BIOCHEMICAL REFERENCE INTERVALS IN GERIATRICS

BIOCHEMICAL REFERENCE INTERVALS IN GERIATRICS: A SYSTEMATIC REVIEW AND EXAMINATION OF THE INFLUENCE OF MORBIDITY ON CREATININE REFERENCE INTERVALS

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TITLE: Biochemical Reference Intervals in Geriatrics: A Systematic Review and Examination of the Influence of Morbidity on Creatinine Reference Intervals

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ABSTRACT

Reference intervals are important estimates used to determine whether an individual is healthy or unhealthy. They are the most widely used decision making tool in medicine and heavily influence doctor's decisions regarding patient care. Despite the abundance of reference interval research in the field of clinical chemistry, age-related reference intervals have yet to be well-established for elderly populations. Many physiological and biochemical changes have been documented to occur with age however limited attempts have been made to quantify these changes. As a result, it is typical in clinical practice to assess geriatric patient data using an adult reference interval. Such practices can result in over-medicalization, unnecessary medical procedures and/or missed diagnoses. This thesis aims to address this gap in literature by summarizing what geriatric reference intervals are available and by investigating how reference intervals are affected by the presence of morbidity, a common characteristic of the elderly.

The first chapter of the thesis introduces the reader to reference intervals, summarizes the current guidelines used in their determination and provides a rationale for the use of age-related reference intervals in geriatrics. Chapter 2 presents a systematic review that summarizes all available reference intervals for populations ≥65 years of age and the methodology used in their determination. Despite extreme variability in methodology, evidence suggests that geriatric reference intervals are significantly different from those of adults for many analytes. Chapter 3 presents a study that evaluates the effect morbidity has on reference intervals. In this study data from the National Health and Nutrition Examination Survey (NHANES) was used to calculate age-specific

reference intervals for creatinine, a marker of kidney function known to increase with age. Findings suggest that the presence of morbidity significantly increases the upper limit for creatinine in elderly populations. Finally, the concluding chapter summarizes the overall findings of the thesis, proposes areas for future research and reinforces the importance of the above findings.

NOTE: This Master's thesis is written in the style of a PhD "sandwich" thesis, with two journal articles. As such some material, especially introductory information, may be repetitive in nature.

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LIST OF ABBREVIATIONS & SYMBOLS

ANOVA	Analysis of Variance
BIRNH	Belgian Interuniversity Research on Nutrition
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CI(s)	Confidence Interval(s)
CLSA	Canadian Longitudinal Study on Ageing
CLSI	Clinical Laboratory Standards Institute
COPD	Chronic Obstructive Pulmonary Disease
CV	Coefficient of Variation
CVD	Cardiovascular Disease
ELISA	Enzyme-Linked Immunosorbent Assay
GC-MS	Gas Chromatography – Mass Spectrometry
GFR	Glomerular Filtration Rate
HBP	High Blood Pressure
HIMS	Health in Men Study
HPLC	High-Performance Liquid Chromatography
IFCC	International Federation of Clinical Chemistry
IDMS	Isotope Dilution Mass Spectrometry
IMUSCE	Italian Multicentre Study on Centenarians (IMUSCE)
IRMA/RIA	Radioimmunoassy
LC-MS	Liquid Chromatography – Mass Spectrometry

MCQ	Medical Conditions Questionnaire
NCCLS	National Committee for Clinical Laboratory Standards
NHANES	National Health and Nutrition Examination Survey
NORIP	Nordic Reference Interval Project
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors study
RI(s)	Reference Interval(s)
SD	Standard Deviation
SHIP	Study of Health in Pomerania
VITA	Vienna Transdanube Aging study
WHO	World Health Organization
kPa	Kilopascals
IU/L	International Units per litre
μ	Mean
μmol/L	Micromoles per litre
μg/L	Microgram per litre
μkat/L	Microkatal per litre
mEq/L	Milliequivalent per litre
mIU/L	Milli International Units per litre
mmol/L	Millimoles per litre
mOsm/kg	Milliosmoles per kilogram
nmol/L	Nanomoles per litre
ng/L	Nanogram per litre

pmol/L	Picomoles per litre
g/L	Gram per litre
SI units	The International System of Units

1 INTRODUCTION

1.1 Reference Intervals

1.1.1 What are They and How are They Used?

Reference intervals or reference ranges are one of the most widely used clinical decision making tools. They can simply be described as a range of test values that are considered to be acceptable for a healthy population (1). Specifically reference intervals (RIs) are estimates that provide the limits within which a test result is considered normal and the cut-off at which the result is considered abnormal (2). Typically the limits are defined by the range of values that 95% of the reference sample falls between. In this case 2.5% of the reference sample will have test values that fall below the lower limit and 2.5% will have values that exceed the upper limit (3).

RIs may be applied to a variety of different tests including anthropomorphic measures, measures of dietary intake and most commonly the measurement of biochemical markers in bodily fluids (4). Usually these values are health associated and used in a diagnostic or treatment process but they can also be used to reflect physiological states such as pregnancy (5). Specifically this thesis will be examining RIs of biochemical tests performed on human blood fractions.

In the practice of laboratory medicine, a measured or observed laboratory test for an individual is compared with a RI for the purpose of making medical decisions (6). If a patient's laboratory value falls outside of the recommended interval, the result is flagged for further examination (1). Thus the use of RIs is known to be a comparative-decision

making process (6). This decision making process often influences physician decisions regarding hospital admittance and discharge, treatment initiation, treatment cessation and treatment monitoring (1).

1.1.2 The History of Reference Intervals

The topic of RIs first began with the advent of automation in the early 1960s (7). Technical and scientific advances expanded the need and desire for clinical laboratory services; introducing new analytes for measurement, sophisticated analytical methods and high throughput instrumentation (8). As such, the need for RIs to be able to interpret lab test results grew stronger.

Around this time the term ‘normal values’ was used to describe what we know as the RI. The term normal was deemed to be too ambiguous of a term as it took on different meanings in various settings. Normal in a statistical sense implies that the observed data follows a normal distribution which is not always the case for biological data (8). The epidemiological definition of normal implies that if a value is not within the normal range it is abnormal, infrequent or atypical. Contrastingly the clinical definition of normal implies that normal is synonymous with healthy or harmless (8). All of these definitions have different implications when calculating and interpreting RIs and for these reasons a Scandinavian research group coined the term ‘reference values’ at the beginning of the 1970s (9).

The discussion of consolidating terminology regarding reference values was well received and sparked the formation of the International Federation of Clinical Chemistry (IFCC) (5). This panel of experts began introducing a series of recommendations for RI

determination that were internationally accepted (10) and they have since joined up with the Clinical and Laboratory Standards Institute (CLSI). Both groups have worked separately and together to provide international guidelines for the process of determining RIs and are in general agreement as to the proper procedures (4). The recommendations of these guidelines aim at instituting a standard protocol that meets the minimum requirements necessary for determination of a reliable RI (6). When calculating RIs it is important to follow these guidelines' recommendations, however it has been recognized by the institutions that there are instances where the requirements are hard to fulfill, particularly in pediatric and elderly populations (11).

1.2 The Current Guidelines and Limitations of Reference Interval Determination

1.2.1 Definitions

The CLSI has provided a flow diagram that helps to define the RI (Figure 1-1). Reference individuals are selected from the general population to form a *reference population* (11). A set of a priori defined inclusion and exclusion criteria are then applied to the *reference population* and the *reference sample* is formed (11). The inclusion and exclusion criteria should ensure that all included individuals are 'apparently healthy'.

The laboratory test of interest is then performed and the results obtained are considered the *reference values.* The culmination of reference values defines the *reference distribution* which is examined using proper statistical methods prior to the calculation of the *reference limits*. The *reference limits* are said to define the *reference interval*. Any *observed value* from the general population can then be compared to the

reference interval. If the *observed value* falls within the *reference interval* it is considered to be representative of the *reference sample* or 'normal'. Any *observed value* that falls outside of the *reference interval* is not considered to be representative of the 'apparently healthy' *reference sample* and is flagged as such.

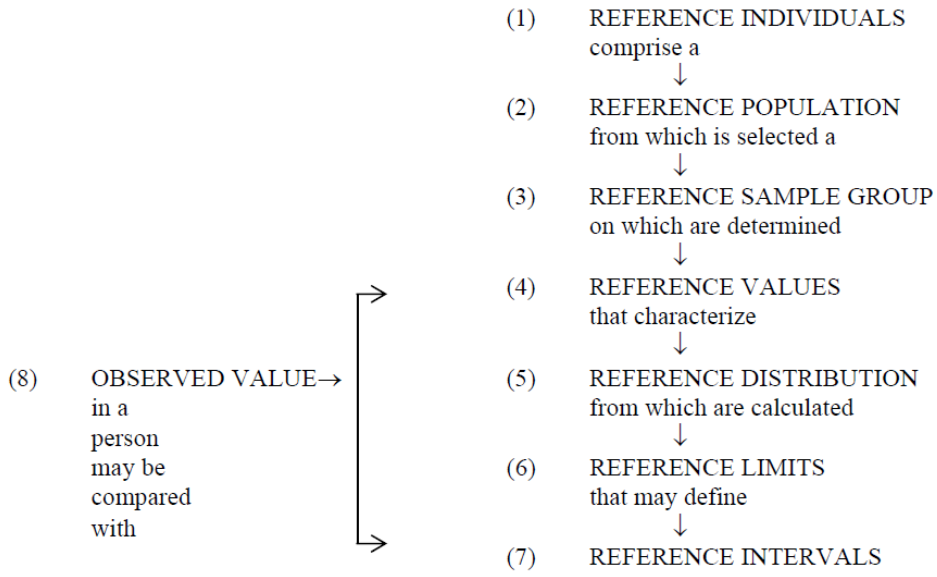

Figure 1-1: Definitional Schematic Used in Reference Interval Determination (11)

1.2.2 Selection of the Reference Sample

Selecting subjects to be part of the reference sample is the first step in RI determination and often the hardest. The goal is to select a 'healthy' sample from the population however this can be very difficult considering 'health' is a relative term that lacks a universal definition (11). The World Health Organization (WHO) defines health as "a state of physical, mental, and social wellbeing and not merely the absence of disease or infirmity" (12). Realistically this definition is impractical for the use of RI determination.

In 1975 the Scandanavian Committee on Reference Values attempted to generate a list of pathological conditions that should exclude a person from being considered 'healthy' (13). However this recommendation was also deemed impractical, especially when applied to older individuals (1). Using data from the National Health and Nutrition Examination Survey (NHANES) Horn and Pesce were able to show that applying this definition of health to an elderly population is not feasible (Figure 1-2). They demonstrated that the prevalence of morbidity increases with age, as expected, and that to obtain a healthy reference sample for persons aged 70-80 one must exclude 9 out of every 10 subjects (1).

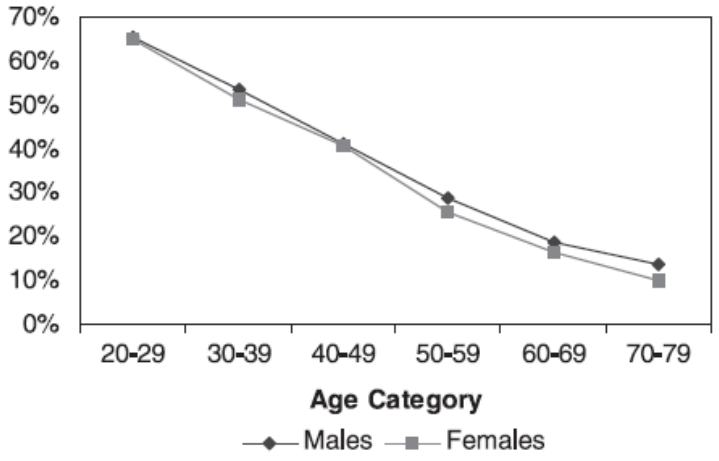

Figure 1-2: Percentage of Subjects in the 'Healthiest' Category (1)

As a result the practice of RI determination has adopted the definition that a 'healthy' reference sample is free of any disease specifically related to the analyte being measured (14-19). When establishing RIs for a new analyte or analytical method the typical protocol is to first develop a list of known conditions and biological factors that affect the analyte concentration of interest. From there a list of exclusion and partitioning

criteria are established and applied to the reference population available (11). For example, to determine the reference interval for hemoglobin it is suggested to exclude individuals with iron deficiency, marked vitamin B_{12} deficiency, inflammation or chronic respiratory disease, tumors and/or genetic abnormalities of hemoglobin synthesis because all are known factors to affect hemoglobin concentration (5). The analyte of interest is then measured in the included persons and used for RI determination. Unfortunately exclusion criteria for a particular analyte are not necessarily the same across all studies due to ambiguity in reporting and purposeful strict or lenient selection (5).

Obtaining large and representative samples for the purposes of RI determination can sometimes be very difficult; particularly for pediatric and geriatric populations. Often it is more feasible, less costly and less time consuming to use previously collected blood test results (14-19). It is common to obtain laboratory test results from clinical laboratory information systems of hospitals or community clinics (11). This sampling technique is only valid assuming that the population is "normal" which is not typical of hospital data, although more normal results will be found in community based laboratories. Statistical methods have been developed to extract reference populations from clinical laboratory data though it is recommended to sample from individuals who are relatively healthy (11). This may include sampling from blood donors, individuals undergoing regular screening, patients undergoing minor surgery or individuals undergoing genetic screening (11). This may also include using samples collected in large epidemiological studies.

1.2.3 Controlling for Pre-Analytical and Analytical Variation

The width of a RI is affected by intra- and inter-individual variability as well as the analytical variability of the measurement system (5). Therefore, when establishing RIs it is important to consider all of the pre-analytical and analytical variables that can affect the measured result of the analyte (11). Standardization of subject preparation, sample collection and processing, the analytical system and instrumentation help to limit variability and are ideally controlled when determining RIs.

Pre-analytical characteristics can be separated into two main categories; biological and methodological (11). Biological characteristics to consider that affect analyte concentrations include things like age, sex, ethnicity etc. and may indicate the need for partitions separating the reference sample into subgroups (6). Other biological factors such as diet and lifestyle are harder to control for. Methodological factors that influence inter-individual variability include characteristics such as patient positioning, patient fasting status, time of collection, the environment, and processing of specimens prior to measurement (11). These factors are easier to control by standardization of collection and processing procedures and do not often require separate RIs by partition.

Analytical variability also needs to be controlled when establishing RIs (5). Laboratories should provide valid and detailed data describing the analytical method used including between-run analytical imprecision, the limit of detection, interference characteristics and traceability of the results (11). Extensive work has also gone into ensuring quality control and assurance of the instruments, samples and reagents being used clinically. Unfortunately inter-laboratory differences and intra-laboratory changes

are known to affect the interpretation of analytical tests and make it difficult to compare RIs from different studies (11). For these reasons the IFCC has initiated harmonization procedures to potentially be able to share and compare reference data from different laboratories (14-19).

1.2.4 Analysis of Reference Values

The first step in calculating RIs based on the measured values of the reference sample is to examine the reference distribution (11). If the analyte measurements follow a Gaussian distribution (confirmed by a statistical test of normality) the classical parametric method of RI calculation may be applied (4). This method sets limits at the 2.5^{th} and 97.5^{th} percentiles ($\mu \pm 2SD$) as the reference interval. Normally distributed test results are often hard to come by due to outlier data points (1). If the data is skewed it is possible to transform the data, usually using a log transformation; however this involves transforming the data back for clinical interpretation (4). In literature the parametric method is used often because it is a statistically simple method to understand and employ. Unfortunately many studies inappropriately apply this method to data where the normality assumption is not met (6).

The non-parametric approach is the preferred and recommended method proposed by the IFCC and CLSI as it does not hold any assumptions about the underlying distribution of the data. Using this method the sample measurements are rank ordered and the 2.5^{th} and 97.5^{th} percentiles are set as the RI (1). Although this method does not assume normality it does require a minimum of 120 subjects per interval being calculated for calculation of confidence intervals (CIs) around the upper and lower limits of the RI

(11). For instance, if two RI are required (i.e. male and female) a minimum of 240 subjects would be necessary; 120 males and 120 females. Sample sizes less than 120 subjects using this method tends to bring in the use of the extreme values on either end of the distribution making it more susceptible to bias from influence of outliers (1).

To deal with smaller sample sizes the robust approach was developed which also does not make any assumptions about the underlying distribution of the data (1). It is used to resist outlier influence by down-weighting values the further away they are from the central values (1). Though quite efficient, robust methods are often not as suitable for populations that approximate a Gaussian distribution and are more useful for distributions that are heavy tailed.

As demonstrated above, outlier data is a common problem for any study. Exclusion criteria are used to exclude individuals with known disease but the presence of extreme outliers may indicate individuals whose ill-health is currently unknown. Inclusion of these data points can affect the point estimates calculated when determining RIs. Methods of outlier detection have been developed such as the one recommended by the CLSI; the Dixon outlier test. This test compares the difference between an extreme observation and the next largest or smallest value (D) and the range of all observed values (R) (11). With this method, if there are multiple outlier data points on either side of the distribution, extreme outliers will go undetected (1). More importantly, even if an outlier is detected with this method, guidelines suggest to keep all data points in the dataset unless there is evidence proving some sort of systematic error or bias in that specific measurement (11).

1.2.5 Reporting of Reference Intervals

Two other considerations are important when reporting RIs; the partitioning of subgroups and the calculation of 90% confidence intervals around the upper and lower limits of the RI.

