly et al., 1983; Brune et al., 1989). The negative effects of tea and coffee taken with meals are particularly striking (Hallberg and Rosander, 1982; Morck et al., 1983). Calcium also appears to inhibit nonheme iron, although this is somewhat controversial. A review paper by Whiting (1995) outlined numerous studies that demonstrated an inhibitory effect with both dietary and supplemental calcium. This phenomenom appears most pronounced with studies of single-meal effects. Recent papers (Reddy

and Cook, 1997; Tidehag et al., 1995) that examined the effect of calcium on nonheme iron absorption in the context of the whole diet did not find the same effect. It is possible that an adaptive response over a longer period of time may lessen the inhibiting effects of calcium (Minihane and Fairweather-Tait, 1998).

The ability of a factor to enhance nonheme iron absorption refers to its ability to improve absorption relative to the overall inhibitory action of food (Carpenter, 1992). Nonheme iron absorption is two to four times greater when meat, poultry, or fish is consumed at the same meal (Cook and Monsen, 1976) but this effect is only apparent with muscle tissue, and not milk, cheese or eggs (Morris, 1998). Suggested theories for this "meat factor" include: cysteinyl residues or carboxylate groups of the proteins may interact with nonheme iron in the gut lumen and maintain iron in a soluble form, increased gastric acid production with meat ingestion, and interactions between phytates and meat-derived peptides that minimize the inhibitory effects of phytate (Baynes and Stipanuk, 2000). Organic acids such as citric acid, malic acid, lactic acid, and ascorbic acid, found in various fruits and vegetables, are also known enhancers of iron absorption (Baynes and Stipanuk, 2000). One mg of ascorbic acid is approximately equal to 1 g of meat, fish, or poultry in its ability to enhance nonheme iron absorption (Monsen et al., 1978), but these positive effects on absorption of nonheme iron are limited only to intake with meals (Morris, 1998).

b) Heme iron

In contrast, a limited number of factors are known to affect the bioavailability of heme iron. Meat is also an enhancer of heme iron absorption, since proteolytic digestion products may prevent the formation of insoluble heme aggregates (Carpenter, 1992). Supplemental calcium also appears to have an inhibitory effect on heme iron absorption when consumed with meals (Hallberg et al., 1991; Hallberg et al., 1992).

The above findings have limited application to elderly individuals. Most studies of iron bioavailability have been conducted with younger subjects, and findings need to be replicated with elderly individuals (Johnson et al., 1994). Non-dietary factors such as

achlorhydria may also exert significant adverse effects on iron absorption in the elderly (Johnson et al., 1994). Furthermore, most previous studies are based on single-meal studies of iron availability, and these effects are probably less pronounced in the context of a mixed, western diet (Baynes and Stipanuk, 2000; Cook et al., 1991). The iron content of typical Western diets is considered to be highly bioavailable, with estimates of iron biovailability ranging from 14 to 17% and the overall iron content approximating 6 mg /1000 kcal (Baynes and Stipanuk, 2000).

2.53 Assessment of Dietary Intake

In order to study the role of diet as a determinant of iron status in the elderly, an accurate assessment of dietary intake is essential. This poses many challenges in elderly individuals. Changes in cognitive function, hearing, vision, memory, or speech present unique problems and may limit the situations where dietary recall or questionnaires are practical (Lee & Nieman, 1996). These impairments are also likely to be more prevalent in patients following a stroke. Although there is currently no gold standard for assessing nutrient intake (Briefel et al., 1992), commonly used tools include 24-hour recalls, food records, and food frequency questionnaires.

a) Twenty-Four Hour Recall

The 24-hour recall is the most widely used assessment tool. A trained interviewer conducts the recall using standardized forms and visual references such as food models; the purpose is to define and quantify food intake during a previous 24-hour period (Willett, 1990). Although this method is well-suited to assessing the average intake of groups, it is not optimal for examining relationships between diet and disease since nutrient values may not represent typical or long-term intake (Willett, 1990).

b) Food Records

Food records are detailed descriptions of types and amounts of foods consumed over a specific period (usually 3 or 7 days). Foods may be weighed or measured during this time. As detailed record keeping involves a large subject burden, this method is best suited to studies with a small number of literate and highly motivated subjects (Willett, 1990).

c) Food Frequency Questionnaire (FFQ)

Food frequency questionnaires (FFQ) are designed to estimate nutrient intake over a period of time such as weeks or months, and are thus appropriate for studying the relationship between diet and disease (Briefel, 1992; Willett, 1990). A FFQ is comprised of a food list and food frequency sections. Some questionnaires may contain additional information about portion size. Intake is determined by multiplying the frequency of each item by the portion size and nutrient composition (Briefel et al., 1992). Nutrients are summed across foods, and adjustment factors are used to approximate usual daily nutrient intake (Briefel et al., 1992; Harlan & Block, 1990). FFQs may be either self-administered or interviewer-administered.

There are also limitations inherent in the use of FFQs. As foods are grouped into large categories, information is not available for individual food items. Information about specific food brands or method of preparation is also not available. The application of FFQ results to different ethnic subgroups may be limited, as food preparation, recipe ingredients, or nutrient values may vary considerably (Briefel et al., 1992). Furthermore, some individuals may not be able to accurately describe their usual portion size resulting in large intra-person variation. A high level of subject motivation is needed to ensure accuracy with this method (Willett, 1990).

Reliability and validity are also important considerations in the selection of nutrient assessment tools. The reliability or reproducibility of results with FFQs is generally considered to be better than 24 hour recalls, and comparable to food records (Zulfiki & Yu, 1992). Validity or accuracy of FFQs is usually established by a comparison of reported mean nutrient intake with (a) observed intake, (b) results from lab analysis of duplicate food portions, (c) results from another method of dietary assessment, or (d) biochemical indicators of nutrient status (Willett, 1990). As even minor changes may affect the performance of a FFQ, each newly developed tool should be validated separately (Willett, 1990).

The **Clue II questionnaire**, which was utilized in the current study, is a 63-item food frequency questionnaire. It was developed by Block Dietary Systems (Berkely, California) and collects information about food frequency and portion size. This questionnaire was validated for dietary iron intake using self-reported dietary intakes from 116 men ranging in age from 30 to 60 years. In a comparison with serum ferritin, a biochemical measure of iron stores, meat intake was found to be a significant predictor of serum ferritin ($p=0.047$) (G. Block, personal communication). Since meat is an excellent source of heme iron (Hallberg, 2001) and vitamin supplement users were not included in this analysis, the Clue II questionnaire appears to be a good indicator of dietary iron. This questionnaire is a slightly modified version of the widely used Block Questionnaire (Brief 87 version), which has been validated for other nutrients including energy, protein, vitamin C, and fibre (Block et. al., 1990).

3.0 Methods

3.1 Subjects

Ethics approval was obtained from the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation (Appendix A). The data for this study were obtained prospectively from elderly patients ≥ 65 years of age who were admitted sequentially to the neurology service at Royal University Hospital, Saskatoon, Saskatchewan with a primary clinical diagnosis of stroke or transient ischemic attack (TIA) between May 23 and December 23, 1999 (n=94). Exclusion criteria included: patients with a previous clinical history of stroke, patients with intracerebral or subarachnoid hemorrhage, and those with stroke due to a brain tumour. Physician patient lists for the Neurology service were reviewed on a daily basis, and provided information about new admissions and patient diagnosis. Consent was obtained from patient or next-of-kin (Appendix A).

3.2 Laboratory Data

Bloodwork was obtained within 24 hours of admission. The following indices were measured: serum ferritin, serum transferrin receptor (sTfR), serum iron, transferrin saturation, total iron binding capacity (TIBC), white blood cell count, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, and mean platelet volume.

For sTfR, serum samples were collected, stored at -70° C, and subsequently sent to the laboratory of Dr. S. Zlotkin (The Hospital for Sick Children Research Institute, Toronto, Ontario) for analysis using an enzyme immunoassay kit (Ramco Laboratories, Houston, Texas). The reference range used for sTfR cited by Ramco Laboratories was based on serum samples from 119 males and 120 males, 19 to 40 years of age, in the Kansas City area. The other laboratory tests were conducted by the Department of

Laboratory Medicine, Saskatoon District Health, and the reference ranges used were the reference ranges cited by the kit manufacturers, modified for local usage as necessary (L. Massey, personal communication). Serum ferritin was determined by a kit that utilizes microparticle enzyme immunoassay technology (Abbott Laboratories, Abbott Park, Illinois). Serum iron was determined by a kit that complexes ferrous iron with FerroZine Iron Reagent (SYNCHRON LX® Systems, Beckman Coulter Inc., Brea, California), and the change in absorbance is monitored at 560 nm. TIBC was derived by combining values for unsaturated iron binding capacity and serum iron. Unsaturated iron binding capacity was measured by a kit (Diagnostic Chemicals, Charlottetown, P.E.I) as the difference between the amount of unbound ferrous iron and the total amount of iron added to a serum sample. A ferroin type compound (Ferene®) was used to chelate unbound ferrous iron and absorbance was measured at 593 nm. Transferrin saturation is calculated by dividing the value obtained for serum iron by TIBC. The STKS Coulter Counter (Beckman Coulter Inc., Brea, California) was used to obtain the following hematological measurements: white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, and mean platelet volume.