In many cases it is assumed that the RI is the same for sub-groups (i.e. male and female), mostly because acquiring the 120 minimum subjects for each sub-group can become quite difficult (1). Contrastingly it may be the case that groups are separated without any analysis to test that they are actually different. If a partition is thought to be necessary based on physiological evidence it is recommended to test the need for partitions using a statistical method (11). Described by the IFCC and CLSI is the Harris and Boyd method (11). This method examines whether there is a statistically significant difference between partitions based on the standard normal test (20). Unfortunately this method is subject to the assumption of normality, is unable to account for different prevalence of a particular characteristic within sub-classes and is poor at reflecting one-tailed distributions (11). For instance, two subgroups (i.e. male and female) may have comparable lower limits and standard deviations but very different upper limits and the difference may go undetected.

One alternative but relatively more subjective method of assessing the needs for partitions is to examine CI overlap. The 90% CI of the upper and lower limits of a RI reflects the precision of that estimate (1). The 90% CI can be calculated non-parametrically however for reliable and accurate CIs the sample size should exceed the minimum 120 subjects. This is because with 120 subjects the CI will include the lowest

observed data point as part of the lower limit confidence interval and the highest observed data point as part of the confidence interval of the upper limit (1). When using the robust approach, the bootstrap method is used to calculate the 90% CIs (21). Boot-strapping involves resampling of the data with replacement of values from the dataset to create a "pseudo-sample". From each pseudo sample a reference is derived and the process is repeated a number of times (21). A distribution is then created from the intervals derived and the 5^{th} and 95^{th} quantiles are used to create the CI. This reinforces the influence of outliers when sample size is limited. Despite being recommended by the IFCC and the CLSI, CIs often go unreported in literature (22). This could be due to the required sample size using the non-parametric approach or shortcoming in statistical knowledge.

1.3 Geriatric Clinical Chemistry

The terms elderly, geriatric and older persons are used to describe the same general type of population subgroup. Since age is a very dynamic process chronological age cut-offs may not always be the most accurate method of defining the sub-group, however for the purposes of RI calculation it is the most practical. The most commonly used age cut-off for elderly/geriatric/older persons is 65 years of age (23) primarily because many physiological changes become evident at or near this point and this is a common age of retirement. The age of 65 is not a stead-fast rule however it does provide insight into the population referenced by the terms elderly/geriatric/older. The necessity for geriatric RIs is suspected to arise from increased pre-analytical variation and physiological changes with age that affect biochemical parameters.

1.3.1 Biological Variability in Geriatrics

Many of the pre-analytical variables known to affect analyte concentrations of an adult population also affect the elderly population and may even have a stronger or lesser effect. For instance, differences seen between males and females in adults may begin to magnify or diminish with increasing age. One example of this is the difference in alkaline phosphatase between men and women which tends to decrease in older age groups (6).

Other sources of biological variation tend to occur with age (6). The difference in diet of elderly persons versus younger persons may affect blood tests; many older persons are known to suffer from malnutrition, protein insufficiency and caloric deficits. Changes in sleep pattern, stress, mobility, difficulties in blood collection and accumulated effects of various lifestyle behaviours also begin to increase the biological variability seen in elderly patients.

One major source of heterogeneity within elderly subjects is the development of morbidity and the concept of biological age. It is common for persons older than 65 years of age to have at least one chronic illness (6). It is also very common for two individuals of the same chronological age to have very different health states. Due to difficulty in defining biological age, chronological age is typically the method used to classify subgroups in RI determination.

The increase in biological variability impact RI calculations by increasing the number or type of partitions needed (11). This inherently increases the number of individuals needed during sample recruitment and obtainment. An increase in biological variability will also affect the validity and reliability of the point estimates of the RI.

Lastly increased biological variability may decrease the precision of the RI estimates, causing the CIs to be wider (5).

1.3.2 Changes in Physiology with Age

The care of older persons is very different from adult persons due to a combination of biological changes that occur during the aging process (24). In 2006 Goldsmith defined biological aging as "time-sequential deterioration that occurs in most living beings including weakness, increased susceptibility to disease and adverse environmental conditions, loss of mobility and agility, and age-related physiological changes" (25). Many have proposed theories that attempt to describe different possible mechanisms of aging including the stochastic theory of aging, the gene theory, the vascular theory, the neuroendocrine theory and the immunological theory (26). Though there is much debate about the cause of aging, all of the proposed theories agree that disease is distinctly different from aging and that one's pathology changes with age causing more susceptibility to disease and the inability to deal with external stressors (24).

Many studies have attempted to define what constitutes the normal aging process. Most evidence comes from cross-sectional studies comparing younger age groups to older age groups but this data may reflect differences in lifestyle, medication use or even diet rather than just differences with age (24). Many changes that are associated with aging result from a gradual loss in function. Unfortunately the loss of function does not often become functionally significant until the damage is quite extensive (24).

Physiological and morphological changes are known to affect body composition as one ages. Between the ages of 30 and 70 ones height may decrease by as much as 5 cm as a result of thinning of the intravertebral discs and flattening of the arch of the foot (26). Total body water decreases with age and the fat compartment approximately doubles to 30% of one's body weight. Fat also begins to become more centrally distributed adding to adipose tissue (26). Lean body mass is also known to decrease by as much as 40% into the 8^{th} decade of life. These changes in addition to many others contribute to functional changes seen in the elderly.

Major organ systems also begin to change both in morphology and function (26). Within the gastroinstestinal system, absorption of some micronutrients is impaired, motility is decreased, and hepatic size, mass and blood flow are decreased. The cardiovascular system changes dramatically, including increases in atrium size, increases in heart muscle thickness, thickening and calcification of heart valves, decreased gas exchange and increased blood pressure (26). Changes to the kidney and bladder include decreased kidney size, a decline in creatinine clearance, loss of nephrons, decreased ability to handle sodium and potassium load and a decrease in function of the glomeruli. The neuroendocrine system is also known to decrease hormone production and produce changes in sleep patterns and thermoregulation (26). Given the number of `normal` changes that occur with age it is not surprising that some changes are seen in blood chemistry of older adults.

1.3.3 Analyte Concentrations Change with Age

Abnormal laboratory findings in elderly patients are often attributed to 'old age' (24). It is true that abnormal findings are common in geriatric patients but it is often hard to distinguish between age associated changes and changes that are the result of underlying disease (24). A summary of laboratory parameters that are reported to change with age are presented in Table 1-1.

Table 1-1: Laboratory Parameters Expected to Change with Age
(Adapted from 6)

Biochemical Test	Age-associated Change
Alkaline phosphatase	Increased
Cholesterol	Increased
Cortisol	Peaks earlier (6-8 AM)
Creatinine clearance	Decreased
Estrogen	Decreased
Gastrin	Increased
Protein, total	Slightly decreased
Testosterone	Decreased
Thyroid-stimulating hormone	Slightly increased

Mild asymptomatic elevations of alkaline phosphatase are common in the elderly (24) however other liver function tests are suspected to stay unchanged. Kidney function impairment is known to decrease creatinine clearance and cause a build up of creatinine in the blood despite daily production of creatinine declining with old age (24). Sex hormones such as estrogen and testosterone begin to decrease as sexual maturity is surpassed. Decreased values of albumin, iron and protein may indicate malnutrition (which is common in the elderly) and/or gastrointestinal blood loss (24). Many other

biochemical markers are not suspected to change with age; in particular electrolytes such as sodium, potassium and chloride.

Despite the known and suspected changes in analyte concentration with age, they are seldom quantified by standard RI determination protocols. In most situations the physician or clinical chemist's interpretation of the laboratory test is based on the RI provided by the laboratory where the reference sample is typically aged 20-50. Age-related RIs for geriatric populations are often not provided (6). As a result RIs obtained from young adult populations are used for interpretation of a laboratory test from an elderly individual. The inappropriateness of this interpretation may lead to undiagnosed disease, increase the risk of mortality and/or lead to unnecessary examinations or diagnostic procedures.

1.4 Preface

To better grasp the scope of RIs in the geriatric population a systematic review of the literature was performed (Chapter 2). Through this examination we were able to identify major sources of methodological variation that occurs between studies. Methodologies for selecting the reference population, pre-analytical and analytical treatment of the samples, as well as RI calculations and reporting differed significantly across studies. Despite the variation in differences between geriatric and adult RIs were demonstrated for a variety of analytes.

Given that studies differed greatly in the way they selected their reference population and that morbidity is common in the aging population, a study was performed

to determine whether or not the presence of morbidity has an effect on RIs, specifically RIs for creatinine (Chapter 3). Results of this study suggested that the presence of morbidity significantly increases the upper limits for creatinine in older age groups but not younger age groups. Quantifying the effect of morbidity provides a new method of RI determination in the elderly that uses less stringent exclusion criteria and controls for the presence of disease through partitioning.

1.5 References

(1) Horn, P. S., & Pesce, A. J. (2003). Reference Intervals: An Update. *Clinica Chimica Acta, 334*(1-2), 5-23.

(2) Sasse, E. A. (2002). Objective Evaluation of Data in Screening for Disease. *Clinica Chimica Acta, 315*(1-2), 17-30.

(3) Marshall, W. J. (1995). *Clinical Biochemistry: Metabolic and Clinical Aspects*. New York: Churchill Livingstone.

(4) Horn, P. S., & Pesce, A. J. (2005). *Reference Intervals: A User's Guide*. Washington, DC: American Association for Clinical Chemistry. Chapters 1-3.

(5) Ceriotti, F., Hinzmann, R., & Panteghini, M. (2009). Reference Intervals: The Way Forward. *Annals of Clinical Biochemistry, 46*(1), 8-17.

(6) Faulkner, W. R., & Meites, S. (1994). *Geriatric Clinical Chemistry: Reference Values*. Washington, DC: AACC Press. Chapters 1-5.

(7) Marks, V. (1985). *Clinical Biochemistry Nearer the Patient*. Edinburgh: Churchill Livingstone.

(8) Tietz, N. W., Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2006). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (5th ed.). St. Louis, Mo.: Elsevier Saunders. Section I – Establishment and Use of Reference Intervals.

(9) Dybkaer, R., & Grasbeck, R. (1973). Theory of Reference Values. *Scandinavian Journal of Clinical and Laboratory Investigation, 32*, 1-7.

(10) Henny, J., Petitclerc, C., Fuentes-Arderiu, X., Petersen, P. H., Queralt, Ã. J. M., Schiele, F., et al. (2000). Need for Revisiting the Concept of Reference Values. *Clinical Chemistry and Laboratory Medicine, 38*(7), 589-595.

(11) Clinical and Laboratory Standards Institute (CLSI). (2008). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline. CLSI document C28-A3. 3rd ed. Wayne P.A.: Clinical and Laboratory Standards Institute.

(12) World Health Organization. Constitution in Basic Documents. Geneva: WHO, 1948.

(13) Alstrm, T., Grasbeck, R., Hjelm, M., and Skandsen, S. Committee on Reference Values, Scandanavian Society for Clinical Chemistry and Clinical Physiology. Recommendations Concerning the Collection of Reference Values in Clinical Chemistry and Activity Report. *Scandanavian Journal of Clinical Laboraotry Investigation, 35 (Suppl. 144)*, 1-74.

(14) Petitclerc, C., & Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 2. Selection of Individuals for the Production of Reference Values. *Clinica Chimica Acta*, *170*(2-3), S1-S11.Sasse, E. A. (2002). Objective Evaluation of Data in Screening for Disease. *Clinica Chimica Acta*, *315*(1-2), 17-30.

(15) Solberg, H. (1987). Approved Recommendation (1986) on the Theory of Reference Values. Part 1. The Concept of Reference Values. *Clinica Chimica Acta*, *165*(1), 111-118.

(16) Solberg, H. (1988). Approved Recommendation (1987) on the Theory of Reference Values. Part 3. Preparation of Individuals and Collection of Specimens for the Production of Reference Values. *Clinica Chimica Acta*, *177*(3), S3-S11.

(17) Solberg, H. (1991). IFCC Recommendation-Theory of Reference Values Part 4. Control of Analytical Variation in the Production, Transfer, and Application of Reference Values. *Clinica Chimica Acta*, *202*(3), S5-S12.

(18) Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 5. Statistical Treatment of Collected Reference Values. Determination of Reference Limits. *Clinica Chimica Acta*, *170*(2-3), S13-S32.

(19) Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 6. Presentation of Observed Values Related to Reference Values. *Clinica Chimica Acta*, *170*(2-3), S33-S42.

(20) Lahti, A. (2004). Partitioning of Nongaussian-Distributed Biochemical Reference Data into Subgroups. *Clinical Chemistry*, *50*(5), 891-900.

(21) Horn, P., Pesce, A., & Copeland, B. (1998). A Robust Approach to Reference Interval Estimation and Evaluation. *Clinical Chemistry*, *44*, 622-631.

(22) Daly, C. H., Liu, X., Grey, V. L., & Hamid, J. S. (2013). A Systematic Review of Statistical Methods used in Constructing Pediatric Reference Intervals. *Clinical Biochemistry*, *46*(13-14), 1220-1227.

(23) Sieber, C.C. The Elderly Patient-Who is That?. (2007). *Internist, 48,* 192-194.

(24) Kane, R. L. (2009). *Essentials of clinical geriatrics* (6th ed.). New York: McGraw-Hill Medical. Chapters 1-3.

(25) Goldsmith, T. C. (2008). Aging, Evolvability, and the Individual Benefit Requirement; Medical Implications of Aging Theory Controversies. *Journal of Theoretical Biology, 252*(4), 764-768.

(26) Dharmarajan, T. S., & Norman, R. A. (2003). *Clinical geriatrics*. Boca Raton: Parthenon Pub. Group.

2 GERIATRIC REFERENCE INTERVALS: A SYSTEMATIC REVIEW

2.1 Abstract

Importance: Applying adult reference intervals (RIs) to the elderly population may result in over-medicalization and unnecessary diagnostic procedures. Even still, geriatric RIs have not been established and no standardized method currently exists for calculating age-related RIs.

***Objective*:** To provide a comprehensive review of age-related RIs for the elderly and summarize sources of variation. Secondly, to determine if elderly RIs are significantly different from adult RIs.

Evidence Review: A search was conducted on EMBASE and Medline for articles between January 1989 and June 2013. Studies were selected if they: (a) were English primary articles, (b) performed a clinical chemistry test on a blood fraction, (c) had a population sub-group consisting of individuals ≥65 years of age, and (d) calculated a reference interval for the subgroup ≥65 years of age.

Findings: Of 985 articles screened, 62 studies met inclusion criteria. From these studies a total of 1082 RIs for 93 unique analytes were captured. The minimum number of RIs provided for any analyte was 1 with a maximum for any analyte of 76. Studies differed considerably in reference sample selection, analytical methods and reference interval calculation protocols. A total of 56 papers (90.3%) performed a statistical test to determine whether RIs calculated for age partitions ≥65 years of age were significantly different from partitions <65 years or if there changes with analyte concentration with

increasing age. From these papers at least one result was available for 82 analytes of which 68 analytes (82.9%) were reported to increase or decrease in concentration with increasing age. Multiple studies reported increases in alkaline phosphatase, creatinine, follicle-stimulating hormone, glucose, ϒ-glutamyltransferase, lactate dehydrogenase, low-density lipoprotein cholesterol, luteinizing hormone, magnesium, sex hormone binding globulin, thyroid-stimulating hormone and urea with age. Multiple studies also report decreases in albumin, total bilirubin, total calcium, estradiol, insulin-like growth factor-I, iron, free and total testosterone, and vitamin B_{12} with age.

Conclusion and Relevance: A multitude of RIs for persons ≥65 years of age are available in the literature. Despite considerable variation in methods of analytical measurement and RI calculation, evidence suggests significant changes in RIs with age for many analytes. There is therefore a need to standardize methods of RI determination for geriatric populations.

2.2 Introduction

Laboratory test results play an important role in clinical decision making. They are reported to influence approximately 60-70% of decisions made in medicine including but not limited to medication administration, hospital admittance and hospital discharge (1). Reference intervals (RIs) provide clinicians with a normal range of values or cut-off points to assess a patient's status, typically bound or set by the central 95th percentile. Whether a patient's result is considered 'in range' or 'out of range' will determine diagnostic and/or therapeutic outcomes (2).

The validity of such RIs is influenced by many factors including selection of a reference sample, sample size, analytical factors such as instrumentation, sex, and age, demographic and lifestyle factors and even statistical approaches used in RI calculation (3-8). The Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS), has outlined the need for standardization of RI determination and has provided a guideline for establishing RIs (9). The guideline suggests the use of a non-parametric calculation of the 2.5th and 97.5th percentiles using a minimum of 120 healthy samples in the reference group.

Unfortunately, the suggested method by the CLSI was primarily developed for use in the general adult population and poses many problems when establishing RIs for the elderly. Firstly, selecting a 'healthy' reference population of older persons is very challenging. Typically, in regards to RIs, 'healthy' refers to the absence of disease (2). Applying this definition to an elderly population where the presence of at least one disease is normal and expected, results in the exclusion of many individuals. This

effectively reduces the sample size of the reference population and furthermore begs the question of whether this definition of 'healthy' is really applicable to this age group. Secondly, the concentration of various biochemical markers or analytes tends to increase or decrease with age (10). This can result in the necessity for multiple age partitions. Obtaining the recommended 120 individuals for each age partition, especially for older age groups where sample size is already limited, can be very difficult, time consuming and costly.