3.3 Interpretation of Hematological Parameters

On the basis of available hematological data, patients were classified into the following groups (Figure 3.1): a) iron deficiency anemia (IDA), b) iron deficiency, c) anemia of chronic disease (ACD), d) combination (ACD coexisting with IDA), e) other anemia, and f) normal iron status. For statistical purposes, the total number of patients with iron deficiency anemia (IDA) or "IDA present" group included those with IDA and IDA coexisting with anemia of chronic disease (ACD). "IDA absent" was comprised of the other groups.

The prevalence of excess iron stores was also determined using the following criteria established by Fleming et al. (2001): serum ferritin > 300 g/L (males), serum ferritin > 200 g/L (females).

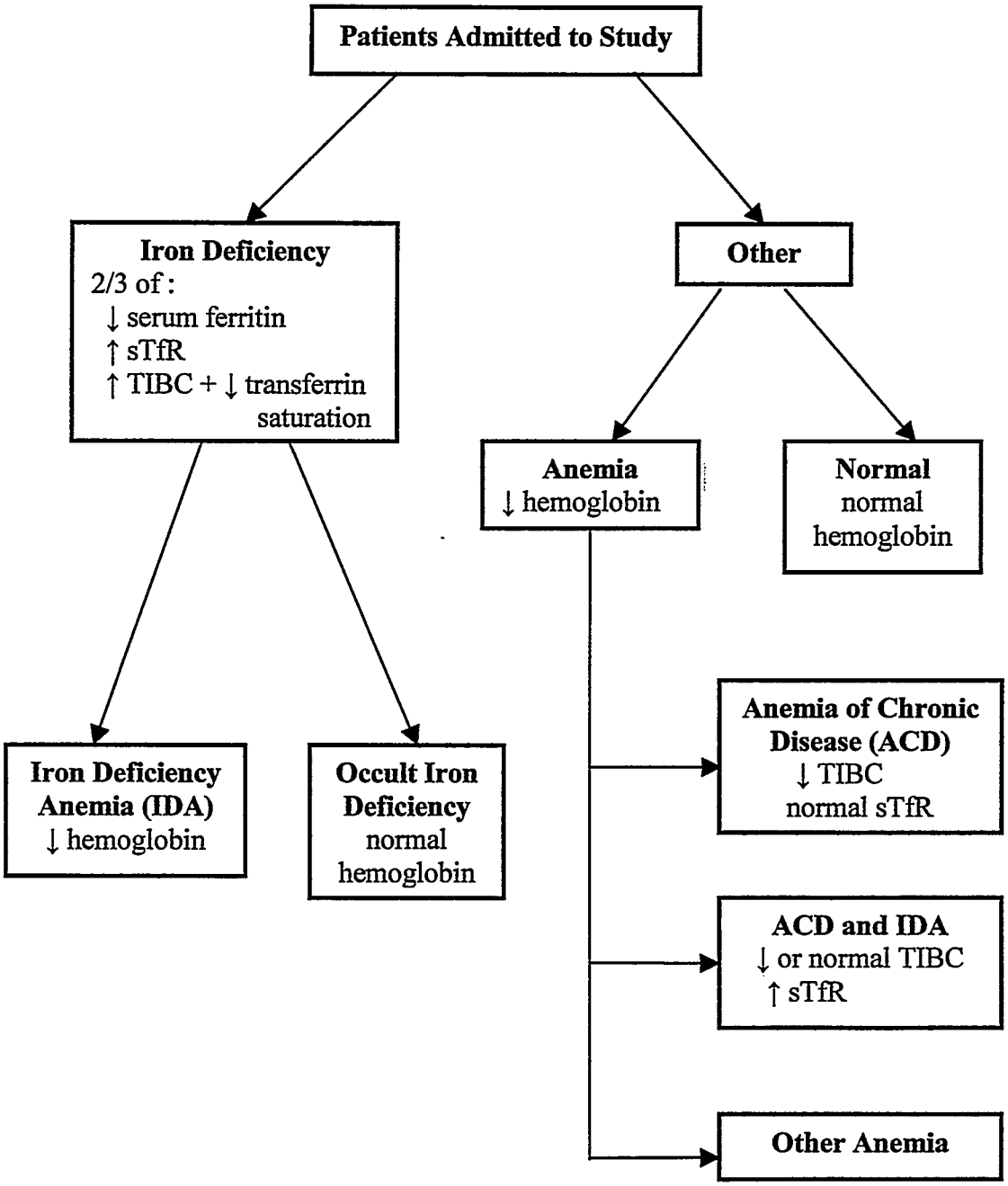

Figure 3.1. Classification of Iron Status

3.4 Medical Information

Medical charts were reviewed in order to document three types of information. First, the presence of known risk factors for stroke such as age, gender, race, hypertension, smoking status, carotid stenosis, atrial fibrillation, previous TIA, diabetes mellitus, family history, hyperlipidemia, and cardiac disease, was noted. Hypertensive patients were considered to be those with a known history of hypertension, hypertensive medication usage, or subsequent discharge from hospital on hypertensive medications. Patients with serum cholesterol >5.2 mmol/L, documented history of hyperlipidemia, or lipid lowering medication usage were considered to have hyperlipidemia. Cardiac disease included conditions such as coronary artery disease, angina, myocardial infarction, cardiomyopathy, congestive heart failure, mitral stenosis/calcification, or left ventricular hypertrophy. Family history of stroke included individuals with stroke occurrence in one or more birth parents. The second type of information included potential causes of iron deficiency anemia (i.e. gastrointestinal blood loss, non-steroidal anti-inflammatory drugs (NSAID) or anticoagulant use, genitourinary disease), and the third type included the existence of possible causes of anemia of chronic disease (i.e. chronic inflammation, liver disease, infection, or neoplasm).

3.5 Dietary Data

Dietary intake was assessed by the CLUE II Health Habits and History Questionnaire (Brief 89 version). This is a 63-item food frequency questionnaire (Appendix B). The patient and/or surrogate source were interviewed regarding usual dietary intake, and data was obtained for 58 patients. Pictures of food portions were used to facilitate responses (Appendix B). These questionnaires were scanned and analyzed by Block Dietary Data Systems (Berkeley, California), and final reports containing nutrient data for individual patients were forwarded. The nutrient database used by Block Dietary Data Systems is comprised of data from the United States Department of Agriculture and values from the literature (T. Block, personal communication). Dietary heme iron intake was determined as follows: a) if an iron containing food came entirely from an animal source, all of the iron was considered heme iron, and b) for mixed dishes,

the heme/non-heme distribution was considered to be proportional to the animal/plant distribution of the dish (T. Block, personal communication).

3.6 Statistics

Statistics were performed using SPSS 10.0.5 for Windows (release 27 Nov. 1999). A cut-off of $p<0.05$ was utilized for all statistics.

In order to describe the characteristics and risk factor profile of subjects enrolled in the study, frequencies and percentages were calculated for the following factors: age groupings, gender, race, smoking status, history of previous TIA, family history of stroke (i.e. birth parent), and presence of carotid stenosis, atrial fibrillation, diabetes mellitus, elevated cholesterol, or cardiac disease. Frequencies and percentages for admitting diagnosis (stroke versus TIA) were also determined.

After patients into were classified into iron status groupings (Figure 3.1), frequencies and percentages were determined for each group. Information that had been collected from the medical charts regarding potential causes of IDA such as use of non-steroidal anti-inflammatory drugs, anticoagulants, gastrointestinal disease, or genitourinary disease was tabulated in 2 x 2 table form. Fisher's Exact Test (non-parametric) was used to determine statistically significant differences between the two groups of IDA (i.e. present or absent).

Descriptive statistics and testing for gender differences were performed for both laboratory and dietary data. Laboratory indices included: sTfR, total iron binding capacity (TIBC), transferrin saturation, and hemoglobin. Dietary variables included: energy, protein, dietary iron, supplemental iron, total iron (diet + supplements), dietary vitamin C, supplemental vitamin C, total vitamin C (diet + supplements), fiber, heme iron, and alcohol. Descriptive statistics included mean with standard deviation and median for males and females, and mean, standard deviation, median, and minimum/maximum values for both genders together. The t-test statistic (parametric) or Mann-Whitney U test (non-parametric) were used to test for differences between genders.

IDA prevalence in the study group was compared with NHANES III (Looker et al., 1997) results in two ways. The first was by comparison with published data. IDA prevalence obtained for this study was compared with published prevalence figures from NHANES III for free-living elderly subjects (n=3,067) (Looker et al., 1997). As the latter data were only specified for the age groups of 50-69 years and ≥ 70 years, IDA prevalence was calculated for those subjects from this study who were ≥ 70 years (n=81) in order to perform this comparison. The Single Sample Proportion Test (Altman, 1991) was used to compare the two prevalence figures.

The second comparison was a case-control analysis. IDA prevalence from this study was compared with 94 age and gender-matched, non-Hispanic controls from areas in the Midwestern U.S.A with populations <1,000,000, who did not have a previous history of stroke. These controls were selected from the NHANES III database (U.S. Department of Health and Human Services, 1996). Fisher's exact test (non-parametric) was used to test for a difference in IDA prevalence between the two groups.

For diet questionnaire respondents, information was collected regarding type of respondent and vitamin supplement use. Frequencies and percentage of total were determined for categories for diet questionnaire respondent type, iron supplement use, and vitamin C supplement use. For patients who reported taking multivitamin supplements, the iron content was assumed to be 5 mg/tablet which approximates the iron content of a "senior's type" of multivitamin.

Mean individual daily intake of key nutrients (energy, protein, dietary iron, total iron, vitamin C, and total vitamin C) were compared with current nutrient recommendations. The prevalence of inadequacy was calculated by percentage of individuals with mean nutrient intakes below the mean requirement divided by the total number of respondents in that age and gender category. Energy and protein intakes were compared with the 1990 Nutrition Recommendations (RNIs) (Health and Welfare Canada, 1990), and protein requirements were determined as the RNI minus 2 standard deviations. Intakes of dietary iron, total iron, dietary vitamin C, and total vitamin C were compared with estimated average requirement (EAR) for iron and vitamin C (Institute of Medicine, 2001; Institute of Medicine, 2000).

Mean nutrient intakes for diet questionnaire respondents from this study were also compared with intakes for individuals 65-74 years of age from the Saskatchewan Nutrition Survey released in 2000. Mean intakes for subjects in this study were calculated for both those 65-74 years of age, and for all respondents.

Associations between dietary variables and outcome measures of iron status were examined through multivariate analysis. Those dietary variables that would be expected to influence iron status were considered for analysis. The following variables were converted into categorical variables to facilitate analysis and interpretation: iron supplement (0, 5+ mg/day), vitamin C supplement (<45 mg/d, ≥45 mg/d), and total vitamin C (0-110 mg/d, >110-170 mg/d, >170-250 mg/d, >250 mg/d). Initial bivariate analysis was used to examine the association between single predictor variables (age, BMI, gender, kilocalories, protein, dietary iron, supplemental iron, total iron, dietary vitamin C, supplemental vitamin C, total vitamin C, fiber, heme iron, and alcohol), and outcome measures of iron status (sTfR, TIBC, hemoglobin, and IDA [present/absent]) (Appendix C). During bivariate analysis linear regression was used to examine associations of single predictor variables with the continuous outcome variables TfR, hemoglobin, TIBC; logistic regression was used to examine associations between single predictor variables and the categorical outcome variable iron deficiency anemia (present/absent). Those variables that were found to be significant predictors at $p<0.20$ were considered in subsequent model building: a) supplemental iron and total vitamin C were included for sTfR; b) BMI, gender, supplemental iron, dietary vitamin C, and alcohol were included for hemoglobin; c) BMI, supplemental iron, total iron, supplemental vitamin C, total vitamin C, and heme iron were included for TIBC; and d) protein, dietary vitamin C, and heme iron were included for IDA. For multivariate linear regression analysis, forward stepwise selection procedure was used and stepwise criteria for entry were set as follows: probability of F to enter ≤0.20, probability of F to remove ≥ 0.30. For logistic regression analysis the relevant variables derived from bivariate analysis were protein, dietary vitamin C, and heme iron. These variables were entered into the model through purposeful selection (i.e. investigator controlled).

4. Results

4.1 Characteristics and Risk Factor Profile of Subjects

The overall rate of consent in this study was 92%. Frequencies and percentages of age, gender, and race groupings are included in Table 4.1. The majority of individuals were ≥ 75 years of age. There were an equal number of men and women enrolled in the study. Race was primarily white; 3 individuals were of Filipino, Cantonese, or First Nations background.

Table 4.1. Demographic characteristics of subjects (n=94).

Characteristic		Frequency	Percent
Age (years)	65-74	35	37.2
	75+	59	62.8
Gender	Male	47	50.0
	Female	47	50.0
Race	White	91	96.8
	Other[1]	3	3.2

[1] Includes Filipino, Cantonese, and First Nations individuals.

Table 4.2 describes the risk factor profile of this sample of stroke and TIA patients. Many subjects (65 %) have hypertension. A small percentage of subjects (19%) were smokers in the year preceding this hospital admission. Of those patients who underwent carotid ultrasound or angiogram while in hospital, 63% were found to have some degree of carotid stenosis. A small percentage of patients (19%) had documented atrial fibrillation. History of previous TIA was documented in 21% of cases. A substantial portion (30%) of patients had diabetes mellitus. Family history of stroke (one or more birth parents) was documented in 4% of patients. Elevated total serum cholesterol was noted in 55% of patients, however cholesterol information was not available in 36% of cases. Presence of cardiovascular disease was noted in 51% of patients.

Table 4.2. Risk factor profile of subjects.

Risk factor		Total N (missing)	Frequency	Valid Percent
Hypertension[1]		94 (0)		
	Yes		61	64.9
	No		33	35.1
Smoking Status		94 (0)		
	Yes		18	19.1
	No		76	80.9
Carotid Stenosis		71 (23)		
	Yes		26	36.6
	No		45	63.4
Atrial Fibrillation		94 (0)		
	Yes		18	19.1
	No		76	80.9
Previous TIA		94(0)		
	Yes		20	21.3
	No		74	78.7
Diabetes Mellitus		94 (0)		
	Yes		28	29.8
	No		66	70.2
Family History of Stroke		94 (0)		
	Yes		4	4.3
	No		90	95.7
Elevated Cholesterol[2]		60 (34)		
	Yes		33	55.0
	No		27	45.0
Cardiac Disease[3]		94 (0)		
	Yes		48	51.1
	No		46	48.9

[1] Patients with documented history of hypertension, hypertensive medication usage, or subsequent discharge from hospital on hypertensive medications.

[2] Patients with serum cholesterol >5.2 mmol/L, documented history of hyperlipidemia, or lipid lowering medication usage.

[3] Patients with documented history of coronary artery disease, angina, myocardial infarction, cardiomyopathy, congestive heart failure, mitral stenosis/calcification, or left ventricular hypertrophy.

The admitting diagnosis of subjects (stroke vs. TIA) is presented in Table 4.3. The majority (84%) presented with ischemic stroke; only 16% presented with TIA. Diagnosis is not significantly different between genders (X^2=0.078, p>0.05).

Table 4.3. Patient diagnosis.

	Females	Males	Total (%)
N	47	47	94 (100.0)
Diagnosis			
Stroke	39	40	79 (84.0)
TIA	8	7	15 (16.0)

4.2 Iron Status of Subjects

The iron status of stroke and TIA patients is summarized in Table 4.4. The prevalence of iron deficiency anemia is 6.4% in this group (n=6), comprised of patients with IDA (n=5) and those with anemia of chronic disease coexisting with IDA (n=1). Figure 4.1 reviews the classification scheme used to obtain these groupings. An equally high number (6.4% of subjects) were grouped into the "other anemia" category.

Table 4.4. Iron status of subjects.

	Females	Males	Total (% of total)
N	47	47	94 (100.0)
Iron deficiency anemia (IDA)	3	2	**5 (5.3)**
Iron deficiency	1	1	2 (2.1)
Anemia of chronic disease (ACD)	1	2	3 (3.2)
Combination (ACD + IDA)	0	1	**1 (1.1)**
Other Anemia	0	6	6 (6.4)
Normal	42	35	77 (81.9)