As a result, elderly laboratory tests are currently assessed using adult RIs (11). Since certain analyte concentrations tend to increase or decrease with age, it is inappropriate to apply cut-offs derived from an adult population to the elderly as this can result in further invasive testing, over-medicalization or undiagnosed disease. Unfortunately the availability of age-specific RIs for the elderly is sparse as they are not often reported or are intermixed with adult RIs (11).

To assess what RIs do exist for the elderly population a systematic review was performed; defining elderly as anyone above the age of 65 using the widely accepted chronological definition (12). The methodologies used to calculate geriatric RIs within the articles included in the review were evaluated to identify current issues that occur when applying CLSI guidelines to RI determination in older populations. A secondary objective of the study was to identify any differences in elderly RIs compared to adult RIs. Any reported increases or decreases in analyte concentration with age were summarized within the review.

2.3 Methods

2.3.1 Literature Search

A literature search on EMBASE and Medline databases was performed to identify articles published between January 1989 and June 2013. A preliminary literature search revealed a number of articles reporting elderly RIs published in 1989 into the early nineties validating the search start date. This preliminary literature search also identified useful search terms. Separate searches were performed using the terms "reference intervals", "reference ranges", "reference values" and "reference parameters" crossed with (operator AND) "elderly", "old", "geriatric", "older adult" with the field limit "humans" (full search criteria located in Appendix A). A total of 985 articles, 982 with removal of duplicates, were found using these search criteria and imported into DistillerSR (Evidence Partners Incorporated, Ottawa ON) for review.

2.3.2 Inclusion/Exclusion Criteria

Title and abstract screening was performed to select for articles that were in English, were primary research articles, performed a test on a blood fraction, measured a clinical chemistry analyte, and included persons ≥65 years of age (Figure 2-1). Full text screening was then performed to ensure the remaining articles calculated a RI for at least one subgroup that consisted only of individuals ≥65 years of age.

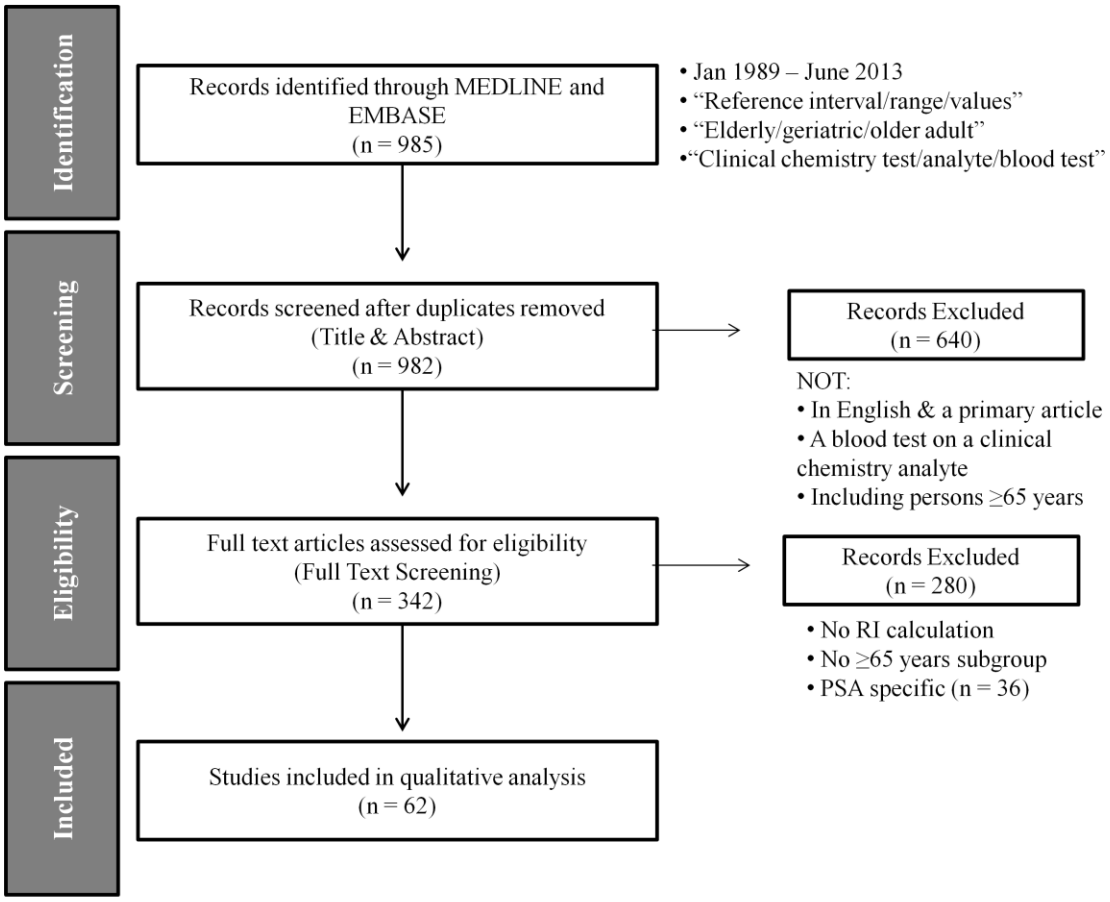

Figure 2-1: Analytical Framework

2.3.3 Data Extraction

Three data extraction forms (Appendix B) were created to 1) evaluate the reference sample selection, 2) record the analytical processes and 3) capture the RI(s) established for persons ≥65 years of age. The purpose of each study was identified using searches for the key terms "objective", "aim", "goal" and "purpose". Study purposes were then categorized into one of three groups; to establish RIs in general, to establish elderly RIs specifically or to test a new method of measurement or calculation. The reference sample form also captured the country of publication, the number of persons included in

the study, the method of participant recruitment or sample obtainment, inclusion and exclusion criteria, and any other available information about the population regarding age, sex, ethnicity and clinical history characteristics. The analytical methods form consisted of fields pertaining to pre-analytical, analytical and post-analytical characteristics. This included the type of blood fraction that was tested, whether participants were fasting or underwent any other special treatment, what clinical analyzer or equipment was used for analyte measurement, other instrumentation details and storage procedures if applicable.

Any RI for a clinical chemistry analyte that was calculated for an age group ≥65 years was collected. The type of RI calculation used by each study and whether sex and/or age partitions were used was also captured. If a study did not specifically state the RI calculation method, it was inferred based on other information. Specifically, if RIs were reported as means plus or minus standard deviations it was assumed that a parametric method was used. If the interval was stated as the 2.5^{th} and 97.5^{th} percentiles it was assumed that a non-parametric method was used.

Also recorded was whether any statistical tests were performed on the data to evaluate changes in analyte concentration with increasing age. If a statistical test was performed or a trend with age was evaluated using subjective measures (i.e. graphical depictions or numerical comparisons) the type of test was recorded for each study. The results of the statistical test was then recorded (whether the analyte was shown to increase, decrease or not change with age) along with any associated significance levels typically in the form of p-values. In addition, if a study only included persons ≥65 years

of age in their study they were identified as such. Trends in age may be harder to identify when only evaluating an elderly sample.

It is important to note that one study may report multiple RIs for one analyte depending on the stratification used. For example a study reporting RIs for testosterone may have calculated a RI for males aged 40-49 and a separate interval for males aged 50-59. Every RI that was established for a subgroup consisting only of persons aged 65 and older (no matter the partition cut-off) was recorded (Appendix C). In addition, for studies that performed a statistical test examining changes with age, the RI interval for the youngest age strata was captured to allow numerical comparisons of RIs between young and old age groups (Appendix D).

2.3.4 Data Analysis

All data was exported from DistillerSR into Microsoft Excel 2007 for data management. To compare all of the RIs captured for each analyte, the RIs were converted into the SI unit of measurement (13). A table was created to describe the study details such as where each paper was published, how reference populations were selected and what type of RI calculation methods were used. The number of intervals captured for each analyte was calculated as well as the minimum, maximum and median number of RIs that came from one study for that analyte. If three or more studies performed a statistical test examining trends in analyte concentration with age, the proportion of studies that reported an increase, decrease or no change was calculated and reported as a percentage.

2.4 Results

2.4.1 Scope

The search strategy described identified 985 articles. Title and abstract screening resulted in the selection of 342 studies (Figure 2-1). After full text screening a total of 98 studies (Figure 2-1) were found to have met the full text screening eligibility criteria. Any study whose purpose was to calculate age-specific RIs for Prostate-specific antigen (n=36) were then excluded. The sheer volume of these studies indicated that age-specific RIs for this analyte have been well established and that it is not necessary to investigate this further. Therefore a total of 62 papers were selected for final inclusion in the review.

Studies selected were published in a variety of countries world-wide with the majority of publications (74.2%) coming from Europe, 21.0% coming from the United States and none coming from Canada (Table 2-1). An influx of publications regarding geriatric RIs occurred in the early to mid-90s (32.3% prior to 2000) with another influx between 2000 and 2009.

Most of the studies' (64.5%) main purpose was to establish a new method of measurement/calculation or calculate RIs in general (Table 2-1). Only one-third of studies (35.5%) aimed to establish RIs specifically for the elderly. Despite this, a total of 1082 geriatric RIs were captured from all 62 papers. These RIs were for 93 unique analytes that represent a broad range of physiological tests including markers of kidney and liver function, hormones, metabolites, lipids and enzymes. Each analyte for which a geriatric RI was captured is listed in Table 2-2 along with the total number of RIs captured for that analyte and the minimum, maximum and median number of RIs reported for that analyte

within a singular study. There was a minimum of 1 RI reported per analyte and a maximum of 76. The 76 RIs that were reported for 1 analyte were for creatinine, a marker of kidney function that was investigated by 17 (27.4%) of the 62 studies.

Characteristics of the study population were well reported among all 62 studies but varied considerably by analyte. All studies reported how study participants were recruited or how blood samples were obtained however many were unclear about the exclusion criteria used to select their reference population (Table 2-1). Three studies simply did not provide any data on their inclusion and exclusion criteria.

All studies included in the systematic review commented on the population size selected for their study however 10 studies (16.1%) did not report the number of persons within each age and gender partition. This made it difficult to assess whether these studies were reaching the recommended 120 individuals per partition as recommended by the CLSI when using a non-parametric calculation method. Pre-analytical characteristics including whether or not a patient was fasting and post-analytical characteristics such as whether samples were stored or discarded were sparsely reported (<40% of the time). However, analytical characteristics such as the type of assay or instrumentation used were reported for each study.

The method of RI calculation used was reported by each study or was able to be inferred based on presentation of the data (Table 2-1). The type of stratifications used by each study was fairly straightforward to capture but there was often little justification for why particular age or gender strata were used. Barely ever was a statistical test performed to test whether the partitioning was even necessary (<40% of the time). Lastly it was

noticed that very few studies (21.0%) reported confidence intervals (CIs) around the lower and upper bound of the reported RIs. This is one of the criteria set out by the CLSI guideline and lack of CIs made it particularly difficult to statistically compare RIs across studies.

Upon initial inspection of the data it became apparent that for any given analyte it would be difficult to compare RIs from multiple studies. This was deemed to be the result of four contributing factors; 1) selection of the reference sample, 2) pre-analytical and analytical variation 3) different applications of RI calculations 4) differences in reporting of RIs. To demonstrate how variable the reported RIs were for a particular analyte, the RIs for albumin were plotted in Figure 2-2. Despite the large variation in methodology across included studies, certain trends became apparent when evaluating studies that reported changes in analyte concentration with increasing age.

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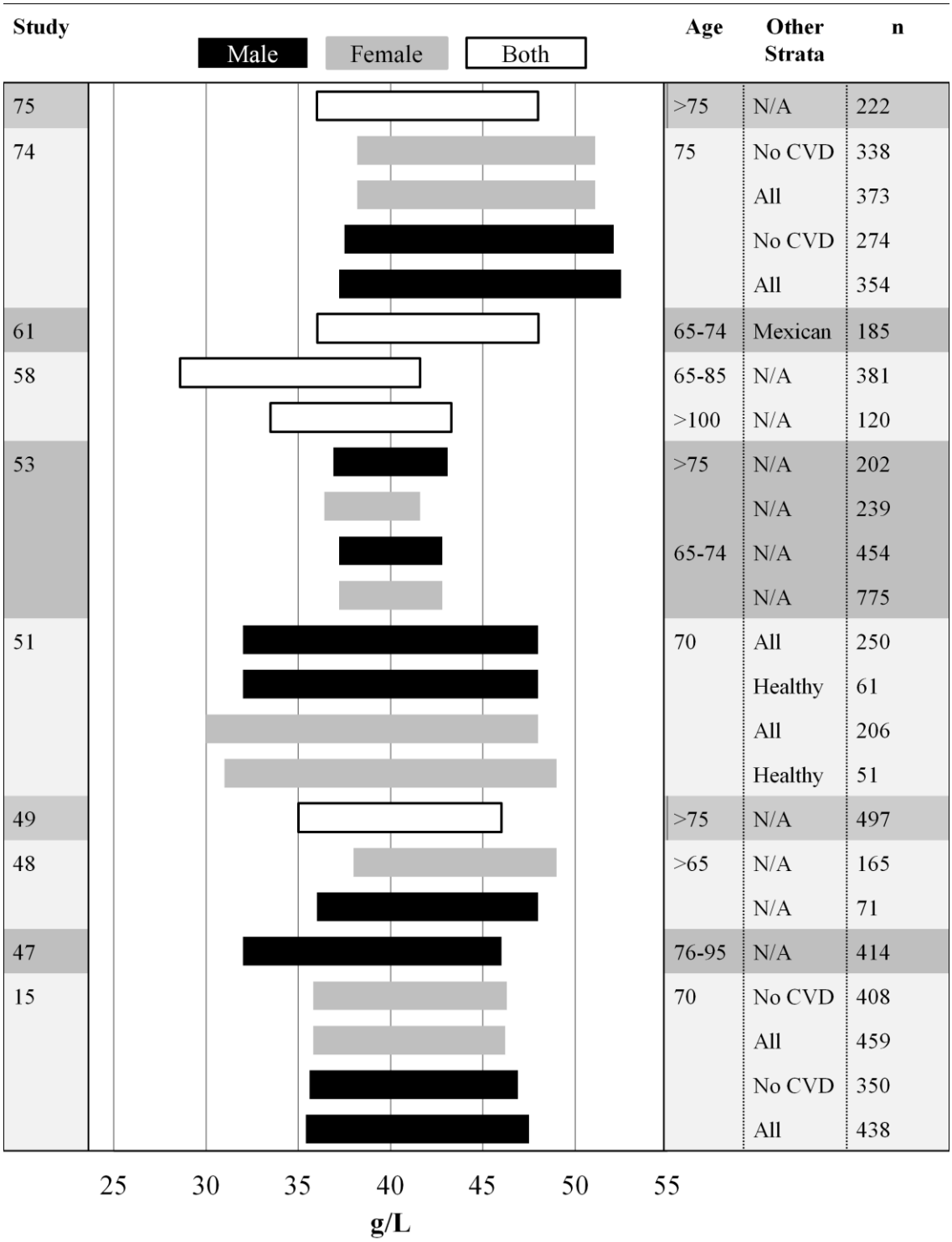


Figure 2-2: Reference Intervals for Albumin – A Display of Variability
All reference intervals for albumin were plotted. Sex partitioning was indicated by colour whereas age partitioning and any other portioning was indicated in table format.

2.4.2 Reference Sample Selection

A total of 19 studies (30.6%) only sampled persons greater than 65 years of age, all of which were performed with the purpose of calculating RIs specifically for the elderly (Table 1). The four methods of participant recruitment or sample obtainment were recruitment from a large databank (n=6, 9.7%), from a larger study (n=23, 37.1%), from the community (n=17, 27.4%) or a hospital/clinic (n=16, 25.8%). Three of the studies that recruited from a large data bank used results from hospital laboratories. Recruitment from databanks and larger studies typically resulted in larger sample sizes. The larger studies that samples or participants were recruited from include the:

i) Belgian Interuniversity Research on Nutrition (BIRNH)
ii) Cardiovascular Health Study (76)
iii) Framingham Offsrping Study (77)
iv) Health in Men Study (HIMS) (78)
v) Italian Multicentre Study on Centenarians (IMUSCE)
vi) Lieto study
vii) National Health and Nutrition Examination Survey (NHANES) (79)
viii) Nordic Reference Intervals Project (NORIP) (80)
ix) Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (81)
x) Study of Health in Pomerania (SHIP) study (82)
xi) Swedish "Aging in women in men" study
xii) Vienna Transdanube Aging (VITA) study
xiii) VITAGE project

Most notably, the lack of a clear definition for what a "healthy elderly" person is significantly affected the exclusion criteria utilized by each study (Table 2-1). Many studies aimed to include persons they often termed "apparently healthy" by excluding anyone diagnosed with a morbidity common to elderly subjects i.e. diabetes, cancer, cardiovascular disease etc. However 12.9% of studies did not use any exclusion criteria.

Furthermore 29.0% of studies actually excluded persons based on their laboratory values for particular analytes based on the suspicion that undiagnosed disease may be present.

2.4.3 Pre-Analytical and Analytical Variation

It was rare to find that two studies used the same analyzer, assay or piece of laboratory equipment to measure the same analyte. For example out of the 17 studies that measured creatinine, only 3 used the same instrument and method. The various analyzers, assays and/or laboratory equipment used in each study were reported in Table 2-3 along with the manufacturer of the equipment. Many different analytical methods were used for the measurement of one analyte making comparison across studies even more difficult.