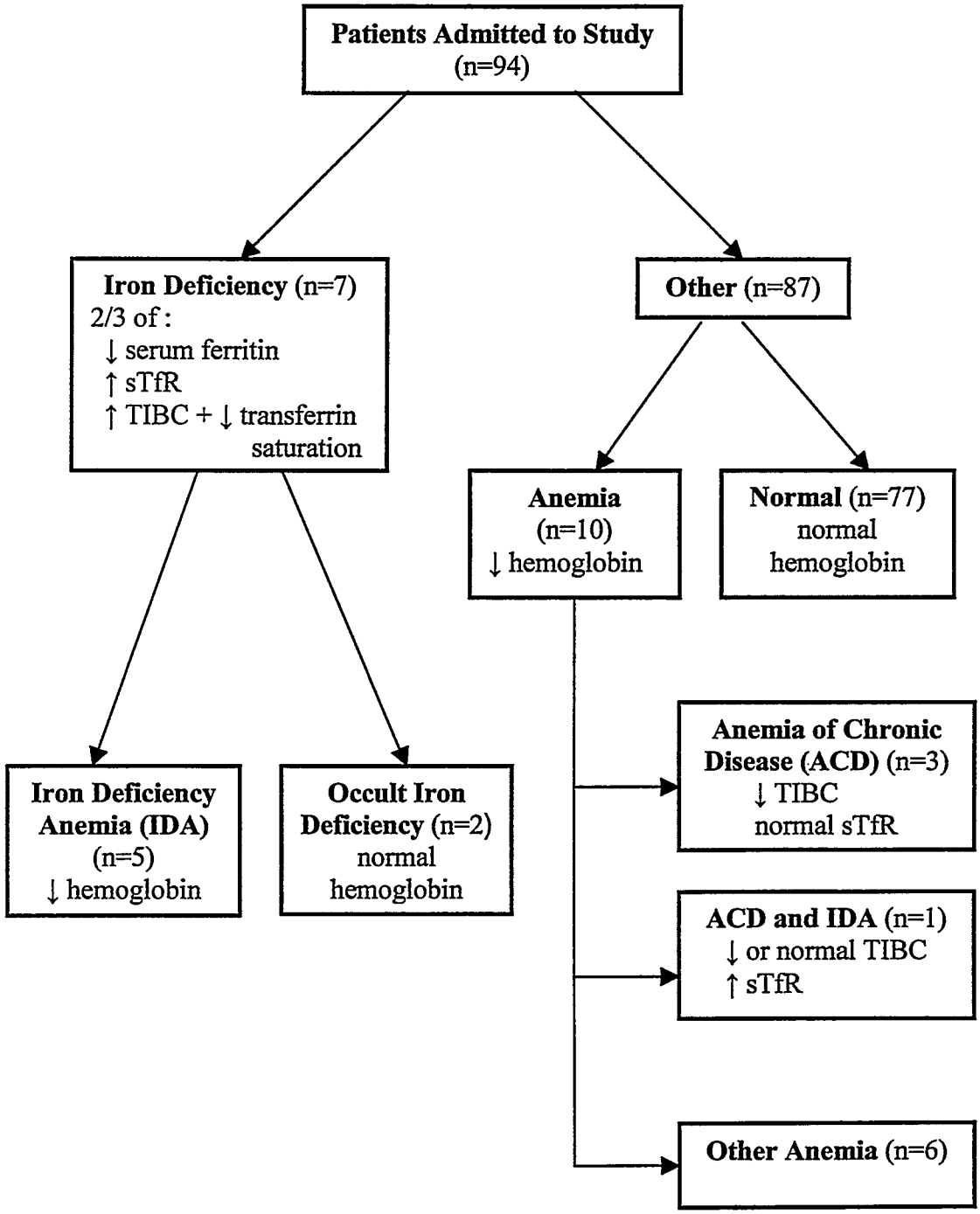

Figure 4.1. Results of Classification of Iron Status

Information regarding other possible causes of IDA such as medication usage and coexisting medical concerns are found in Table 4.5. Many patients (n=50) were using non-steroidal anti-inflammatory drugs, and 44 patients were taking anticoagulant medications. Ten patients had documented gastrointestinal disease, and 10 patients had genitourinary disease. None of these variables were significantly associated with the presence of iron deficiency anemia using Fisher's Exact Test.

Table 4.5. Potential causes of iron deficiency anemia.

		Iron Deficiency Anemia			Fisher's Exact Test[1]
		Absent	Present	Total	p-value
NSAID[2] use	No	40	4	44	
	Yes	48	2	50	0.414
Anticoagulant[3] use					
	No	46	4	50	
	Yes	42	2	44	0.681
GI Disease[4]					
	No	78	6	84	
	Yes	10	0	10	1.000
Genitourinary Disease[5]					
	No	79	5	84	
	Yes	9	1	10	0.501

Abbreviations: NSAID, non-steroidal inflammatory drug; GI, gastrointestinal
[1] Fisher's exact test due to cells with count <5.
[2] Includes ASA, celocoxib, diclofenac, ibuprofen, nabumetone.
[3] Includes ASA, warfarin, heparin, diclofenac sodium, clopidogrel bisulfate, ticlopidine.
[4] Includes peptic ulcer disease, gastroesophageal reflux, hemorrhoids, colon cancer, diverticular disease.
[5] Includes hematuria, urinary tract infection, urolithiasis, and hyperplasia, cancer or recent surgery of prostate.

Descriptive statistics for laboratory data and differences between genders are contained within Table 4.6. Significant differences in ferritin ($p<0.05$), and highly significant differences in TIBC and hemoglobin ($p<0.01$) are apparent between genders. Although mean and median values fall within the reference range for serum transferrin receptor, a high number (n=27) or 30% of individuals have values below the lower cut-point of 2.9 μg/mL (Figure 4.2).

Table 4.6. Gender effects on haematological parameters.

Variable (reference range)	Female Valid N	Female Mean (SD)	Female Median	Male Valid N	Male Mean (SD)	Male Median	Both Genders Valid N	Both Genders Mean (SD)	Both Genders Median	Minimum, Maximum	Difference Test Statistic	Difference P-value
Serum Transferrin Receptor (2.9-8.3 µg/ml)	45	4.8 (2.6)	4.0	46	4.9 (3.6)	3.9	91	4.8 (3.1)	4.0	1.0, 22.5	1007.5[2]	0.83
Serum Ferritin (Female 20-120µg/L, Male 20-400 µg/L)	47	90 (71)	83	47	210 (402)	97	94	150 (294)	90	6, 2669	817[2]	**0.048**
Total Iron Binding Capacity Female 34-51 µmol/L, Male 41-63 µmol/L)	47	56 (11)	57	47	50 (11)	52	94	53 (11)	53	19, 82	2.8[1]	**0.007**
Transferrin Saturation (Female 14-51%, Male 25-56%)	47	21 (12)	19	47	25 (13)	23	94	23 (12)	21	3, 67	915.5[2]	0.14
Hemoglobin (Female 110-160 g/L, Male 135-180 g/L)	47	132 (15)	133	47	147 (14)	147	94	139 (16)	138	83, 175	-4.9[1]	**p<0.001**

[1] T-test for independent samples. [2]Mann-Whitney test for non-parametric data.

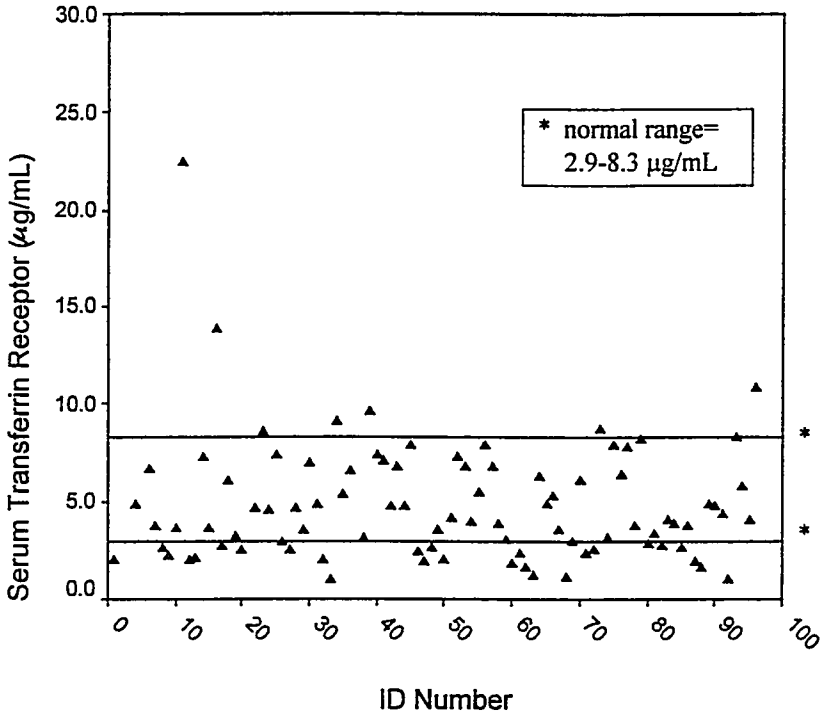

Figure 4.2. Distribution of serum transferrin receptor values.

4.3 Prevalence of IDA

4.31 Comparison of IDA Prevalence with Published Data

Overall prevalence of IDA is 6.4% in this study. Table 4.7 highlights comparison data with NHANES III prevalence figures for gender-specific age groups ≥ 70 years. The prevalence of IDA in this study is significantly higher than that found in NHANES III for both men and women ≥ 70 years.

Table 4.7. Prevalence of IDA in males and females ≥ 70 years: comparison with NHANES III data.

Gender	Iron and Stroke Study	NHANES III	p-value[1]
Male	8.1% (n=37)	2%	**0.038**
Female	6.8% (n=44)	1%	**0.010**

[1]Single-sample proportion test (non-parametric)

4.32 Case-Control Comparison.

The 6.4% prevalence of IDA in this group of stroke and TIA subjects is higher than the 1.1% prevalence of IDA found in an equal number (n=94) of age and gender-matched, non-Hispanic white controls from the NHANES III database (Table 4.8). This difference is not statistically significant using Fisher's exact test (p=0.118).

Table 4.8. Prevalence of IDA: stroke and TIA study subjects versus age and gender-matched, non-Hispanic white controls from NHANES III[1].

	IDA present Frequency (%)	IDA absent Frequency (%)	Fisher's Exact Test[2] (p-value)
Iron and stroke/TIA study	6 (6.4%)	88 (93.6%)	0.118
NHANES III	1 (1.1%)	93 (98.9%)	

[1]n=94
[2]non-parametric

4.4 Dietary Data

Dietary information was available for 58 patients (62% of all patients), which included 26 females and 32 men. Reasons for not administering questionnaires to 36 subjects included: impaired medical status without suitable surrogate information source (n=21), language barrier without surrogate information source (n=3), early hospital discharge with unsuccessful follow-up (n=8), subject declined to participate (n=3), and subject resided in long term care and was not involved in meal preparation (n=1). Characteristics of questionnaire responders were compared with those of non-responders. There was no significant difference between the two groups for the following categorical variables using Chi-square analysis: gender, diagnosis (stroke or TIA), iron deficiency anemia, and iron status. There was no significance difference ($p>0.05$) using independent samples T-test to compare the two groups for the following continuous variables: age, serum transferrin receptor, ferritin, total iron binding capacity, and hemoglobin. Significant differences in body weight and transferrin saturation were apparent between the two groups (Table 4.9).