Pre-analytical and analytical variation was also determined to be a large source of variation. Sample collection varied by study with differences in patient position, patient fasting and type of sample collected. Sample processing and storage was also quite different across studies. Studies that were part of larger epidemiological studies often used samples that had been frozen and stored for future analysis where other studies performed processing same day or used already acquired lab test results. The abundance of analytical factors that attribute to variation in analyte measurement complicates comparison of RIs across studies.

2.4.4 Reference Interval Calculations

Methods of RI calculation were classified by three primary methods; parametric, non-parametric and robust. A total of 32.2% of studies used non-parametric methods, 50% parametric, 8% robust and 9.7% used more than one method (Table 2-1). Less than

half (41.9%) of studies used the recommended number of 120 individuals in the majority of their strata, majority being defined as >80%.

2.4.5 Reporting of Reference Intervals

Over 60% of studies stratified RIs for at least one analyte by sex and another 11.3% only included one sex removing the need for stratification by sex. Studies varied considerably in the partitions used for age. The majority of studies (77.4%) used five or ten year intervals to separate age groups. However it was common for studies to use five or ten year intervals and then set an age cut-off for older partitions where sample size starts to dwindle i.e. >70 or >80 (Table 2-1). Approximately one-quarter of included studies (24.2%) stratified by some other variable, typically ethnicity. The maximum number of studies that used the same gender and age stratification for the same analyte was 2. Very few studies (21.0%) reported confidence intervals around the lower and upper bound of the reported RIs.

2.4.6 Differences in Analyte Concentration with Age

A total of 56 papers (90.3%) performed a statistical test to determine whether RIs calculated for age partitions ≥65 years of age were significantly different from partitions <65 years or if there changes with analyte concentration with increasing age. From these papers at least one result was available for 82 analytes of which 68 analytes (82.9%) were reported to increase or decrease in concentration with increasing age (Appendix C). Figure 2-4 illustrates the percentage of studies that reported increases, decreases or no change in analyte concentration for analytes that had results from three or more studies.

Greater than 60% of studies reported increases in alkaline phosphatase, creatinine, follicle-stimulating hormone, glucose, ϒ-glutamyltransferase, lactate dehydrogenase, low-density lipoprotein cholesterol, luteinizing hormone, magnesium, sex hormone binding globulin, thyroid-stimulating hormone and urea with age (Figure 2-4). Greater than 60% of studies reported decreases in albumin, total bilirubin, total calcium, estradiol, insulin-like growth factor-I, iron, free and total testosterone, and vitamin B_{12} with age.

Analytes that were reported to not change with age in greater than 60% of studies included aspartate aminotransferase, chloride, high-density lipoprotein cholesterol, potassium, total protein, sodium and triglycerides. Conflicting results were reported for alanine aminotransferase, cholesterol, and creatine kinase.

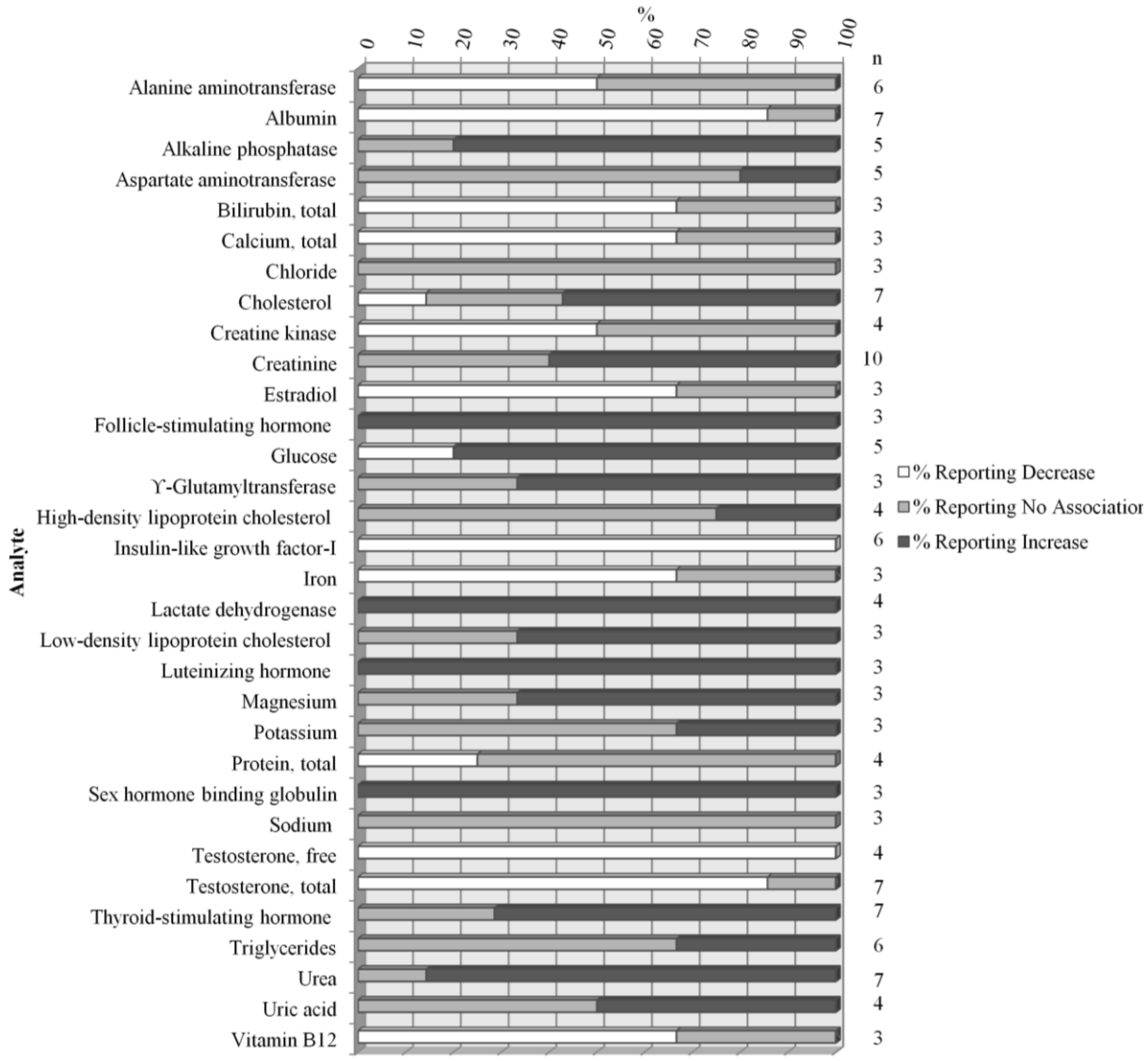


Figure 2-3: Percentage of Studies Reporting Increases, Decreases or No Change in Reference Intervals with Age

Data for this figure was obtained from Table 2-4: Analyte Concentrations Change with Age. If at least three studies reported a result for whether there was a change with age the analyte was included in the figure. The percentage of studies that reported an increase, decrease or no association with age were calculated and plotted above.

2.5 Discussion

Age-specific RIs for persons in older age groups are not typically used in diagnosis and monitoring of therapy, instead adult RIs are used to assess the normality of an older person's blood test result (11). This review highlights the fact that literature is not lacking reports of age specific RIs for persons ≥65 years of age for common laboratory tests however the quality of the reports that do exist are lacking.

Less commonly tested analytes had fewer reported RIs as expected. The majority of RIs reported in the literature were produced by European nations, which is suspected to be in part because these nations were the producers of large epidemiological studies from which RI projects were developed. Despite the multitude of RIs available in literature it is impossible to consolidate, compare or utilize these RIs in practice due to missing or ambiguous data and extreme variability in methodology.

2.5.1 Selecting a 'Healthy' Elderly Reference Sample

Reference sample selection seems to be a large area for debate when considering the elderly population. In practice when calculating RIs according to the Clinical Laboratory Standards Institute (CLSI) guidelines, it is important to select a healthy reference population (9). Unfortunately the definition of a "healthy elderly" person is very subjective. As discussed, it is common after the age of 65 to have acquired some sort of morbidity. Does that mean that someone above the age of 65 with morbidity should then be considered 'normal'? Some of the studies included in our systematic review suggest the answer to this question is yes and that no exclusion criteria should be used when selecting a geriatric reference population (47). However, the question then becomes

are the changes seen in RIs due to morbidity or normal physiological changes with age, and how does that affect clinical decision making? Unfortunately, if the opposite approach is taken, and the geriatric population was to be treated like the adult by excluding all persons with morbidity the sample size in most cases is very limited and leads to biased and unreliable estimates. This has proven to be one of the main sources of conflict in determining geriatric RIs and is something that needs further investigation in order to consolidate the definition of "healthy elderly".

Another approach commonly used was to exclude persons based on abnormal laboratory values due to expected disease. This approach limits the heterogeneity of the population by excluding persons who may not have disease but have 'abnormal' test results due to age or other physiological consequences. Perhaps these abnormal values seen in persons ≥65 years of age are due to physiological changes, and the suspected disease is in fact suspect. One study included in this review by Huber, 2006 (31) actually tried to "remove" the effect of aging by studying a reference population who were all the same age. Development of criteria for how to select a geriatric reference population is necessary for the calculation of geriatric RIs, and when doing so it is important to keep in mind methods that will be representative of the population but not too restrictive.

Sample size requirements also pose an issue when determining RIs for the elderly. It is difficult and not always feasible to obtain the recommended 120 individuals per partition for elderly when they are selected as 'healthy' and when physiological changes require more partitions with age. In order to circumvent this problem studies have taken

to using data from large epidemiological studies as opposed to selecting persons for the sole purpose of RI determination.

One of the only epidemiological studies that was created for the purposes of determining RIs was the Nordic Reference Interval Project (80). This study uses samples from their bio-bank that other researchers can request blood samples from. This method of sample recruitment is used by many other studies such as the Study of Health in Pomerania (SHIP) (82).

2.5.2 Pre-Analytical and Analytical Variation in Geriatric Populations

Due to the time frame chosen for this review (1989-2013) it seems that there is a lot of variation in analytical methods for measuring certain analytes. With the booming development of technology, analytical methods have changed drastically in the last 10 years. New analyzers are being made and new more accurate and specific methods are being created for the measurement of analytes. For some analytes comparisons of RIs calculated years ago are not relevant today. A total of five articles have been identified since the initial search was performed that meet the outlined search criteria. It has been reasoned that the inclusion of these articles would not dramatically impact the findings of this study as they did not capture any analytes that were not already included in this analysis and were still subject to the same sources of variation.

2.5.3 Lack of Standardization for Calculating and Reporting Geriatric Reference Intervals

No standard method for calculating RIs in the geriatric population exist today. The best method whether it is parametric, non-parametric or robust is still being investigated

and discussed in particular by Pottel, H., et. al. (25). In addition, no guideline to what age limits should be used has been described. For instance, it is not clear whether a strict cut-off at 65 is appropriate or what age partitioning should be used. Regardless, the importance of statistical testing for partitioning of any age group has been outlined by Harris and Boyd (85). This is particularly true considering the number of studies that demonstrated age-dependent changes in analyte concentration.

A recent study published by Daly, et. al. outlined the need for consistency of reporting RIs. This systematic review also pointed out the lack of reporting CIs around RI limits and limited testing for differences among partitions (83). Although our review wasn't focused on the statistical methods used in establishing RIs for geriatric populations, it did come to similar conclusions regarding the current literature. There is a significant amount of RI literature that does not specify the sample size of each partition, and even those that do, do not often meet the recommended guideline provided by CLSI. Few studies referenced the CLSI (or NCCLS) guideline and even those that did, did not always use the guideline appropriately. Screening for outliers was not assessed in this study although it is suspected that this will be an important topic to consider in the future when thinking about geriatric RIs since biological variation tends to increase in older adults (84).

2.5.4 Geriatric Reference Intervals are Different from Adult Reference Intervals

Despite the inability to statistically compare RIs from various studies, aside from coefficients of variation, it was clear that some agreement has been made about particular analytes shown in Figure 3. These changes tend to also coincide with suspected

physiological changes. Limited changes with age were detected for metabolites such as sodium, potassium, and chloride. Decreased levels of total calcium may be due to the diminished conversion of vitamin D to 1,25-hydroxyvitamin D, decreased absorption in the gut and decreased serum albumin (13). Increased glucose levels with age are suspected to be a result of a pathological process and have been reported by many studies (47,53,56,57). The only liver enzyme reported to change was alkaline phosphatase which is expected to increase with age especially in women due to hormonal changes (56,57). Other liver function tests such as bilirubin are expected to change based on decreased muscle mass in older individuals (13). A decrease in renal function with age is suspected to be due in part to decreased number of glomeruli, decreased renal blood flow and/or persistent vasoconstriction (13). These physiological changes with age can help to explain increased serum creatinine, urea and uric acid. Many changes in various hormone levels are suspected and expected particularly for sex hormones like testosterone. In particular decreased testosterone production in the elderly has been linked to diminished testicular function (13).

Notably there are many physiological changes that occur in old age. In addition the presence of morbidity becomes more common. It is important then to establish what laboratory values are considered “normal” for the elderly as this may not be the same for adult populations. This is especially true considering the abundance of evidence that suggests analyte concentrations change with age.

2.6 Conclusion

A multitude of RIs are available in the literature for persons greater than 65 years of age. Unfortunately no standardized method currently exists for selecting a reference population, or calculating RIs for this population. This in combination with poor reporting of methodologies, lack of confidence intervals for RI estimates and potential analytical variation makes it very difficult to consolidate findings and apply them to current situations. It is therefore recommended that a method for reporting and calculating RIs of an elderly population be established.

Table 2-1: Study Characteristics and Study Design

	Purpose			Analytes		Sample Recruitment						Exclusion Criteria									RI Calculation Method			Stratifications						
Study Reference	Establish RI for new method	Establish RI for elderly	Establish RI in general	Single	Multiple	Country	Sampled only persons > 65	Recruited from large databank	Recruited from larger study	Recruited from community	Recruited from hospital/clinic	Presence of morbidity	Abnormal laboratory values	On meds affecting analyte	Morbidity affecting analyte	Disease not affecting analyte	On meds (in general)	Lifestyle factors	None	Not provided	Non-parametric	Parametric	Robust	Sex	age - Primarily 5 yr intervals	Age - Primarily 10 yr	Age - > cut off	Age - Other	Other strata	Majority of strata have n>120
14	✓			✓		USA		✓				✓	✓								✓					✓	70			✓
15		✓			✓	SWE	✓		✓				✓			✓					✓		✓	✓				✓	✓	✓
16	✓				✓	USA				✓		✓	✓	✓	✓		✓					✓		✓		✓				NP
17			✓		✓	DEU			✓						✓	✓					✓			✓	✓				✓	
18	✓				✓	JPN					✓				✓						✓			✓		✓	70			
19			✓	✓		DNK				✓						✓						✓		✓		✓				
20			✓		✓	NOR			✓				✓	✓	✓							✓		M		✓				NP
21	✓				✓	NLD				✓		✓				✓	✓					✓						✓		NP
22			✓		✓	NOR			✓				✓		✓							✓						✓		NP
23	✓				✓	DEU			✓					✓		✓		✓			✓	✓	✓	M	✓		74			
24			✓	✓		DNK			✓				✓			✓	✓						✓			✓	70		✓	
25	✓			✓		BEL		✓			✓	✓	✓								✓		✓	✓	✓					✓
26	✓				✓	DEU			✓						✓	✓						✓	✓	✓	✓		74			
27			✓		✓	M			✓				✓	✓	✓	✓						✓					65	✓		
28		✓			✓	M	✓			✓				✓	✓								✓				65			
29	✓		✓	✓		M					✓	✓		✓	✓							✓		✓		✓	70			
30	✓				✓	FIN			✓					✓	✓		✓				✓			M	✓		75			✓
31		✓			✓	AUT	✓		✓			✓				✓					✓	✓		✓				✓		✓
32			✓	✓		USA			✓										✓			✓		✓			70	✓	✓	✓
33		✓			✓	FIN	✓		✓					✓	✓							✓		✓			65		✓	✓
34			✓	✓		CHN					✓	✓	✓			✓					✓			✓		✓				
35		✓		✓		SWE	✓		✓										✓			✓					70			NP
36			✓	✓		DEU				✓				✓	✓	✓					✓			✓			75	✓		NP