Table 4.9. Differences in transferrin saturation and body weight between diet questionnaire respondents and non-respondents.

	Mean (Standard Deviation) Non-Respondents	Respondents	Total	T-test statistic	p-value
Transferrin Saturation (%)	20 (12)	25 (13)	94	-2.2	0.029
N	36	58			
Body Weight (kg)	67.6 (15.4)	77.9 (18.0)	89	-2.7	0.009
N	31	58			

Information regarding questionnaire respondent type is included in Table 4.10. Most of the questionnaires were interviewer-administered; due to difficult circumstances (i.e. early hospital discharge) three were self-administered. Respondent type included the patient (n=35), patient with assistance of a relative (n=9), and surrogate source (n=10).

Table 4.10. Diet questionnaire respondent type

	Female (frequency)	Male (frequency)	Total (% of total)
Questionnaire respondent:			
Patient	20	15	35 (60.3)
Patient with assistance	2	7	9 (15.5)
Surrogate source	4	10	14 (24.1)
All categories	26	32	58

A total of 44 subjects (76%) used at least one vitamin/mineral supplement. Data detailing iron and vitamin C supplement use are contained in Table 4.11. Fourteen patients (24%) were taking iron supplements in the form of a multivitamin. Most were taking only 5 mg of iron per day, and one patient consumed 2 multivitamins per day (10 mg iron). None of these patients were taking additional iron supplements. Of all 94 subjects studied, there was documentation of only one person taking an iron supplement of 300 mg ferrous sulfate/day; this person did not complete a diet questionnaire. Thirty of the 58 patients (51.7%) were taking supplemental vitamin C; most of these (83.3%) took up to 500 mg/day, and one person took 2000 mg/d.

Table 4.12 contains descriptive statistics for dietary intake data. Mean intakes of energy, protein, dietary iron, total vitamin C, heme iron, and alcohol were significantly higher for males versus females.

A comparison of dietary intakes of questionnaire respondents versus the most current available nutrient recommendations is contained within Table 4.13. Prevalence of inadequacy is featured for all nutrients. Many subjects reported inadequate energy intakes, with the highest prevalence of inadequacy (86%) found within the female, 65-74 years subgroup. Subsequent linear regression analysis of energy intake (kcal/kg) with body mass index (BMI)[1], a measure of body composition, reveals a significant inverse relationship between the two variables (β= -0.284, p=0.001). A higher prevalence of protein inadequacy (22%) was noted with males versus females (15%); a significant inverse relationship was also noted between protein intake (g/kg) and body mass index (β=-6.448, $p<0.01$). For total iron intake and total vitamin C intake, a higher percentage of females (12% and 19% respectively) were found to have inadequate intakes than males (6% and 3% respectively).

Table 4.11. Iron and Vitamin C supplement use.

	Female (frequency)	Male (frequency)	Total (% of total)
Iron Supplements			
5 mg/d	9	4	13 (92.9)
10 mg/d	0	1	1 (7.9)
all categories	9	5	14
Vitamin C Supplements			
0-500 mg/d	11	14	25 (83.3)
501-1000 mg/d	1	3	4 (13.3)
1001-1500 mg/d	0	0	0
1501-2000 mg/d	0	1	1 (3.3)
all categories	12	18	30

[1] BMI = weight (kg) ÷ [height (m)]^2

Table 4.12. Mean dietary intake of iron-related nutrients by gender.

	Female[1]		Male[2]		Both Genders[3]			Difference in Intake	
	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Minimum, Maximum	Test Statistic	P-value
Energy (kcal)	1717.9 (470.1)	1718.4	2062.4 (541.7)	2026.2	1908.0 (535.1)	1812.8	898.7, 3934.7	-2.6[4]	**0.013**
Protein (g)	63.6 (17.8)	62.1	81.8 (25.9)	82.4	73.6 (24.2)	72.1	32.4, 177.5	-3.0[4]	**0.004**
Dietary Iron (mg)	11.1 (3.7)	10.6	13.5 (4.0)	13.3	12.4 (4.0)	12.6	4.3, 23.6	-2.3[4]	**0.025**
Iron Supplement (mg)	1.7 (2.4)	0	0.9 (2.4)	0	1.3 (2.4)	0	0, 10.0	341.5[5]	0.12
Total Iron (mg)	12.9 (4.4)	12.1	14.4 (4.5)	13.7	13.7 (4.5)	12.8	5.2, 23.6	336.5[5]	0.21
Dietary Vitamin C (mg)	110.3 (57.9)	108.2	116.1 (51.1)	104.3	113.6 (53.9)	105.4	17.2, 249.9	-0.4[4]	0.69
Vitamin C Supplement (mg)	84.6 (211.8)	0	238.5 (417.3)	60	169.5 (346.9)	44.3	0, 2000.0	329[5]	0.15
Total Vitamin C	194.9 (216.8)	136.3	354.6 (423.1)	200.2	283.0 (352.7)	171.7	17.2, 2189.2	291[5]	**0.05**
Fiber	14.7 (6.4)	14.0	16.1 (4.9)	16.0	15.5 (5.6)	14.5	4.6, 28.5	-1.0[4]	0.34
Heme Fe (mg)	2.5 (0.8)	2.5	4.0 (1.8)	3.8	3.3 (1.6)	3.0	1.0, 10.8	162.5[5]	**p<0.001**
Alcohol (g)	1.7 (4.5)	0	11.9 (21.4)	0.5	7.3 (16.9)	0	0, 95.1	272[5]	**0.009**

[1]N=26. [2]N=32. [3]N=58. [4]T-test for independent samples. [5]Mann-Whitney test for non-parametric data.

Table 4.13. Mean daily intakes of iron-related nutrients and inadequacy of intakes compared with mean requirement levels.

	Iron and Stroke Study		Mean Requirement Level	
Nutrient	Males (% Inadequacy[1])	Females (% Inadequacy[1])	Males	Females
N	32	26		
Energy (kcal/kg)				
65-74 years	24.9 (68.8)	24.6 (85.7)	31[2]	29[2]
75+ years	24.5 (75.0)	26.4 (31.6)	29[2]	23[2]
All ages	(71.9)	(46.2)		
Protein (g/kg)	0.98 (21.9)	0.96 (15.3)	0.70[2, 3]	0.69[2, 3]
Diet Iron (mg)	13.5 (2.5)	11.1 (5.8)	6[4]	5[4]
Total Iron (mg) (including supplements)	14.4 (2.0)	12.9 (2.4)	6[4]	5[4]
Vitamin C (mg)	116.1(21.9)	110.3(23.1)	75[4]	60[4]
Total Vitamin C (mg) (including supplements)	354.6(3.1)	194.9(19.2)	75[4]	60[4]

n=58

[1] "Probability of Inadequacy" for iron was calculated by dividing the total sum of the probabilities of inadequate intake for each subject, by the number of patients in each gender category. "Percentage of Inadequacy" for energy, protein and vitamin C was calculated by dividing the number of patients with intakes less than the mean requirement level by the total number of patients in each gender category.

[2] Based on 1990 Canadian Recommended Nutrient Intake (Health and Welfare Canada, 1990)

[3] Mean requirement for protein was calculated using recommended nutrient intake - 2 SD.

[4] Based on Dietary Reference Intakes: Estimated Average Requirement for vitamin C (Institute of Medicine, 2000); Estimated Average Requirement for iron (Institute of Medicine, 2001)

A comparison of mean nutrient intakes of diet questionnaire respondents with results from the Saskatchewan Nutrition Survey is found in Table 4.14. In a direct comparison of the 65 to 74 year age group, little difference was apparent in the mean daily intakes of nutrients related to iron status. A higher fat intake (as a percentage of total energy) was found in our study. A comparison of daily intake for all nutrients in the two studies is shown in Appendix D.

Table 4.14. Comparison of mean daily nutrient intakes: Iron and Stroke/TIA Study subjects versus Saskatchewan Nutrition Survey[1].

	Saskatchewan Nutrition Survey		Iron and Stroke Study	
Nutrients	Males	Females	Males (65-74 yrs)	Females (65-74 yrs)
Valid N	N/A	N/A	16	7
Energy (kcal)	2143	1447	2112	1685
Protein (g)	87	59.2	83.2	60.2
Iron (mg)	15.2	10.7	13.1	10.6
Vitamin C (mg)	111.1	99.7	105.6	111.2
Dietary Fibre (g)	18.2	15	14.3	15.2
Total Fat (%)	32.8	31.1	38.4	36.9
Protein (%)	16	16	15.5	14.3
Carbohydrate (%)	49.7	52	42.1	48.3
Alcohol (%)	1.6	0.8	5.8	0.4

[1] Data from subjects 65-74 years (Saskatchewan Nutrition Survey, 2000).

4.5. Multiple Regression Analysis: Associations between Dietary Intake and Iron Status

The results of the analysis of specific dietary variables as possible predictors of iron status are shown in Tables 4.15 and 4.16. For sTfR, the variables that were selected from bivariate analysis for model building were supplemental iron and total vitamin C. As a result of this analysis, supplemental iron was the only remaining predictor of sTfR, and 5.4% of the variation in sTfR could be attributed to iron supplement use (adjusted R^2=0.054); this was not however a significant relationship as

p>0.05. For hemoglobin, variables that were selected from bivariate analysis for model building included BMI, gender, supplemental iron, dietary vitamin C, and alcohol. As a result of this analysis, gender was the only predictor of hemoglobin, and 26% of the variation in hemoglobin could be attributed to gender; this was a highly significant linear relationship as p<0.01. For TIBC, the variables that were selected from bivariate analysis included BMI, supplemental iron, total iron, supplemental vitamin C, total vitamin C, and dietary heme iron. Supplemental iron and heme iron intake were found to be negative predictors of TIBC; 13.5% of the variation in TIBC could be attributed to the combination of these two variables, and there was a significant inverse relationship between TIBC and both supplemental iron and heme iron intake, as p<0.05 for both variables.

Table 4.15. Multiple linear regression models of iron status: results of forward stepwise selection procedure to determine predictors of iron status.

Variable	β Coefficient (95% Confidence Interval)	SE	T	p-value
Model[1]: sTfR = constant + supplemental iron				
Constant	4.191 (3.239, 5.143)	0.475	8.826	<0.01
Iron supplement	1.727 (–0.249, 3.703)	0.986	1.752	0.085
Adjusted R^2=0.054				
Model[2]: hemoglobin = constant + gender				
Constant	131.808 (126.478, 137.137)	2.661	49.542	<0.01
Gender	16.442 (9.267, 23.618)	3.582	4.590	<0.01
Adjusted R^2 = 0.260				
Model[2]: TIBC[3] = constant + supplemental iron + heme iron				
Constant	60.174 (54.420, 65.928)	2.871	20.959	<0.01
Iron supplement	-7.331 (-13.066, -1.597)	2.861	-2.562	0.013
Heme Iron	-1.606 (-3.122, -0.089)	0.757	-2.121	0.038
Adjusted R^2 = 0.135				

[1] N=56
[2] N=58
[3] TIBC: total iron binding capacity

Two of 58 diet questionnaire respondents had IDA; Table 4.16 contains results from multivariate logistic regression analysis for this outcome measure. Based on bivariate analysis, protein, dietary vitamin C and heme iron were included in multivariate analysis. Dietary vitamin C intake was the only predictor variable that resulted from model building, and this inverse relationship was of borderline significance (p=0.064).

Table 4.16. Multiple logistic regression model of iron deficiency anemia: results of forward elimination procedure.

Variable	β Coefficient	SE	Wald Statistic	Odds Ratio (95% Confidence Interval)	p-value
Model[1]: logit IDA = constant + dietary vitamin C					
Constant	-7.693	2.978	6.676	0.00	0.010
Dietary vitamin C	0.029	0.015	3.435	1.029 (0.998, 1.061)	0.064

[1] N=58

5. Discussion

The primary focus of this study was to document the prevalence of IDA in a group of elderly stroke and TIA patients, and compare this result with previously published prevalence figures. This study is of particular importance since the elderly comprise the group at highest risk for stroke, and previous research has suggested that there is a relationship between IDA and stroke.

IDA was more prevalent in this group of ischemic stroke patients than in subjects from NHANES III. Caution is required in interpretation of our findings since the methods used to define IDA differ between these two studies. In our study, IDA was described as low hemoglobin with two or more abnormal values for sTfR, transferrin saturation combined with TIBC, or ferritin. sTfR was deliberately selected to serve as a sensitive indicator of iron status that would remain useful in the setting of the anemia of chronic disease in elderly individuals. In contrast, with NHANES III, IDA was defined as low hemoglobin with two or more abnormal values for free erythrocyte protoporphyrin, transferrin saturation, or ferritin (Looker et al., 1997). The stratified, multistage probability design of NHANES III differs substantially from this case-series approach. Furthermore, the reference ranges for serum ferritin, transferrin saturation and hemoglobin differ between these two studies.

The first approach we used was to make a gender specific comparison of IDA prevalence from this study with published prevalence figures from NHANES III for free-living subjects 70 years of age and older (Looker et al., 1997). The prevalence of IDA in the current study was significantly higher for both genders. Unfortunately this comparison does not represent a true difference in IDA between individuals with and without previous stroke as the NHANES III sample included individuals with many different diagnoses. Inevitably an unknown number of these subjects would have also had a previous history of stroke. In order to compare IDA prevalence with published figures, it was necessary to limit our comparison group to those ≥ 70 years of age, which in turn reduced the number of eligible subjects in our study to 81, and reduced

the power of the comparison. The NHANES III prevalence figure also included individuals from many different U.S. states, so factors such as altitude, ethnic characteristics, and lifestyle (including diet) may have differed substantially from our prairie-province based sample.

Our second approach, the case-control comparison, has several advantages over the previous comparison, and the results are likely more meaningful. Access to the NHANES III database (U.S. Department of Health and Human Services, 1996) enabled us to randomly select age and gender-matched, non-Hispanic white controls from the Midwestern U.S.A. who had no previous known history of stroke. These measures ensured that the two groups were more comparable, enabled all study subjects (n=94) to be included in the comparison, and provided more useful information about the difference in IDA prevalence in the setting of incident stroke. A higher prevalence of IDA was found in the stroke and TIA study group versus NHANES III controls (6.4% versus 1.1% respectively), but this difference was not statistically significant. A higher number of subjects may be required for this difference to approach statistical significance. Nevertheless, the trend toward a higher IDA prevalence in the stroke and TIA subjects is intriguing and warrants further investigation.

The prevalence of elevated iron stores in this study (8.5%) was slightly higher than the prevalence of IDA. This difference is not as large as that noted by Fleming et al. (2001) who cited prevalence figures of 12.9% and 1.2% for elevated iron stores and IDA respectively for elderly Framingham Heart Study participants. Interestingly, these authors found that elevated serum ferritin was falsely elevated by chronic disease in only 1% of these subjects. It was not feasible to examine the effects of chronic disease on elevated ferritin in the current study for reasons discussed below.

In order to examine prevalence of IDA, patients were classified into iron status groupings. While we used a detailed classification system that incorporated the sensitive marker, sTfR, some degree of misclassification may have been present. The systematic classification scheme and the approach of using low hemoglobin with two out of three abnormal values for sTfR, serum ferritin, and TIBC combined with transferrin saturation, was implemented to limit the influence of any single variable. It was interesting that a total of 6 individuals were classified into the other anemia group,

a number equal to the total number with IDA, but closer examination of the data for these subjects did not reveal any hematological explanations or strikingly different medication usage or medical conditions. If several borderline laboratory values had been apparent for these patients identified with other anemia, this trend could have been explained by questionable cut-off points for hematological indices; however, this did not appear to be the case. The possibility still exists that problematic reference ranges for any of the laboratory indices used in the classification scheme (i.e. hemoglobin, serum ferritin, TIBC, transferrin saturation, and sTfR) could have led to incorrect classification of patients. The reference ranges in use by the hospital at the time of this study were utilized for all laboratory parameters except sTfR. For hemoglobin, the derivation of appropriate reference ranges is particularly difficult in the elderly, as populations are often not randomly selected, small sample sizes are common, and values may decrease by up to 1.0 g/dL over the age of 65 (Lichtman & Williams, 2001). The hemoglobin values used were based on results obtained from approximately 100 normal subjects in Saskatoon, Sask. in the 1970's, but the age and gender distribution of these subjects is uncertain (A. Saxena, personal communication). For serum ferritin and TIBC, reference ranges were based on values cited by the manufacturers of the laboratory kits with further modifications for local usage as indicated. Although some authors have suggested that higher cut-off levels for serum ferritin should be used to identify iron deficiency in the elderly due to aging and chronic disease effects (Holyoake et al., 1993; Guyatt et al., 1990; Patterson et al., 1985), these recommendations have not been widely implemented in clinical practice and were not used in this study. It possible that some patients with true IDA may have been missed due to the use of stringent criteria for serum ferritin.

The manufacturer's cited reference range that we used for sTfR may not be appropriate for the elderly, since an unusually high number of cases (27/ 91) in this study had values below the reference range. Although low sTfR values have been described with conditions such as renal failure, aplastic anemia, and post bone marrow transplant (Ponka and Lok, 1999), these subjects did not exhibit these medical concerns. Although sTfR is reported to be stable across age in adults (Cook, 1999), little research has been done to identify normal ranges for elderly individuals. In fact, if sTfR values

for normal elderly subjects are lower than the reference range used in the study, the prevalence of IDA has been underestimated. Lower than expected sTfR values might be explained by decreased erythropoiesis in the elderly. Lichtman & Williams (2001) reported that the erythropoietin response in non-anemic elderly is the same as that in younger individuals. Matuso et al. (1995) did not find a difference in erythropoietin levels between middle aged and elderly adults either, but they did find a reduced reticulocyte to log-transformed erythropoietin ratio suggesting an impaired red cell production response in the elderly.