	Purpose			Analytes		Sample Recruitment						Exclusion Criteria									RI Calculation Method			Stratifications						
Study Reference	Establish RI for new method	Establish RI for elderly	Establish RI in general	Single	Multiple	Country	Sampled only persons > 65	Recruited from large databank	Recruited from larger study	Recruited from community	Recruited from hospital/clinic	Presence of morbidity	Abnormal laboratory values	On meds affecting analyte	Morbidity affecting analyte	Disease not affecting analyte	On meds (in general)	Lifestyle factors	None	Not provided	Non-parametric	Parametric	Robust	Sex	age - Primarily 5 yr intervals	Age - Primarily 10 yr	Age - > cut off	Age - Other	Other strata	Majority of strata have n>120
37	✓		✓		✓	DEU					✓	✓									✓	✓		✓	✓					
38		✓			✓	NOR	✓			✓					✓			✓			✓			✓			70			
39	✓			✓		M				✓					✓	✓						✓		✓	✓					
40			✓		✓	USA		✓				✓	✓		✓						✓			✓	✓		80			✓
41			✓		✓	KWT				✓				✓	✓							✓		✓				✓		
42			✓	✓		SWE				✓				✓	✓						✓				✓					✓
43		✓			✓	FIN	✓		✓				✓		✓								✓	✓			65		✓	✓
44		✓		✓		FRA					✓		✓	✓	✓						✓			✓			65	✓		
45			✓	✓		USA			✓										✓			✓		✓			70	✓	✓	NP
46		✓			✓	AUS				✓					✓	✓						✓		✓		✓				
47	✓				✓	SWE		✓			✓									✓	✓			M		✓				✓
48		✓			✓	CHE	✓				✓	✓	✓				✓				✓			✓			65		✓	✓
49			✓		✓	USA				✓		✓	✓	✓	✓	✓					✓						75		✓	✓
50		✓			✓	M					✓		✓	✓	✓							✓		✓			65			
51		✓			✓	ISR	✓			✓											✓			✓		✓				✓
52	✓		✓		✓	USA			✓										✓			✓		✓				✓	✓	
53		✓			✓	USA	✓		✓			✓					✓					✓	✓			✓	75			✓
54			✓		✓	FIN		✓			✓		✓		✓							✓		✓		✓	65			NP
55			✓	✓		USA					✓	✓				✓						✓		F		✓	80			
56		✓			✓	M	✓			✓				✓							✓	✓		✓			65		✓	
57		✓			✓	USA	✓				✓		✓								✓			✓			90	✓	✓	
58		✓			✓	ITA	✓		✓			✓				✓						✓					100	✓	✓	✓

	Purpose			Analytes		Sample Recruitment						Exclusion Criteria									RI Calculation Method			Stratifications							
Study Reference	Establish RI for new method	Establish RI for elderly	Establish RI in general	Single	Multiple	Country	Sampled only persons > 65	Recruited from large databank	Recruited from larger study	Recruited from community	Recruited from hospital/clinic	Presence of morbidity	Abnormal laboratory values	On meds affecting analyte	Morbidity affecting analyte	Disease not affecting analyte	On meds (in general)	Lifestyle factors	None	Not provided	Non-parametric	Parametric	Robust	Sex	age - Primarily 5 yr intervals	Age - Primarily 10 yr	Age - > cut off	Age - Other	Other strata	Majority of strata have n>120
59		✓			✓	ITA	✓			✓									✓			✓					65			✓
60			✓		✓	SWE					✓								✓			✓		✓		✓	80			✓
61			✓		✓	USA			✓											✓		✓				✓			✓	
62		✓			✓	GBR	✓				✓								✓			✓		✓			65			NP
63	✓			✓		PRT				✓		✓			✓							✓		✓		✓				
64			✓	✓		ENG					✓		✓									✓		✓		✓	90			
65		✓			✓	USA	✓				✓	✓				✓	✓					✓					65			NP
66			✓		✓	BEL			✓											✓		✓		✓		✓				✓
67		✓			✓	ESP	✓			✓									✓		✓			✓			65			✓
68	✓		✓	✓		CHN				✓				✓	✓						✓			✓	✓					
69	✓				✓	GBR			✓				✓			✓					✓			F		✓				✓
70			✓	✓		TUR					✓					✓	✓					✓		✓	✓		70			
71			✓	✓		M		✓						✓	✓						✓					✓	90			✓
72	✓			✓		AUS					✓			✓	✓						✓				✓		90			✓
73		✓			✓	AUS	✓		✓				✓			✓					✓			M			70			✓
74		✓			✓	SWE	✓		✓				✓			✓							✓	✓				✓	✓	✓
75		✓		✓		JPN					✓								✓							✓	85			✓

NOTE: A check mark indicates that 'yes' this criteria applies to the study of interest whereas an empty cell indicates 'no' this criteria does not apply. When a study did not provide enough information to confirm 'yes' or 'no'; NP was used to indicate the information was 'Not provided'. The International Organization for Standardization's three letter coding system for countries was used to display which country each study was conducted in, with M used to denote multiple countries were involved. If sex strata were not necessary because a study only included male or female subjects this was denoted under the sex stratification as M for 'Male' and F for 'female'. Age strata using an age cut-off were described by indicating at which year they set a cut-off i.e. >65 years of age

Table 2-2: Included Analytes

Analyte	# Of Studies Included In Total # Intervals	Min	Max	Median	Study Reference
Adrenocorticotropic hormone	2	1	1	1	31, 57
Alanine aminotransferase	11	1	8	2	15, 40, 48, 49, 51, 56, 57, 58, 61, 67, 74
Albumin	11	1	4	2	15, 47, 48, 49, 51, 53, 57, 58, 61, 74, 75
Alkaline phosphatase	10	1	8	2.5	15, 40, 48, 49, 51, 56, 57, 61, 66, 74
Amylase	3	1	4	1	15, 48, 57
Androstenedione	3	1	2	2	31, 57, 69
Apolipoprotein A-1	4	1	4	4	15, 56, 60, 74
Apolipoprotein B	4	2	4	4	15, 52, 60, 74
Arginine	1	1	1	1	27
Aspartate aminotransferase	9	1	1	2	31, 40, 48, 51, 56, 57, 58, 61, 67
Asymmetric dimethyl arginine	1	1	1	1	27
Bilirubin, conjugated	2	1	1	1	56, 57
Bilirubin, total	8	1	8	3.5	15, 40, 51, 56, 57, 61, 66, 74
B-type natriuretic peptide	1	1	1	1	28
N-terminal-pro-B-type natriuretic peptide	2	1	3	1.5	28, 34
Calcium, ionized	1	1	1	1	57
Calcium, total	10	1	4	2	15, 31, 48, 51, 57, 61, 65, 66, 67, 74
Carbon dioxide, partial pressure	1	2	2	2	38
Carbon dioxide, total	3	1	3	1	57, 61, 65
Carcinoembryonic antigen	2	1	22	12	22, 57
Chloride	8	1	4	2.5	15, 48, 51, 57, 61, 65, 66, 74
Cholesterol	13	1	4	2	15, 31, 47, 48, 51, 53, 56, 57, 58, 60, 66, 67, 74
Copper	2	2	2	2	41, 57
Cortisol, total	2	1	1	1	31, 57
C-peptide	1	1	1	1	57
C-reactive protein	6	1	8	3	15, 31, 35, 44, 45, 74
Creatine kinase	7	1	6	4	15, 48, 49, 51, 54, 57, 74
Creatinine	17	1	39	2	15, 25, 31, 46, 47, 48, 49, 51, 53, 55, 56, 57, 58, 61, 66, 67, 74
Dehydroepiandrosterone sulfate	2	2	6	4	23, 57
Dehydroepiandrosterone, unconjugated	2	1	1	1	31, 57
Dihydrotestosterone	1	1	1	1	73
Estradiol	4	1	2	1.5	30, 31, 57, 73
Ferritin	2	1	2	1.5	31, 63
α-Fetoprotein	1	22	22	22	22
Fibrinogen	3	1	4	3	31, 53, 50
Folate	1	2	2	2	57
Folic Acid	2	1	4	2.5	31, 51

Analyte	# Of Studies Included In	Total # Intervals	Min	Max	Median	Study Reference
Follicle-stimulating hormone	4	8	2	2	2	20, 30, 31, 57
Glucose	4	12	1	4	3.5	48, 61, 62, 74
Glucose, fasting	7	14	1	4	1	47, 51, 53, 56, 57, 58, 67
ϒ - Glutamyltransferase	9	22	1	4	2	15, 31, 48, 51, 56, 57, 66, 67, 74
Growth hormone releasing hormone	1	6	6	6	6	21
High-density lipoprotein cholesterol	9	21	1	4	2	15, 31, 53, 56, 57, 58, 66, 67, 74
Homoarginine	1	1	1	1	1	27
Homocysteine	3	9	1	5	3	31, 32, 50
Insulin, fasting	2	5	1	4	2.5	53, 57
Insulin-like growth factor I	8	54	1	12	7	17, 19, 21, 26, 31, 37, 39, 70
Insulin-like growth factor binding protein-3	4	30	1	12	8.5	17, 26, 37, 31
Insulin tolerance test	1	6	6	6	6	21
Iron	7	18	1	4	2	15, 31, 43, 48, 51, 57, 74
L-Lactate	1	1	1	1	1	31
Lactate dehydrogenase	9	22	1	4	3	15, 31, 48, 49, 51, 54, 57, 61, 74
Lactate dehydrogenase isozymes (1-5)	2	8	3	5	4	54, 57
Lead	1	2	2	2	2	57
Lipase	3	6	1	4	1	48, 57, 74
Low-density lipoprotein cholesterol	7	17	1	4	17	15, 31, 52, 53, 56, 67, 74
Luteinizing hormone	4	8	2	2	2	20, 30, 31, 57
Magnesium	6	19	1	5	4	15, 48, 51, 57, 64, 74
Methlymalonic acid	2	4	1	3	1.5	14, 50
Neopterin	1	1	1	1	1	56
Osmolality	1	1	1	1	1	57
Oxygen, arterial partial pressure	1	2	2	2	2	38
Oxygen, partial pressure	1	2	2	2	2	38
Oxygen, saturation	1	2	2	2	2	38
Parathyroid hormone	1	1	1	1	1	57
pH	1	1	1	1	1	57
Phosphate	9	23	1	4	2	15, 31, 48, 51, 57, 61, 66, 67, 74
Potassium	11	29	1	4	3	15, 31, 48, 51, 53, 57, 61, 62, 65, 66, 74
Progesterone	2	3	1	2	1.5	31, 57
Prolactin	2	3	1	2	1.5	31, 57
Protein, total	8	16	1	4	2	31, 48, 51, 56, 57, 58, 61, 66
Serum amyloid A	2	3	1	2	1.5	29, 31
Sex hormone binding globulin	3	6	2	2	2	20, 30, 57
Silicon	1	2	2	2	2	36
Sodium	10	25	1	4	2.5	15, 31, 48, 51, 57, 61, 62, 65, 66, 74
Symmetric dimethyl arginine	1	1	1	1	1	27

Analyte	# Of Studies Included In	Total # Intervals	Min	Max	Median	Study Reference
Testosterone, free	4	10	1	6	1.5	16, 30, 57, 69
Testosterone, total	8	21	1	6	2	16, 20, 23, 30, 31, 57, 69, 73
Thyroid-stimulating hormone	11	31	1	7	2	18, 24, 31, 33, 51, 57, 59, 67, 68, 71, 72
Thyroxine 3, free	1	1	1	1	1	31
Thyroxine 3, total	2	1	1	1	1	57, 59
Thyroxine 4, free	3	6	1	3	2	18, 31, 33
Thyroxine 4, total	3	6	1	4	1	51, 57, 59
Transferrin	6	13	1	4	1.5	15, 31, 43, 48, 57, 74
Triglycerides	11	28	1	4	2	15, 31, 48, 51, 53, 56, 57, 58, 60, 67, 74
Urea	13	28	1	4	2	15, 46, 47, 48, 49, 51, 56, 57, 58, 61, 66, 67, 74
Uric acid	10	29	2	4	2	15, 48, 51, 53, 57, 58, 61, 66, 67. 74
Vitamin B_{12}	5	13	1	4	3	31, 42, 50, 51, 57
Zinc	2	3	1	2	1.5	41, 57

Table 2-3: Analytical Methods

Company/Manufacturer	Analyzer/Assay/Equipment	Study Reference
Abbott	ABA-200	52
	Fluorescence polarization immunoassay	32
	IRMA	57
	Spectrum CCx	52
	Architect	15
	IMx	31
Adaltis	RIA	31
Agilent	Chemstation 1100	27
American Monitor Corp.	American Monitor Parallel	57
Amersham Buchler	RIA	50
Amersham Corporation	RIA	57
Baxter	Stratus II	51
Bayer	Advia	35, 67
	DAX	49
BDH	ERIS Selective Multichannel Analyzer	64
Beckman	Array	57
	Enzymatic Aniylase-OS Reagant	57
Becton-Dickinson	BBL fibrometre	53
BIO-RAD Diagnostics	RIA; Quantphase II	42
Cambridge	Clinical Assays	57
Ciba-Coming	CMAGIC	57
Cisbio	CHILA	31
	RIA	31
Dade Behring	BNA II	31, 43, 44, 45
DPC/Siemens	Advia	72
	Chemiluminescence immunoassay	68
	CHILA	31
	Immulite (2000, 2500)	17, 21, 23, 37
	RIA	31, 63
DSL Laboratories	IRMA	31
	RIA	31
Endocrine Sciences	Equilibrium dialysis	57
	RIA	57
GBC Scientific Equipment	Absorption Spectrometre 902	41
Hybritech	Tandem-E, CEA Kit	57
Immunotech	IRMA	70
	RIA	31
IMMUtest Neopterin	RIA	56
INCSTAR Corp.	RIA	57
Kodak	Ektachem 700	53, 56
Kone	Progress Selective	51
Nichols	Advantage	26, 39
Olympus	Olympus Demand	53
Organon Teknika	IRMA	59

Company/Manufacturer	Analyzer/Assay/Equipment	Study Reference
Ortho Clinical Diagnostics	Ortho Vitros FS 5.1	74
Pantex	RIA	1310
Perkin Elmer/Wallac	200 HPLC-2000 MS	14
	Absorption Spectrometre Model 4110	336
	AutoDelfia	21, 162, 247, 301
	Tandem-MS	1677
R&D Systems	ELISA	19
Radiometre	ABL 555 & 625	38
	HemoCue	15, 74
	ICA 1	57
Roche	Cobas Core	47, 31
	Cobas Fara	55
	Elecsys 2010	18, 28, 34
	Ferrozine reaction	43
	Hitachi 704, 717, 747, 917	31, 40, 48, 54, 56
	Integra 800	25
	Modular E	20, 24, 70
Technicon	AutoAnalyzer II	57
	DAX-18	60
	RA 1000, SMA-20, SMA 6/60	46, 57, 65
Thermo Scientific	Finnigan TSQ Quantum Ultra	16
Toshiba	TBA-C8000	75
Trinity Biotech	AMAX 400	31
Union Carbide	Centrificihem 500	61
Varied	Varied	58
N/A	In house ELISA	29
Not provided	Absorption spectrometry	57
	GC-MS	50
	LC-MS	73
	Not provided	21, 28, 56, 57, 62, 66

Table 2-4: Changes in Analyte Concentration with Age

Analyte	Units	Trend With Age	Method of Testing	Significance	Study Ref	
Adrenocorticotropin hormone	**pmol/L**	**None**	T-test	NS	57	
Alanine aminotransferase	**µkat/L**	↓	Graphical Depiction	N/A	40	
		None	Graphical Depiction	N/A	48	
		↓	Numerical Comparison	N/A	49	
		None	Mann-Whitney U test	NS	56	
		None	T-test	NS	57	
		↓	T-test	p<0.001	58	*
Albumin	**g/L**	↓	Univariate Linear Regression	NP	47	
		↓	Graphical Depiction	N/A	48	
		↓	Numerical Comparison	N/A	49	
		↓	ANOVA	p<0.01	53	*
		↓	T-test	p<0.001	57	
		None	T-test	NS	58	*
		↓	T-test	p<0.05	75	
Alkaline phosphatase	**µkat/L**	**None**	Graphical Depiction	N/A	40	
		↑	Graphical Depiction	N/A	48	
		↑	Numerical Comparison	N/A	49	
		↑	Mann-Whitney U test	<0.0101	56	
		↑	T-test	p<0.001	57	
Amylase	**µkat/L**	**None**	Graphical Depiction	N/A	48	
		↑	T-test	p<0.001	57	
Androstenedione	**nmol/L**	↓ **F**	ANOVA	p<0.001	69	
		↓	T-test	NS	57	
Apolipoprotein A-1	**g/L**	**None**	Mann-Whitney U test	NS	56	
		↑ **then** ↓	Graphical Depiction	N/A	60	
Apolipoprotein B	**g/L**	↑ **F**	T-test & Correlation	p<0.05 & p<0.0001	52	
		↑ **then** ↓	Graphical Depiction	N/A	60	
Arginine	**µmol/L**	**None**	Kruskill-Wallis Test	NS	27	
Aspartate aminotransferase	**µkat/L**	**None**	Graphical Depiction	N/A	40	
		None	Graphical Depiction	N/A	48	
		None	Mann-Whitney U test	NS	56	
		↑	T-test	NS	57	
		None	T-test	NS	58	*
Asymmetric dimethyl arginine	**µmol/L**	↑	Kruskill-Wallis Test	p<0.05	27	
Bilirubin, conjugated	**µmol/L**	**None**	Mann-Whitney U test	NS	56	
Bilirubin, total	**µmol/L**	↓	Graphical Depiction	N/A	40	
		None	Mann-Whitney U test	NS	56	
		↓	T-test	p<0.001	57	
B-type natriuretic peptide	**ng/L**	↑	ANOVA & Multivariate Linear	p<0.002 & p<0.002	28	*
N-terminal-pro-B-type natriuretic peptide	**ng/L**	↑	ANOVA & Multivariate Linear	p=0.008 & p<0.001	28	*
		↑	Kruskill-Wallis Test	p=0.035	34	
Calcium, total	**mmol/L**	↓	Graphical Depiction	N/A	48	
		↓	T-test	p<0.002	57	
		None	Numerical Comparison	N/A	65	*
Carbon dioxide, partial	**kPa**	**None**	Multivariate Linear Regression	NS	38	*
Carbon dioxide, partial pressure	**kPa**	**None**	Multivariate Linear Regression	NS	38	*

Analyte	Units	Trend With Age	Method of Testing	Significance	Study Ref	
Carcinoembryonic antigen	**µg/L**	↑	Multivariate Linear Regression	p<0.001	22	
		↑	T-test	NS	57	
Chloride	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		None	T-test	NS	57	
		None	Numerical Comparison	N/A	65	*
Cholesterol	**mmol/L**	↑	Graphical Depiction	N/A	47	
		None	Graphical Depiction	N/A	48	
		None	ANOVA	p<0.01	53	*
		↑	Mann-Whitney U test	<0.0001	56	
		↑	T-test	p<0.001	57	
		↓	T-test	p<0.001	58	*
		↑	Graphical Depiction	N/A	60	
Copper	**µmol/L**	↑	ANOVA & Scheffe	p<0.0001	41	
		↑	T-test	NP	57	
Cortisol, total	**nmol/L**	**None**	T-test	NS	57	
C-peptide	**nmol/L**	↑	T-test	p<0.001	57	
C-reactive protein	**mg/L**	↑	Numerical Comparison	N/A	44	
		↑	z-test & Univariate Linear Regression	p<0.05	45	
Creatine kinase	**µkat/L**	**None**	Graphical Depiction	N/A	48	
		↓	Numerical Comparison	N/A	49	
		None	Multivariate Linear Regression	NS	54	
		↓	T-test	p<0.001	57	
Creatinine	**µmol/L**	↑	Univariate Linear Regression	p<0.05	25	
		None	T-test	NS	46	
		↑	Numerical Comparison	N/A	47	
		↑	Graphical Depiction	N/A	48	
		↑	Numerical Comparison	N/A	49	
		↑	ANOVA	p<0.01	53	*
		None	Univariate Linear Regression	NS	55	
		None	Mann-Whitney U test	NS	56	
		↑	T-test	p<0.01	57	
		None	T-test	NS	58	*
Dehydroepiandrosterone sulfate	**µmol/L**	**↓ M**	Univariate Linear Regression	p<0.01	23	
		↓	T-test	NS	57	
Dehyroepiandrosterone,	**nmol/L**	↓	T-test	NS	57	
Dhihydrotestosterone	**nmol/L**	↓	Multivariate Logistic Regression	p<0.001	73	*
Estradiol	**pmol/L**	**None**	Correlation (Pearson/Spearman)	NS	30	*
		↓	T-test	NS	57	
		↓	Multivariate Logistic Regression	p<0.001	73	*
Ferritin	**µg/L**	↑	Multivariate Linear Regression	NP	63	
		↓ M	Correlation (Pearson/Spearman)	p<0.05	43	*
α-Fetoprotein	**g/L**	↑	Multivariate Linear Regression	p<0.001	22	
Fibrinogen	**µmol/L**	**↓ M ↑ F**	ANOVA	p<0.01	53	*
Folate	**nmol/L**	**None**	T-test	p=0.1	50	
		↓	T-test	NS	57	
Follicle-stimulating hormone	**IU/L**	**↑ M**	Multivariate Linear Regression	p<0.001	20	
		↑	Correlation (Pearson/Spearman)	p<0.001	30	*
		↑	T-test	p<0.001	57	

Analyte	Units	Trend With Age	Method of Testing	Significance	Study Ref	
Glucose	**mmol/L**	↑	Graphical Depiction	N/A	47	
		↑	ANOVA	p<0.01	53	*
		↑	Mann-Whitney U test	p<0.0013	56	
		↑	T-test	p<0.001	57	
		↓	T-test	p<0.001	58	*
Glucose, fasting		**None**	Graphical Depiction	N/A	48	
ϒ- Glutamyltransferase	**µkat/L**	**None**	Graphical Depiction	N/A	48	
		↑	Mann-Whitney U test	<0.0252	56	
		↑	T-test	p<0.01	57	
High-density lipoprotein cholesterol	**mmol/L**	**None**	Mann-Whitney U test	NS	56	
		None	ANOVA	NS	53	*
		↑	T-test	p<0.001	57	
		None	T-test	NS	58	*
Homoarginine	**µmol/L**	**None**	Kruskill-Wallis Test	NS	27	
Homocysteine	**µmol/L**	↑	ANOVA& Tukey Post-hoc	p<0.05	32	
		↑	T-test	p>0.0001	50	
Insulin, fasting	**pmol/L**	**↑M ↓F**	ANOVA	p<0.01	53	*
Insulin-like growth factor-I	**µg/L**	↓	Graphical Depiction	N/A	17	
		↓	Multivariate Linear Regression	p<0.05	19	
		↓	Multivariate Linear Regression	p<0.001	21	
		↓	Univariate Linear Regression &	p<0.001	26	
		↓	Polynomial Regression	p<0.0001	39	
		↓	Kruskill-Wallis Test	p<0.05	70	
Insulin-like growth factor binding protein-3	**mg/L**	↓	Graphical Depiction	N/A	17	
		↓	Univariate Linear Regression &	p<0.002	26	
Insulin tolerance test	**mEq/L**	↓	Multivariate Linear Regression	p=0.02	21	
Iron	**µmol/L**	**None**	Graphical Depiction	N/A	48	
		↓ M	Correlation (Pearson/Spearman)	p<0.05	43	*
		↓	T-test	NS	57	
Lactate dehydrogenase	**µkat/L**	↑	Graphical Depiction	N/A	48	
		↑	Numerical Comparison	N/A	49	
		↑	Multivariate Linear Regression	p<0.05	54	
		↑	T-test	p<0.001	57	
Lactate dehydrogenase isoenzyme 1	**µkat/L**	↑	Multivariate Linear Regression	p<0.05	54	
		↓	T-test	NS	57	
Lead	**µmol/L**	↓	T=test	NS	57	
Lipase	**µkat/L**	**None**	Graphical Depiction	N/A	48	
		↑	T-test	p<0.001	57	
Low-density lipoprotein cholesterol	**mmol/L**	↑	T-test & Correlation	p<0.05 & p<0.0001	52	
		↑	Mann-Whitney U test	<0.0001	56	
		None	ANOVA	NS	53	*
Luteinizing hormone	**IU/L**	**↑ M**	Multivariate Linear Regression	p<0.05	20	
		↑	Correlation (Pearson/Spearman)	p<0.001	30	*
		↑	T-test	p<0.001	57	

Analyte	Units	Trend With Age	Method of Testing	Significance	Study Ref	
Magnesium	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		↑	T-test	NS	57	
		↑	Rank Sum Test	0.05>p> 0.02	64	
Methlymalonic acid	**nmol/L**	↑	Numerical Comparison	N/A	14	
		↑	T-test	p>0.0001	50	
Oxygen, arterial partial pressure	**kPa**	**None**	Multivariate Linear Regression	NS	38	*
Oxygen, partial pressure	**kPa**	**None**	Multivariate Linear Regression	NS	38	*
Oxygen, saturation	**%**	**None**	Multivariate Linear Regression	NS	38	*
Parathyroid hormone	**pmol/L**	↑	T-test	p<0.001	57	
pH	**pH**	↓	T-test	NP	57	
Phosphate	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		↓ M	T-test	p<0.001	57	
Potassium	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		↑	ANOVA	p<0.01	53	*
		None	Numerical Comparison	NS	65	*
Progesterone	**nmol/L**	↓	T-test	NS	57	
Prolactin	**µg/L**	↑	T-test	p<0.001	57	
Protein, total	**g/L**	**None**	Graphical Depiction	N/A	48	
		None	Mann-Whitney U test	NS	56	
		↓	T-test	NS	57	
		None	T-test	NS	58	*
Serum amyloid A	**mg/L**	↑	T-test	p<0.01	29	
Sex hormone binding globulin	**nmol/L**	**↑ M**	Multivariate Linear Regression	p<0.001	20	
		↑ M	Correlation (Pearson/Spearman)	p<0.001	30	
		↑	T-test	NS	57	*
Silicon	**µmol/L**	↓	Mann-Whitney U test	p<0.000	36	
Sodium	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		None	T-test	NS	57	
		None	Numerical Comparison	NS	65	*
Symmetric dimethyl arginine	**µmol/L**	↑	Kruskill-Wallis Test	p<0.05	27	
Testosterone, free	**pmol/L**	**↓ M**	Graphical Depiction	N/A	16	
		↓	Correlation (Pearson/Spearman)	p<0.001	30	*
		↓ F	ANOVA	p<0.001	69	
		↓	T-test	NS	57	
Testosterone, total	**nmol/L**	**↓ M**	Graphical Depiction	N/A	16	
		↓ M	Multivariate Linear Regression	p<0.001	20	
		↓ M	Univariate Linear Regression	p<0.01	23	
		None	Correlation (Pearson/Spearman)	NS	30	*
		↓	T-test	NS	57	
		↓ F	ANOVA	p<0.001	69	
		↓	Multivariate Logistic Regression	p<0.001	73	*
Thyroid-stimulating hormone	**mIU/L**	↑	Univariate Linear Regression	p<0.00001	18	
		↑	Numerical Comparison	N/A	24	
		None	Correlation (Pearson/Spearman)	p=0.69	33	*
		↑	T-test	p<0.05	57	
		None	T-test	p>0.05	68	
		↑	Kruskill-Wallis Test	p<0.001	71	
		↑	Univariate Linear Regression	p<0.01	72	

Analyte	Units	Trend With Age	Method of Testing	Significance	Study Ref	
Thyroxine 3, total	**nmol/L**	↓	T-test	NS	57	
Thyroxine 4, free	**pmol/L**	↓ **M**	Univariate Linear Regression	p<0.000001	18	
		↑	Correlation (Pearson/Spearman)	p<0.001	33	*
Thyroxine 4, total	**nmol/L**	↓	T-test	NS	57	
Transferrin	**g/L**	**None**	Graphical Depiction	N/A	48	
		↓	T-test	p<0.001	57	
Triglycerides	**mmol/L**	**None**	Graphical Depiction	N/A	48	
		None	ANOVA	NS	53	*
		↑	Mann-Whitney U test	<0.0915	56	
		↑	T-test	p<0.05	57	
		None	T-Test	NS	58	*
		None	Graphical Depiction	N/A	60	
Urea	**mmol/L**	**None**	T-test	NS	46	
		↑	Graphical Depiction	N/A	47	
		↑	Graphical Depiction	N/A	48	
		↑	Numerical Comparison	N/A	49	
		↑	Mann-Whitney U test	p<0.0022	56	
		↑	T-test	p<0.001	57	
		↑	T-test	p<0.001	58	*
Uric acid	**mmol/L**	↑	Graphical Depiction	N/A	48	
		None	ANOVA	NS	53	*
		↑**F**	T-test	NS	57	
		None	T-test	NS	58	*
Vitamin B_{12}	**pmol/L**	↓	ANOVA & Tukey	p<0.01	42	
		None	T-test	p=0.52	50	
		↓	T-test	NS	57	
Zinc	**µmol/L**	↑	ANOVA & Scheffe Post-hoc	p<0.011	41	
		↓	T-test	NS	57	

2.7 References

(1) Forsman, R. W. (1996). Why is the Laboratory an Afterthought for Managed Care Organizations. *Clinical Chemistry*, *5*, 813-816.

(2) Horn, P. S., & Pesce, A. J. (2005). *Reference Intervals: A User's Guide*. Washington, DC: American Association for Clinical Chemistry.

(3) Solberg, H. (1987). Approved Recommendation (1986) on the Theory of Reference Values. Part 1. The Concept of Reference Values. *Clinica Chimica Acta*, *165*(1), 111-118.

(4) Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 2. Selection of Individuals for the Production of Reference Values. *Clinical Chimica Acta*, *170*, S3-S12

(5) Solberg, H. (1988). Approved Recommendation (1987) on the Theory of Reference Values. Part 3. Preparation of Individuals and Collection of Specimens for the Production of Reference Values. *Clinica Chimica Acta*, *177*(3), S3-S11.

(6) Solberg, H. (1991). IFCC Recommendation-Theory of Reference Values Part 4. Control of Analytical Variation in the Production, Transfer, and Application of Reference Values. *Clinica Chimica Acta*, *202*(3), S5-S12.

(7) Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 5. Statistical Treatment of Collected Reference Values. Determination of Reference Limits. *Clinica Chimica Acta*, *170*(2-3), S13-S32.

(8) Solberg, H. (1987). Approved Recommendation (1987) on the Theory of Reference Values. Part 6. Presentation of Observed Values Related to Reference Values. *Clinica Chimica Acta*, *170*(2-3), S33-S42.

(9) Clinical and Laboratory Standards Institute (CLSI). (2008). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline. CLSI document C28-A3. 3rd ed. Wayne P.A.: Clinical and Laboratory Standards Institute.

(10) McPherson, K., Healy, M., Flynn, F., Piper, K., & Garcia-Webb, P. (1978). The Effect of Age, Sex and Other Factors on Blood Chemistry in Health. *Clinica Chimica Acta*, *84*(3), 373-397.

(11) Faulkner, W. R., & Meites, S. (1994). *Geriatric Clinical Chemistry: Reference Values*. Washington, DC: AACC Press. Chapters 1-5.

(12) Roebuck, J. (1979). When Does "Old Age Begin?": The Evolution Of The English Definition. *Journal of Social History, 12*(3), 416-428.

(13) Tietz, N. W., Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2006). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (4th ed.). St. Louis, Mo.: Elsevier Saunders. Section I & Section II – Analytical Techniques and Instrumentation.

(14) Erdogan, E., Nelson, G. J., Rockwood, A. L., and Frank, E. L. (2010) Evaluation of Reference Intervals for Methylmalonic acid in plasma/serum and urine. *Clinica Chimica Acta, 21-22,* 1827-1829.

(15) Carlsson, L., Lind, L., and Larsson, A. (2010) Reference Values for 27 Clinical Chemistry Tests in 70-year-old Males and Females. *Gerontology, 3,* 259-265.

(16) Salameh, W. A., Redor-Goldman, M. M., Clarke, N. J., Reitz, R. E., and Caulfield, M. P. (2010) Validation of a Total Testosterone Assay using High-turbulence Liquid Chromatography Tandem Mass Spectrometry: Total and Free Testosterone Reference Ranges. *Steroids, 2,* 169-175.

(17) Friedrich, N., Krebs, A., Nauck, M., and Wallaschofski, H. (2010) Age- and Gender-specific Reference Ranges for Serum Insulin-like Growth Factor I (IGF-I) and IGF-Binding Protein-3 Concentrations on the Immulite 2500: Results of the Study of Health in Pomerania (SHIP). *Clinical Chemistry; Laboratory Medicine, 1,* 115-120.

(18) Takeda, K., Mishiba, M., Sugiura, H., Nakajima, A., Kohama, M., and Hiramatsu, S. (2009) Evaluated Reference Intervals for Serum Free Thyroxine and Thyrotropin using the Conventional Outlier Rejection Test without Regard to Presence of Thyroid Antibodies and Prevalence of Thyroid Dysfunction in Japanese Subjects. *Endocrine Journal, 9,* 1059-1066.

(19) Andreassen, M., Nielsen, K., Raymond, I., Kristensen, L. O., and Faber, J. (2009) Characteristics and Reference ranges of Insulin-Like Growth Factor-I Measured with a Commercially Available Immunoassay in 724 Healthy Adult Caucasians. *Scandinavian Journal of Clinical Laboratory Investigation, 8,* 880-885.

(20) Bjerner, J., Biernat, D., Fossa, S. D., and Bjoro, T. (2009) Reference Intervals for Serum Testosterone, SHBG, LH and FSH in Males from the NORIP Project. *Scandinavian Journal of Clinical Laboratory Investigation, 8,* 873-879.

(21) Eskes, S. A., Tomasoa, N. B., Endert, E., Geskus, R. B., Fliers, E., and Wiersinga, W. M. (2009) Establishment of Reference Values for Endocrine Tests. Part VII: Growth Hormone Deficiency. *Netherlands Journal of Medicine, 4,* 127-133.

(22) Bjerner, J., Hogetveit, A., Wold, Akselberg K., Vangsnes, K., Paus, E., Bjoro, T., Bormer, O. P., and Nustad, K. (2008) Reference Intervals for Carcinoembryonic Antigen (CEA), CA125, MUC1, Alfa-foeto-protein (AFP), Neuron-specific Enolase (NSE) and CA19.9 from the NORIP Study. *Scandinavian Journal of Clinical Laboratory Investigation, 8,* 703-713.

(23) Friedrich, N., Volzke, H., Rosskopf, D., Steveling, A., Krebs, A., Nauck, M., and Wallaschofski, H. (2008) Reference Ranges for Serum Dehydroepiandrosterone Sulfate and Testosterone in Adult Men. *Journal of Andrology, 6,* 610-617.

(24) Friis-Hansen, L. and Hilsted, L. (2008) Reference Intervals for Thyreotropin and Thyroid Hormones for Healthy Adults Based on the NOBIDA Material and Determined Using a Modular E170. *Clinical Chemistry Laboratory Medicine, 9,* 1305-1312.

(25) Pottel, H., Vrydags, N., Mahieu, B., Vandewynckele, E., Croes, K., and Martens, F. (2008) Establishing Age/Sex Related Serum Creatinine Reference Intervals from Hospital Laboratory Data Based on Different Statistical Methods. *Clinica Chimica Acta, 1-2,* 49-55.