An alternative hypothesis is that TfR expression might decrease following stroke injury, possibly as a protective mechanism to limit iron uptake at the cellular level. Clinically, several studies have demonstrated that sTfR is not affected by acute and chronic inflammation (i.e. rheumatoid arthritis), nonhaematological malignancies, and liver disease (Ferguson et al., 1992; Nagral et al., 1999; Pettersson et al., 1994; Punnonen et al., 1997). Interestingly, there is no published research that describes sTfR levels in stroke or TIA patients. In contrast, at a cellular level the cytokine response to physiological stress does appear to influence TfR. Nitric oxide and hydrogen peroxide, two reactive oxygen species that are formed during the ischemia and reperfusion response to stroke injury, may be involved via binding to the iron regulatory proteins. Preliminary findings suggest that hydrogen peroxide leads to increases in TfR messenger RNA expression, but nitric oxide may lead to either increases or decreases in TfR messenger RNA, depending on the redox species that predominates (Ponka and Lok, 1999). Overall, the physiological significance of these findings is unknown. In reference to this study, it is questionable whether the stress response to localized brain ischemia would exert effects of significant magnitude to ultimately influence TfR mRNA expression and sTfR values, since 75% of TfR is located on erythroid precursor cells (Baynes, 1996). The resulting direction of any possible change in sTfR in response to stroke is also uncertain (i.e. an increase or decrease in sTfR levels). Ultimately, in order to determine whether the apparent trend toward low sTfR is due to advanced age versus a stroke effect, it would be useful to utilize a control group of age and gender-matched controls that have not had a previous stroke.

Other factors that are known to affect serum iron, TIBC, and ferritin might also influence how individual patients were classified according to iron status. The significant gender influence exhibited with serum ferritin, TIBC, and hemoglobin is predictable since reference ranges differ between genders; interestingly, a similar difference was not apparent for transferrin saturation. Serum iron levels fluctuate with meals and the use of non-fasting values may have affected transferrin saturation values (Gibson, 1990). In this study, one blood sample was obtained at the time of hospital admission for each patient. However, day-to-day biological variation is problematic for serum ferritin and iron, and 3 to 10 determinations may be required for accurate results (Borel et al., 1991). In contrast, one determination is considered to be adequate with hemoglobin and sTfR (Borel et al., 1991; Cooper and Zlotkin, 1996). Serum ferritin is not considered to be a good marker of iron stores in conditions such as inflammation, liver disease, neoplasm, and infection, which may lead to false elevations (Borch-Iohnson, 1995). Previous authors (Fleming et al., 2001) have examined these possible confounding effects of ACD by examining criteria such as elevations in C-reactive protein (an acute-phase reactant) ≥6 mg/L, white blood cell counts above or below the reference range, and abnormal liver enzymes. However, since these data were not collected in the current study, it was not possible to examine these effects. It is however reassuring that Fleming et al. (2001) found that the overall effect of these measures on discerning iron status was insignificant. Also, the use of multiple parameters in our classification scheme should have minimized the impact of any such variations in a single variable.

Several medical conditions and medications are known to contribute to iron deficiency anemia in the elderly (Johnson et al., 1994). However, this study failed to reveal any significant associations between IDA and factors such as NSAID or anticoagulant use, gastrointestinal disease or genitourinary disease. Since this information was collected from the medical charts, any problems with incomplete documentation would have affected the accuracy of these findings. Limited power due to a small number of subjects may also have been problematic.

In a comparison with current recommended nutrient intakes, an alarmingly high number of our subjects were found to have apparently inadequate intakes of key

nutrients that could influence iron status. Numerous subjects had inadequate energy intakes, with the highest prevalence of inadequacy (85.7%) apparent in the female, 65-74 years subgroup. Prevalence of inadequacy for protein, iron, and vitamin C was 21.9%, 6.3%, and 3.1% respectively for males, and 15.3%, 11.5%, and 19.2% respectively for females. These figures are considerably higher than those found in the Nutrition Canada National Survey where corresponding prevalence of inadequacy estimates for males were 7.1% (protein), 4.9% (iron), and 4.0% (vitamin C); for women, inadequacy was documented as 14.5%, 8.4%, and 1.9% respectively (Health and Welfare Canada, 1973). Higher prevalence of inadequacy for these nutrients was apparent in our study despite the use of lower cut-points in the Nutrition Canada National Survey (i.e. <0.5 g/kg/d for protein, <6 mg/d for iron, and <10 mg/d for vitamin C) (Health and Welfare Canada, 1973). Research methodology differed between these two studies, since the Nutrition Canada Survey utilized 24-hour food recalls in 1970-72, and subjects were randomly selected, free-living individuals. Part of this discrepancy may also be due to underreporting of dietary intake in these stroke and TIA subjects, since subsequent linear regression analysis of energy intake with BMI revealed a significant inverse relationship between these two variables (i.e. the thinner individuals appeared to be eating more). Despite these findings, it is interesting that there was little difference in reported intakes of key nutrients in our study versus Saskatchewan data collected in 1993-94 for the 65-74 year age group (Saskatchewan Nutrition Survey, 2000). However, research methodology again differed between these two studies.

A large number of subjects in this study used vitamin and/or mineral supplements that could influence iron status. 76% of subjects reported the use of one or more supplements, 24% used iron supplements (in the form of a multivitamin) and 52% reported using vitamin C supplements on a regular basis during the previous year. Most (83%) of vitamin C supplement users took 500 mg or less per day, and no one exceeded 2000 mg/day. These results are similar to those findings for non-Hispanic white elderly (≥70 years) in NHANES III, where vitamin or mineral supplement use during the previous month was 40.8% for men and 56.1% for women (Balluz et al., 2000). Patterson et al. (1998) cautioned that attempts to measure supplement use at one point

in time may overestimate long-term use, but since these stroke and TIA subjects were interviewed about supplement use over the past year, this problem should have been minimized. However, supplement type and corresponding micronutrient content may have changed during this period, and reported frequency of supplement use may not have been consistent throughout the year. Furthermore, since most subjects did not remember the brand of multivitamin used, the iron content of multivitamins was approximated at 5 mg/tablet, which may not have been an accurate representation for all subjects.

Despite the low dietary intake of iron related nutrients, multivariate linear regression and logistic regression analysis revealed few significant associations between these dietary variables and iron status indicators. Comparison of these findings with published literature is difficult since there are few studies that describe associations between diet and iron status in elderly individuals. Hence, research in other adult age groups was also considered. Model building did not reveal any variables significantly associated with sTfR, a parameter that is indicative of functional iron deficiency. Beguin et al. (1997) reported sTfR increases that were significantly associated with a very-low-energy all-protein diet containing inadequate amounts of iron in obese individuals (15-66 years). Another study conducted by Zhu and Haas (1998) found that iron-supplementation (45 mg/day) of 37 marginally iron-deficient women (19-35 years) resulted in a progressive and significant decrease in sTfR. In our study, supplemental iron and heme iron were found to be significant negative predictors of TIBC. This inverse association is expected, since TIBC reflects the amount of iron that transferrin will bind and is found to rise with iron deficiency (Lee, 1999). These findings are similar to those of Milman et al. (1990) who reported significant correlations between dietary iron and TIBC in 92 healthy elderly; food recall was used to determine dietary intake over the previous week. Furthermore, Borch-Iohnson et al. (1990) reported daily iron supplement usage (18-20 mg iron/day) resulted in a significant decrease in TIBC in menstruating women with low iron stores. In this study, no dietary variable emerged as a significant predictor of hemoglobin, although a significant association was apparent with gender. This gender difference is expected since hemoglobin levels are physiologically higher in males than females (Lee et al., 1999). Previous findings are

conflicting: Yearick et al. (1980) found a positive correlation between dietary iron and hemoglobin using 3 day food records, but Payette and Gray-Donald (1991) reported an unexpected inverse association between dietary iron and hemoglobin in elderly men, and a positive relationship between alcohol and hemoglobin in both genders using 7-day food records. Hemoglobin is considered to be a late-stage indicator of iron status, since values drop when iron stores have been depleted and circulating iron levels remain depressed. Therefore, it is possible that any dietary inadequacies in our group of patients may not have been prolonged enough to effect changes in hemoglobin. Dietary vitamin C was the only predictor of IDA, but this relationship was of borderline significance. The fact that a positive association was found between dietary vitamin C and presence of IDA suggests that this relationship was spurious. Vitamin C is a known enhancer of nonheme iron absorption (Baynes and Stipanuk, 2000), and theoretically should be associated with a lower risk of IDA. Overall, the fact that dietary data was obtained for a relatively small number of subjects (i.e. 58 patients) translated into limited power for the model building exercises. Associations may have become significant if a larger number of subjects had been available.

It appears that the dietary data obtained for 58 questionnaire respondents is a good representation of the entire group of subjects. When characteristics of questionnaire responders were compared with non-responders, no significant differences were found for diagnosis, presence of IDA, iron status, age, TfR, ferritin, TIBC, and hemoglobin. The significant difference between the two groups for transferrin saturation cannot be readily explained since no difference was apparent with other laboratory indices. The mean body weight of questionnaire respondents was significantly higher than that of non-respondents, but this difference could be attributed in part to a higher proportion of men in the respondent group versus non-respondent group (55% versus 42%).

Dietary data was obtained directly from the patient in 44 out of 58 cases, but cognitive impairment may have influenced these results. Cognitive impairment may be present in 29% to 51% of stroke patients, depending on the assessment tool used (Martinez-Lage and Hachinksi, 1998). During the course of this study, the decision to pursue dietary information from a patient was dependent on physician and/or nursing opinion regarding a patient's level of cognition. However, the use of an objective

cognitive assessment tool to select appropriate candidates for questioning would have likely ensured more accurate questionnaire responses. Surrogate sources of information were necessary in 14 cases in which the subject was unable to participate. Willett (1998) suggests that agreement between proxy sources and subjects is usually adequate for studies that aim to compare mean intakes between two groups, but may be problematic in situations such as this study where associations between diet and outcome measures are being investigated. The direction of such possible bias is unclear (Willett, 1998). Patient numbers were too small to permit separate analysis of the effect of surrogate sources.

The shortened 63-item food frequency questionnaire was used in this study. This method was selected to provide information about dietary intake over the previous year, and to minimize subject burden associated with lengthy questionnaires. Food frequency questionnaires provide detailed information about diet, and are appropriate for estimating mean nutrient intakes (Lee and Nieman, 1996). The use of visual aids (food photographs) should have improved subject recall, and questionnaire administration by a single interviewer should have reduced variability. However, other factors may have been sources of possible bias. Food frequency questionnaires have been associated with a tendency for subjects to either overestimate or underestimate usual intake (Willett, 1998); a trend toward underreporting dietary intake was apparent in this study. Willett (1998) cited that underreporting is generally greater among women and obese persons, which provides support for our finding of an inverse relationship between BMI and energy intake in this study. Ultimately underreporting may have led to inaccurate low values for the nutrients of interest in this study, and may have contributed to our findings of few significant associations between diet and iron status. The process of obtaining informed consent may have led to bias in diet questionnaire responses since respondent answers may have been influenced by the knowledge that iron was a nutrient of interest. This questionnaire is best suited for white, middle class people and does not include many foods eaten by specific cultural groups (Lee and Nieman, 1996); thus it may have underestimated nutrient intake in some cases. It is difficult to thoroughly review the issues surrounding the determination of individual mean dietary intakes for our subjects, since this process was performed by Block and Associates

(Berkeley, CA). The fact that US databases were the primary sources of nutrient content information for specific foods should not be problematic, since iron fortification practices for products such as flour, pasta and cornmeal are harmonized between these two countries (E. Chao, Health Canada, personal communication). In contrast, the heme iron intake values calculated for our subjects may be of concern. In the data analysis, it was assumed that 100% of iron in meat, poultry and fish was heme iron (T. Block, personal communication), but this assumption differs from previous determinations that suggest considerable variation in heme iron content. For meat, poultry and fish, Monsen (1988) estimated that heme iron comprises 50% of total iron. Schricker et al. (1982) found the relative percentages of heme iron to be 49, 57, and 62% for raw pork, lamb, and beef respectively. Hendricks et al. (1987) reported values of 71% and 38% for beef and chicken respectively, and suggested an overall value of 58% be used to estimate the heme iron content of red meat, poultry and fish. Thus the values derived for heme iron in this study may be inflated compared with previous published data.

There is little agreement in the literature regarding the role of dietary variables in influencing iron status. This may be due in part to difficulties inherent in the relationship between diet and biochemical indicators of iron status. An individual's iron status is believed to directly alter the absorption of dietary iron (Hallberg, 2001). Furthermore, iron absorption is determined by the balance between enhancing and inhibiting factors, and food combinations may vary substantially for individuals and between respondents. These issues, and the possible sources of bias discussed above, may have contributed to the finding of few significant associations between diet and iron status indicators in this study. It is possible that if a larger number of subjects had provided dietary data, bias might have exerted less influence.

It is difficult to determine whether the stroke and TIA patients included in this study are representative of the larger population of these patients, as there is no known available comprehensive data bank that contains risk factor information specific to this population. Non-respondent bias should not have been a concern with the high overall response rate of 92%. A previously published Stroke Data Bank (Foulkes et al., 1988) collected the medical history information for 1273 adult ischemic stroke patients of all

ages in the US between 1983 and 1986, and risk factor prevalence was documented as follows: hypertension, 21%; atrial fibrillation, 4%; diabetes, 8%; and previous TIA, 5%. In contrast, the corresponding percentages for the stroke and TIA in patients enrolled in this study were: hypertension, 65%; atrial fibrillation, 19%; diabetes, 30%; and previous TIA, 21%. All of these risk factors were more common in our elderly group of stroke and TIA patients, possibly due to advanced age. Nevertheless, direct comparison of these numbers is difficult since the population characteristics and research methodology vary considerably, and the criteria used by Stroke Data Bank for defining these risk factors is not known. In the present study, the accuracy of risk factor information may also be limited because all information was derived from documentation found in the medical charts. The ascertainment of complete medical histories may have been problematic in patients presenting with impaired speech or cognition, and/or altered level of consciousness. Any incomplete or unavailable medical histories would adversely impact the accuracy of risk factor information in these subjects.

The relative numbers of stroke and TIA patients enrolled in this study, 79 and 15 respectively is not an accurate reflection of true population incidence. It is quite likely that many TIA and stroke cases may not have sought medical attention; others may have only received medical attention from their family physician. Many cases that presented to Emergency may not have been admitted to hospital and would not have been enrolled in this study. Diagnosis of TIA is also subject to considerable inter-and intraobserver variation, and the use of the arbitrary 24-hour cut-off point to distinguish between stroke and TIA may inevitably lead to misclassification (Warlow et al., 1996). Throughout this study, patients were diagnosed at the discretion of the attending neurologist and no consistent diagnostic criteria were utilized; hence, the approach taken toward defining stroke and TIA might have differed between practitioners. Based on these factors, the relative numbers of incident TIA cases in the general population is likely higher, and that of stroke lower, than reported. In a retrospective review of medical records in Rochester, Minn. the incident rate of TIA was determined to be 41% of stroke incidence (Brown, 1998); in this study, the overall TIA incidence was 19% of stroke incidence.

This is the first prospective attempt toward documenting IDA prevalence in elderly stroke patients, and findings strongly suggest that IDA is more prevalent in this population. The research methodology used in this study is a **case-series design**, defined as "a prevalence survey of a group of individuals with a particular disease, performed at a single point in time" (Fletcher et al., 1988). The limitations inherent in case-series design include (Fletcher, 1988): a) limited value as a means of studying cause and effect relationships, since it describes both purported cause and effect at one point in time, and b) absence of a comparison group. However these preliminary findings will be very valuable as a basis for further research, and a case-control analysis would be the next step toward examining a causative association between IDA and stroke. As part of a case-control analysis, an equal or larger number of age and gender-matched subjects without a previous known history of stroke would be utilized as control subjects; patients admitted to hospital for elective procedures such as non-cerebrovascular related surgery (i.e. orthopedic or ophthalmic) or free-living elderly residing in the community could be utilized as control subjects. Our study has also been useful in determining the sample size that would be required for a future case control study. Based on a power calculation, a minimum of 200 subjects would be required for both case and control groups.

This research has uncovered several other areas for future potential research efforts. Although this study focused on IDA in elderly stroke patients, it is also possible that other forms of anemia might also be implicated as a risk factor for stroke. The equally high prevalence of other anemia in this study (i.e. 6.4%) emphasizes the potential clinical significance of this group. Macrocytic anemia is one intriguing possibility, since the prevalence of vitamin B_{12} deficiency in ill elderly may be as high as 40% (Seiler, 1999). As discussed previously, further research is necessary to determine whether sTfR values are in fact lower in normal elderly individuals, and to determine whether sTfR might be affected as part of the acute-phase response to stroke. Further studies are also needed to investigate possible mechanisms for increased stroke risk in the setting of IDA.

6.0 References

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Appendix A

Ethics Approval

Patient Consent Form

University of Saskatchewan
Advisory Committee on Ethics in Human Experimentation

June 1, 1998

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	EC #
P. Paterson	Pharmacy & Nutrition	98 108

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

Royal University Hospital

CO-INVESTIGATORS

C. Boyle, Medicine
S. Whiting, Pharmacy and Nutrition

SPONSORING AGENCIES

Application of the Ralston Brothers Medical Research Fund

TITLE:

Prevalence of Iron Deficiency Anemia in Acute Stroke Patients: A Case Series

APPROVAL DATE	TERM (YEARS)	AMENDED:	MODIFICATION OF:
June 1, 1998	3		

CERTIFICATION.

The protocol and consent form for the above-named project have been reviewed by the Committee and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

APPROVED.

H.E. Emson MA MD FRCPC
Chair
University Advisory Committee on
Ethics in Human Experimentation

This Certificate of Approval is valid for the above term provided there is no change in the experimental procedures, subject to annual reapproval.

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