(26) Friedrich, N., Alte, D., Volzke, H., Spilcke-Liss, E., Ludemann, J., Lerch, M. M., Kohlmann, T., Nauck, M., and Wallaschofski, H. (2008) Reference Ranges of Serum IGF-1 and IGFBP-3 levels in a General Adult Population: Results of the Study of Health in Pomerania (SHIP). *Growth Hormone Research, 3,* 228-237.

(27) Meinitzer, A., Puchinger, M., Winklhofer-Roob, B. M., Rock, E., Ribalta, J., Roob, J. M., Sundl, I., Halwachs-Baumann, G., and Marz, W. (2007) Reference Values for Plasma Concentrations of Asymmetrical Dimethylarginine (ADMA) and other Arginine Metabolites in Men after Validation of a Chromatographic Method. *Clinica Chimica Acta, 1-2,* 141-148.

(28) Alehagen, U., Goetze, J. P., and Dahlstrom, U. (2007) Reference Intervals and Decision Limits for B-type Natriuretic Peptide (BNP) and its Precursor (Nt-proBNP) in the Elderly. *Clinica Chimica Acta, 1-2,* 8-14.

(29) Wu, T. L., Tsai, I. Chen, Chang, P. Y., Tsao, K. C., Sun, C. F., Wu, L. L., and Wu, J. T. (2007) Establishment of an in-house ELISA and the Reference Range

for Serum Amyloid A (SAA): Complementarity Between SAA and C-reactive Protein as Markers of Inflammation. *Clinica Chimica Acta, 1-2,* 72-76.

(30) Eskelinen, S., Vahlberg, T., Isoaho, R., Kivela, S. L., and Irjala, K. (2007) Biochemical Reference Intervals for Sex Hormones with a New AutoDelfia Method in Aged Men. *Clinical Chemistry Laboratory Medicine 2007, 2,* 249-253.

(31) Huber, K. R., Mostafaie, N., Stangl, G., Worofka, B., Kittl, E., Hofmann, J., Hejtman, M., Michael, R., Weissgram, S., Leitha, T., Jungwirth, S., Fischer, P., Tragl, K. H., and Bauer, K. (2006) Clinical Chemistry Reference Values for 75-year-old Apparently Healthy Persons. *Clinical Chemistry Laboratory Medicine 2006, 11,* 1355-1360.

(32) Ganji, V. and Kafai, M. R. (2006) Population Reference Values for Plasma Total Homocysteine Concentrations in US Adults after the Fortification of Cereals with Folic Acid. *American Journal of Clinical Nutrition, 5,* 989-994.

(33) Eskelinen, S., Suominen, P., Vahlberg, T., Lopponen, M., Isoaho, R., Kivela, S. L., and Irjala, K. (2005) The Effect of Thyroid Antibody Positivity on Reference Intervals for Thyroid Stimulating Hormone (TSH) and Free Thyroxine (FT4) in an Aged Population. *Clinical Chemistry Laboratory Medicine, 12,* 1380-1385.

(34) Shi, X., Xu, G., Xia, T., Song, Y., and Lin, Q. (2005) N-terminal-pro-B-type Natriuretic Peptide (NT-proBNP): Reference Range for Chinese Apparently Healthy People and Clinical Performance in Chinese Elderly Patients with Heart Failure. *Clinica Chimica Acta, 1-2,* 122-127.

(35) Evrin, P. E., Nilsson, S. E., Oberg, T., and Malmberg, B. (2005) Serum C-reactive Protein in Elderly Men and Women: Association with Mortality, Morbidity and Various Biochemical Values. *Scandinavian Journal of Clinical Laboratory Investigation, 1,* 23-31.

(36) Bisse, E., Epting, T., Beil, A., Lindinger, G., Lang, H., and Wieland, H. (2005) Reference Values for Serum Silicon in Adults. *Analytical Biochemistry, 1,* 130-135.

(37) Elmlinger, M. W., Kuhnel, W., Weber, M. M., and Ranke, M. B. (2004) Reference Ranges for Two Automated Chemiluminescent Assays for Serum Insulin-like Growth Factor I (IGF-I) and IGF-Binding Protein 3 (IGFBP-3). *Clinical Chemistry Laboratory Medicine, 6,* 654-664.

(38) Hardie, J. A., Vollmer, W. M., Buist, A. S., Ellingsen, I., and Morkve, O. (2004) Reference Values for Arterial Blood Gases in the Elderly. *Chest, 6,* 2053-2060.

(39) Brabant, G., von zur, Muhlen A., Wuster, C., Ranke, M. B., Kratzsch, J., Kiess, W., Ketelslegers, J. M., Wilhelmsen, L., Hulthen, L., Saller, B., Mattsson, A., Wilde, J., Schemer, R., Kann, P., and German KIMS Board. (2003) Serum Insulin-like Growth Factor I Reference Values for an Automated Chemiluminescence Immunoassay System: Results from a Multicenter Study. *Hormone Research, 2,* 53-60.

(40) Bock, B. J., Dolan, C. T., Miller, G. C., Fitter, W. F., Hartsell, B. D., Crowson, A. N., Sheehan, W. W., and Williams, J. D. (2003) The Data Warehouse as a Foundation for Population-based Reference Intervals. *American Journal of Clinical Pathology, 5,* 662-670.

(41) Abiaka, C., Olusi, S., and Al-Awadhi, A. (2003) Reference Ranges of Copper and Zinc and the Prevalence of Their Deficiencies in an Arab Population aged 15-80 Years. *Biological Trace Element Research, 1,* 33-43.

(42) Wahlin, A., Backman, L., Hultdin, J., Adolfsson, R., and Nilsson, L. G. (2002) Reference Values for Serum Levels of Vitamin B12 and Folic Acid in a Population-based Sample of Adults Between 35 and 80 Years of Age. *Public Health Nutrition, 3,* 505-511.

(43) Takala, T. I., Suominen, P., Isoaho, R., Kivela, S. L., Lopponen, M., Peltola, O., Rajamaki, A., and Irjala, K. (2002) Iron-replete Reference Intervals to Increase Sensitivity of Hematologic and Iron Status Laboratory Tests in the Elderly. *Clinical Chemistry, 9,* 1586-1589.

(44) Herbeth, B., Siest, G., and Henny, J. (2001) High sensitivity C-reactive protein (CRP) Reference Intervals in the Elderly. *Clinical Chemistry Laboratory Medicine, 11,* 1169-1170.

(45) Wener, M. H., Daum, P. R., and McQuillan, G. M. (2000) The Influence of Age, Sex, and Race on the Upper Reference Limit of Serum C-reactive Protein Concentration. *Journal of Rheumatology, 10,* 2351-2359.

(46) Erasmus, R. T., Ray, U., Nathaniel, K., and Dowse, G. (1997) Reference Ranges for Serum Creatinine and Urea in Elderly Coastal Melanesians. *Papua New Guinea Medical Journal, 2,* 89-91.

(47) Kroll, J. and Saxtrup, O. (1998) On the Use of Patient Data for the Definition of Reference Intervals in Clinical Chemistry. *Scandinavian Journal of Clinical Laboratory Investigation, 6,* 469-473.

(48) Boulat, O., Krieg, M. A., Janin, B., Burckhardt, P., Francioli, P., and Bachmann, C. (1998) Clinical Chemistry Variables in Normal Elderly and Healthy Ambulatory Populations: Comparison with Reference Values. *Clinica Chimica Acta, 2,* 127-135.

(49) Mold, J. W., Aspy, C. B., Blick, K. E., and Lawler, F. H. (1998) The Determination and Interpretation of Reference Intervals for Multichannel Serum Chemistry Tests. *Journal of Family Practice, 3,* 233-241.

(50) Joosten, E., Lesaffre, E., and Riezler, R. (1996) Are Different Reference Intervals for Methylmalonic Acid and Total Homocysteine Necessary in Elderly People?. *European Journal of Haematology, 3,* 222-226.

(51) Hammerman-Rozenberg, R., Cohen, A., Ginsberg, G., Maaravi, Y., Ebstein, R. P., and Stessman, J. (1996) Laboratory Reference Values for the 70 Year Olds. *Israel Journal of Medical Sciences, 8,* 611-620.

(52) Contois, J. H., McNamara, J. R., Lammi-Keefe, C. J., Wilson, P. W., Massov, T., and Schaefer, E. J. (1996) Reference Intervals for Plasma Apolipoprotein B Determined with a Standardized Commercial Immunoturbidimetric Assay: Results from the Framingham Offspring Study. *Clinical Chemistry, 4,* 515-523.

(53) Robbins, J., Wahl, P., Savage, P., Enright, P., Powe, N., and Lyles, M. (1995) Hematological and Biochemical Laboratory Values in Older Cardiovascular Health Study Participants. *Journal of the American Geriatrics Society, 8,* 855-859.

(54) Kairisto, V., Hanninen, K. P., Leino, A., Pulkki, K., Peltola, O., Nanto, V., Voipio-Pulkki, L. M., and Irjala, K. (1994) Generation of Reference Values for Cardiac Enzymes from Hospital Admission Laboratory Data. *European Journal of Clinical Chemistry Clinical Biochemistry, 10,* 789-796.

(55) Sokoll, L. J., Russell, R. M., Sadowski, J. A., and Morrow, F. D. (1994) Establishment of Creatinine Clearance Reference Values for Older Women. *Clinical Chemistry, 12,* 2276-2281.

(56) Stulnig, T., Mair, A., Jarosch, E., Schober, M., Schonitzer, D., Wick, G., and Huber, L. A. (1993) Estimation of Reference Intervals from a SENIEUR

Protocol Compatible Aged Population for Immunogerontological Studies. *Mechanisms of Ageing & Development, 1-3,* 105-115.

(57) Tietz, N. W., Shuey, D. F., and Wekstein, D. R. (1992) Laboratory Values in Fit Aging Individuals--Sexagenarians through Centenarians. *Clinical Chemistry, 6,* 1167-1185.

(58) Lio, D., Malaguarnera, M., Maugeri, D., Ferlito, L., Bennati, E., Scola, L., Motta, M., and Caruso, C. (2008) Laboratory Parameters in Centenarians of Italian Ancestry. *Experimental Gerontology, 2,* 119-122.

(59) Maugeri, D., Speciale, S., Santangelo, A., Motta, M., and Panebianco, P. (1999) Altered Laboratory Thyroid Parameters in Elderly People. *Journal of Endocrinological Investigation, 10,* Supp.

(60) Jungner, I., Marcovina, S. M., Walldius, G., Holme, I., Kolar, W., and Steiner, E. .(1998) Apolipoprotein B and A-I values in 147576 Swedish Males and Females, Standardized According to the World Health Organization-International Federation of Clinical Chemistry First International Reference Materials. *Clinical Chemistry, 8,* Pt 1: t-9.

(61) Najjar, M. and Carter-Pokras, O. (1992) Clinical Chemistry Profile Data for Hispanics, 1982-84. *Vital & Health Statistics - Series 11: Data From the National Health Survey, 241,* 1-53.

(62) Lawrence, C. J. and Trewin, V. F. (1991) The Construction of Biochemical Reference Ranges and the Identification of Possible Adverse Drug Reactions in the Elderly. *Statistics in Medicine, 6,* 831-837.

(63) Vicente, C., Porto, G., and de, Sousa M. (1990) Method for Establishing Serum Ferritin Reference Values Depending on Sex and Age. *Journal of Laboratory & Clinical Medicine, 6,* 779-784.

(64) Duncanson, G. O. and Worth, H. G. (1990) Determination of Reference Intervals for Serum Magnesium. *Clinical Chemistry, 5,* 756-758.

(65) Garry, P. J., Hunt, W. C., VanderJagt, D. J., and Rhyne, R. L. (1989) Clinical Chemistry Reference Intervals for Healthy Elderly Subjects. *American Journal of Clinical Nutrition, 50,* 1219-1230.

(66) Kornitzer, M. and Bara, L. (1989) Clinical and Anthropometric Data, Blood Chemistry and Nutritional Patterns in the Belgian Population According to Age and Sex. *For the B.I.R.N.H. Study Group. Acta Cardiologicam, 2,* 101-144.

(67) Millan-Calenti, J. C., Sanchez, A., Lorenzo-Lopez, L., and Maseda, A. (2012) Laboratory Values in a Spanish Population of Older Adults: A Comparison with Reference Values from Younger Adults. *Maturitas, 4,* 396-401.

(68) Li, C., Guan, H., Teng, X., Lai, Y., Chen, Y., Yu, J., Li, N., Wang, B., Jiang, F., Wang, J., Fan, C., Wang, H., Zhang, H., Teng, W., and Shan, Z. (2011) An Epidemiological Study of the Serum Thyrotropin Reference Range and Factors that Influence Serum Thyrotropin Levels in Iodine Sufficient Areas of China. *Endocrine Journal, 11,* 995-1002.

(69) Haring, R., Hannemann, A., John, U., Radke, D., Nauck, M., Wallaschofski, H., Owen, L., Adaway, J., Keevil, B. G., and Brabant, G. (2012) Age-specific Reference Ranges for Serum Testosterone and Androstenedione Concentrations in Women Measured by Liquid Chromatography-tandem Mass Spectrometry. *Journal of Clinical Endocrinology & Metabolism 2012, 2,* 408-415.

(70) Bayram, F., Gedik, V. T., Demir, O., Kaya, A., Gundogan, K., Emral, R., Ozturk, A., Uysal, A. R., and Corapcioglu, D. (2011) Epidemiologic Survey: Reference Ranges of Serum Insulin-like Growth Factor 1 Levels in Caucasian Adult Population with Immunoradiometric assay. *Endocrine, 2,* 304-309.

(71) Vadiveloo, T., Donnan, P. T., Murphy, M. J., and Leese, G. P. (2013) Age- and gender-specific TSH Reference Intervals in People with no Obvious Thyroid Disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS). *Journal of Clinical Endocrinology & Metabolism, 3,* 1147-1153.

(72) Kahapola-Arachchige, K. M., Hadlow, N., Wardrop, R., Lim, E. M., and Walsh, J. P. (2012) Age-specific TSH Reference Ranges have Minimal Impact on the Diagnosis of Thyroid Dysfunction. *Clinical Endocrinology, 5,* 773-779.

(73) Yeap, B. B., Alfonso, H., Chubb, S. A., Handelsman, D. J., Hankey, G. J., Norman, P. E., and Flicker, L. (2012) Reference Ranges and Determinants of Testosterone, Dihydrotestosterone, and Estradiol Levels Measured using Liquid Chromatography-tandem Mass Spectrometry in a Population-based Cohort of Older Men. *Journal of Clinical Endocrinology & Metabolism, 1,* 4030-4039.

(74) Ryden, I., Lind, L., and Larsson, A. (2012) Reference Values of Thirty-one Frequently used Laboratory Markers for 75-year-old Males and Females. *Upsala Journal of Medical Sciences, 3,* 264-272.

(75) Kubota, K., Kadomura, T., Ohta, K., Koyama, K., Okuda, H., Kobayashi, M., Ishii, C., Fujiwara, Y., Nishiora, T., Ohmae, Y., Ohmae, T., and Kitajima, M. (2012) Analyses of Laboratory Data and Establishment of Reference Values and Intervals for Healthy Elderly people. *Journal of Nutrition, Health & Aging, 4,* 412-416.

(76) The Cardiovascular Health Study. (n.d.). *CHS*. Retrieved June 24, 2014, from https://chs-nhlbi.org

(77) Offspring Cohort. (n.d.). *Framingham Heart Study*. Retrieved June 24, 2014, from http://www.framinghamheartstudy.org/participants/offspring.php

(78) WACHA - HIMS. (n.d.). *WACHA - HIMS*. Retrieved June 24, 2014, from http://www.wacha.org.au/hims.html

(79) National Health and Nutrition Examination Survey. (2014, May 29). *Centers for Disease Control and Prevention*. Retrieved June 22, 2014, from http://www.cdc.gov/nchs/nhanes.htm

(80) NORIP: Nordic Reference Interval Project. (n.d.). *NORIP: Nordic Reference Interval Project*. Retrieved June 24, 2014, from http://pweb.furst.no/norip/

(81) PIVUS - Prospective Investigation of the Vasculature in Uppsala Seniors. (n.d.). - *Uppsala universitet*. Retrieved June 24, 2014, from http://www.medsci.uu.se/pivus/

(82) Research Network Community Medicine - SHIP - Documentation. (n.d.). *Research Network Community Medicine - SHIP - Documentation*. Retrieved June 24, 2014, from http://www.medizin.unigreifswald.de/cm/fv/english/ship_en.html.

(83) Daly, C. H., Liu, X., Grey, V. L., & Hamid, J. S. (2013). A Systematic Review of Statistical Methods used in Constructing Pediatric Reference Intervals. *Clinical Biochemistry*, *46*(13-14), 1220-1227.

(84) Kruger, A. (1987). The Limits of Normality in Elderly Patients. *Bailliere's Clinical Haematology*, *1*(2), 271-289.

(85) Harris, E., & Boyd, J. (1990). On Dividing Reference Data into Subgroups to Produce Separate Reference Ranges. *Clinical Chemistry*, *36*(2), 265-270.

3 THE INFLUENCE OF MORBIDITY ON CREATININE REFERENCE INTERVALS

3.1 Abstract

Background: The majority of elderly individuals suffer from at least one chronic condition. This makes selection of a healthy reference population for calculation of reference intervals (RIs) very difficult in geriatric populations. If all persons with disease are excluded it becomes hard to obtain adequate sample sizes for RI calculation. In addition the reference sample is then no longer representative of the population to which it will be applied. If persons with morbidity are to be included in reference samples to obviate these issues, it is important to establish what effect morbidity has on RIs.

Objective: To determine the effect of morbidity on RIs, specifically geriatric RIs for creatinine.

Methods: Data for participants aged 20-80 years who completed the Medical Conditions Questionnaire (MCQ) and provided a blood sample were obtained from the most recently available (2009-2010) National Health And Nutrition Examination Survey (NHANES). Visual assessment of the data and piecewise linear regression was used to evaluate the increase in creatinine concentration with age. A Mann-Whitney U test was performed to confirm any differences in creatinine concentration after a given age. Multivariable linear regression was then performed to assess the effect number of morbidity has on creatinine levels after adjusting for age, sex and ethnicity. Interaction terms for age and number of morbidity were created and tested for statistical significance. RIs were for creatinine were then calculated using a non-parametric approach when sample sizes were greater than 120

and using a robust method when sample sizes were less than 120 but greater than 30. RIs were stratified by age and number of morbidity and visualized using a Forest plot.

Results: Visual assessment of the data suggested a significant increase in creatinine concentration after age 60. Piecewise linear regression demonstrated a 0.136 μmol/L increase in creatinine levels with the addition of one year in persons aged 20-59 compared to a 0.832 μmol/L increase in persons aged 60-80. A Mann-Whitney U test confirmed the difference in median creatinine concentration between ages 20-59 and 60-80 ($p<0.001$). Number of morbidity was shown to be a significant predictor of creatinine concentration ($p<0.001$) after accounting for age, sex and ethnicity. The interaction terms between age and number of morbidity were significant ($p<0.001$) which indicates that morbidity has a modifying effect with age. Subsequently, age-related RIs stratified by number of morbidity showed that the upper limit for creatinine increases significantly with increasing number of morbidity in older persons.

Conclusions: When calculating RIs it is important to consider the nature of the population to which they will be applied. In the case of geriatrics it is important to consider the effect morbidities have on blood chemistry and account for this variable during RI calculation.

3.2 Introduction

The ambiguity surrounding health definitions is one of the largest areas of concern for RI studies, especially in the elderly population. In any study defining what is considered healthy is a difficult task (1). Defining "health" is particularly important for RI studies as it leads to the establishment of exclusion criteria by which the reference sample is selected. In the field of medicine the definition of "healthy" was adopted from the disease model and often refers to someone who is free of disease (2). As such persons are often excluded from the reference sample based on the presence of morbidity or illness. Applying these criteria to elderly populations drastically reduces sample sizes and jeopardizes the representativeness of the reference sample.

An alternative definition would be using the term 'healthy aging', a definition of health often used in geriatrics (3). The term "healthy aging" takes on more than just the pathological definition of health and refers more to health as a measure of emotional, physical and social wellness (3). This type of definition implies functionality as a measure of health in the elderly and attempts to define persons by their biological age. This definition although it may be more biologically relevant, is ambiguous and impractical to apply to the field of RIs (4). For these reasons, in the practice of RI calculation, health typically refers to someone who is free of disease and age is typically defined chronologically (5).

Selecting individuals to use as a reference sample thus becomes very difficult in geriatrics. In order to have sufficient sample sizes for investigation, laboratory data that has been previously collected can be used (5). This data typically comes from hospital

populations who are inherently of poorer health compared to populations sampled from the community. This data also requires the use statistical methods to eliminate individuals that cannot be assumed healthy based on their laboratory results (5). Unfortunately this can also limit sample sizes. For these reasons it is difficult to obtain large samples of elderly persons who are community dwelling and 'healthy' or disease free. An alternative to performing new sampling or using laboratory data is to evaluate the use of other sources such as large epidemiological studies. In this study data from the National Health and Nutrition Examination Survey (NHANES) was used. NHANES samples from the community, observes participants of various ages and provides a large sample size.

NHANES data was used specifically in this study to determine whether the presence of morbidity affects RIs. This is of particular interest for the geriatric population. As previously mentioned, applying a strict definition of health to the elderly often results in insufficient sample sizes for RI calculations. To combat this problem it would be ideal to use less strict exclusion criteria. However to include persons with morbidity in the reference population it must first be established whether morbidity has an effect on RIs. It is known that certain analytes are affected or are indicative of particular disease states (6) but it is unknown whether the presence of morbidity in general affects analyte concentrations or RIs. Given that other sources of biological variability can be controlled by partitioning populations by factors such as age, sex and ethnicity, (5) it is of interest to determine whether the presence of morbidity should also be considered a partitioning factor in the elderly.

Creatinine, a marker of kidney function, was chosen as the analyte of interest for this study. It has been well studied and age-related increases in creatinine have been well documented (7-10). Some studies have even attempted to quantify this increase (7-10). This marker is used to estimate the glomerular filtration rate (GFR) of the kidney (6). It is a waste product that arises after energy production in the muscles. Once produced creatinine is transported through the bloodstream to the kidneys to be filtered out and excreted (6). When the kidney is impaired the ability to excrete creatinine is impaired and creatinine levels in the blood begin to rise. Kidney impairment is common in old age and can be contributed to by the presence of some morbidities, particularly diabetes which is known to cause microvascular complications that can lead to kidney disease (6).

Creatinine although widely used as a clinical marker is also known to be subject to biological variation from age, sex and ethnicity (6). It is suspected that in older age groups, morbidity may also be a source of biological variation. It is of interest to understand how much change in creatinine is due to the aging process and how much can be attributed to the increase in morbidity. More precisely, if it is found that the presence of morbidity does not significantly alter creatinine concentrations than perhaps it is not necessary to select a typical 'healthy' reference sample and more lenient exclusion criteria could be used. Contrastingly, if the presence of morbidity is shown to have an effect on creatinine concentrations there may be a way to control the biological variation by partitioning the data by number of morbidity.

3.3 Methods

Data from NHANES was obtained from http://www.cdc.gov/nchs/nhanes for the 2009-2010 collection period (11). Results from the Medical Conditions Questionnaire (MCQ), Blood Pressure and Cholesterol Questionnaire, Bowel Health Questionnaire along with demographic and laboratory data were merged in SAS® 9.3 (SAS Institute Inc., Cary NC) by subject ID. All further statistical analyses were carried out using SPSS v.20 (IBM Corp., Armonk NY).

3.3.1 Selection of the Reference Sample

Individuals were considered for inclusion in the study if they completed the aforementioned questionnaires and provided a blood sample. Anyone who did not have a creatinine test performed on their blood sample was excluded from the study and any individual with a creatinine result higher than 300 μmol/L was excluded because of suspected measurement error. Any participants who reported to have a disease or were on a medication known to affect renal function were excluded. This included anyone who responded positively to the presence of kidney disease, being on dialysis, the presence of diabetes or the use of insulin.

3.3.2 Analytical Measurement of Creatinine

Creatinine measurements obtained from NHANES were measured using the UniCel DxC 800 analyzer from Beckman Coulter (12). This analyzer uses the Jaffe rate method (kinetic alkaline picreate) to determine the concentration of creatinine which is tractable to an isotope dilution mass spectrometry (IDMS) reference method (12). Coefficients of variation for this method ranged from 1.1 - 5.4% (12).

3.3.3 Descriptive Statistics

Demographic characteristics including the ethnicity, education and marital status of the population were evaluated and reported as percentages. The distributions of morbidity among the included population were also reported as percentages.

Reference curves reporting the 2.5^{th} and 97.5^{th} percentiles with associated 90% confidence intervals (CIs) were created to visually describe any age-related change in creatinine concentration. Non-parametric methods that make no prior assumptions about the underlying distribution of the data were used in RI calculation so no transformation was performed. Based on the reference curves a potential inflection point was identified indicating a potential increase in creatinine concentration after the age of 60. To illustrate this difference in creatinine concentration box-plots were used to describe the data, separating the population into young and old age groups. To determine whether this change was significant, a non-parametric Mann-Whitney U test was performed to compare the median concentration of persons <60 years of age to those ≥60 years of age, stratifying by gender. The apparent increase was also quantified using a piecewise linear regression approach.

3.3.4 Statistical Analyses

Multivariable linear regression analysis was performed to determine whether number of morbidity was a significant predictor of creatinine concentration. Number of morbidity was determined based on the morbidities evaluated in the MCQ. Factors known to affect creatinine were accounted for in the analysis (age, sex and ethnicity). Results were considered significant for p-values less than 0.05. Interaction between number of

morbidity and age was suspected and so appropriate interaction terms were created and tested for statistical significance in the linear regression model.

3.3.5 Reference Interval Calculation and Reporting

Based on the results from the linear regression the included population was stratified by age and number of morbidity. RIs (2.5^{th} – 97.5^{th} percentile) for each stratum were calculated. A non-parametric approach was applied to strata with $n \geq 120$ and a robust approach was applied to strata with $n \geq 30$ but <120. The associated 90% CIs for the lower and upper bound were also reported. Subjective comparison of RIs was performed by visually inspecting Forest plots and comparing confidence interval overlaps between strata. If the 90% CIs did not overlap the two strata were considered different. The width of the CI was also examined to investigate precision of the lower and upper limits of the RI.

3.4 Results

3.4.1 Reporting of Descriptive Statistics

Data for a total of 10 537 participants was available from the 2009-2010 NHANES study. Only 5683 individuals had completed the aforementioned questionnaires and had an acceptable creatinine test result. A total of 740 individuals were excluded due to the presence of kidney disease or diabetes and/or the use of dialysis or insulin. Demographic data of the included study population (n=4943) can be found in Table 3-1 including ethnicity, educational background and marital status. A list of the morbidities

used for this analysis is found in Table 3-2 along with the percentage of the included population that reported to have them.

Table 3-1: Demographic Characteristics of Study Population

		Count (n)	Percent (%)
Ethnicity	Mexican American	890	18.0
	Other Hispanic	458	10.1
	Non-Hispanic White	2479	50.2
	Non-Hispanic Black	805	16.3
	Other	270	5.5
Education	<9th grade	562	11.4
	Highschool (no diploma)	777	15.7
	Highschool (diploma)	1144	23.1
	Some College	1387	28.1
	College Graduate	1062	21.5
	Refused to Answer	5	0.1
Marital Status	Married	2544	51.5
	Widowed	372	7.5
	Divorced	520	10.5
	Separated	167	3.4
	Never Married	908	18.4
	Living w/ Partner	429	8.7
	Refused to Answer	1	0.02

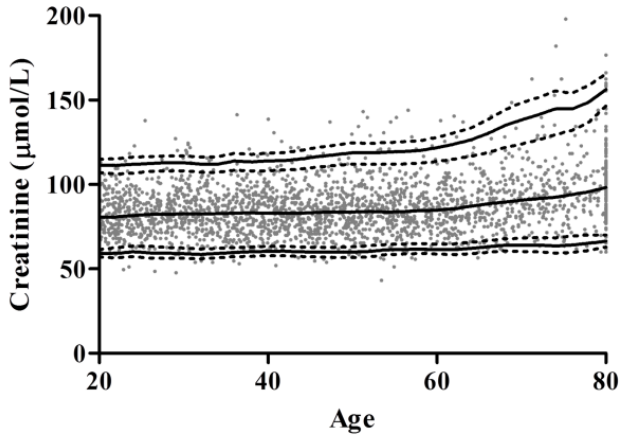
Table 3-2: Distribution of Morbidity among Included Population

	Count (n)	Percent (%)
High Blood Pressure (HBP)	1519	30.7
Arthritis	1265	25.6
Asthma	546	11.0
Thyroid Condition	540	10.9
Cancer	482	9.6
Osteoporosis	347	7.0
Congestive Heart Failure (CHF)	303	6.1
Liver Condition	259	5.2
Anemia	198	4.0
Gout	183	3.7
Coronary Heart Disease (CHD)	179	3.6
Psoriasis	140	2.8

The population consisted of equal proportions male and female aged 20-80 with the average age being 47.6 years (±17.6). Age-related morbidities such as arthritis and hypertension were the most common at 25.6% and 30.7% respectively. This distribution of morbidity was relatively the same among males and females.

An expected age-related increase in creatinine was apparent in the population (Figure 3-1), increasing minimally between the ages of 50-60 years and increasing substantially thereafter. CIs appear to have relatively the same widths between ages 20-60. Contrastingly the CIs began to widen after 60 years of age indicating more variability in creatinine concentration as age increases.

A)



B)

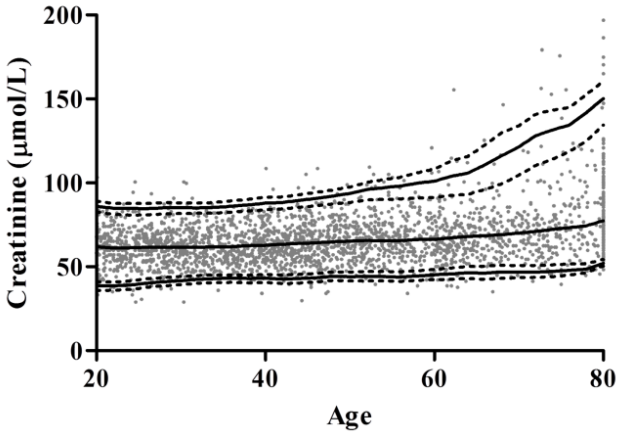


Figure 3-1: Male (A) and Female (B) Reference Curves for Creatinine
Creatinine concentrations (µmol/L) were plotted as a function of age. The solid line between the upper and lower limits represents the median creatinine concentration. Reference limits were calculated using the non-parametric or robust method for every 2 year age group (i.e. 20-21, 22-23 etc.). Reference limits were plotted above and below the median using solid lines. The dotted lines surrounding the upper and lower limits are the 90% confidence intervals around the limit estimates.

Box-plots illustrating the significant increase in creatinine concentration after 60 years of age are displayed in Figure 3-2. Mann-Whitney U tests performed on this data suggested a significant difference between median creatinine levels of persons aged 20-59 compared to persons aged ≥60 for both males and females ($p<0.001$). Similarly,

piecewise linear regression (Figure 3-3) indicated a dramatic change in slope at 60 years of age. Persons aged 20-59 demonstrated a 0.136 μmol/L increase ($p<0.001$) in creatinine for every one year increase in age, while persons aged ≥60 years showed a 0.832 μmol/L ($p<0.001$) increase.

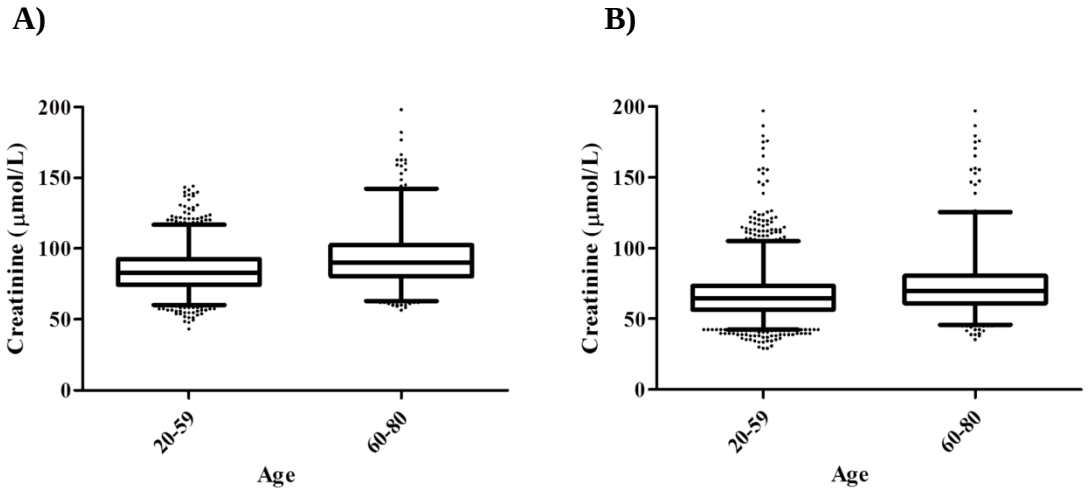


Figure 3-2: Box Plots Describing Differences in Creatinine Concentration Between Young and Old Age Groups for Males (A) and Females (B)

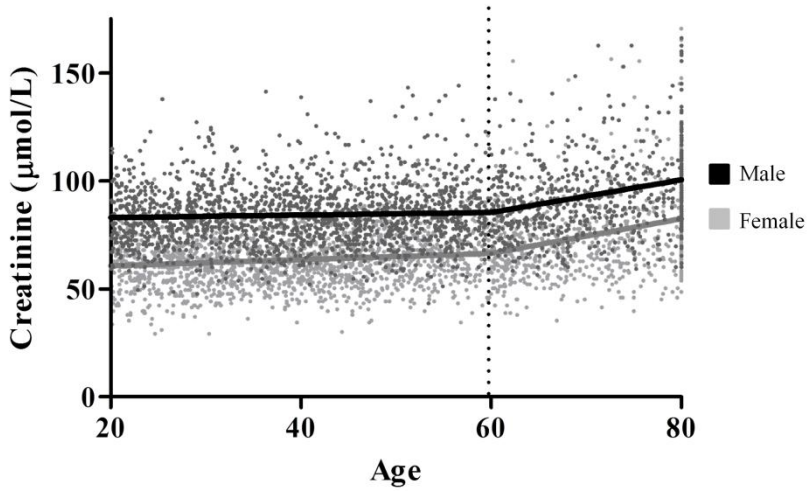


Figure 3-3: Piecewise Linear Regression of Creatinine Concentration by Age

3.4.2 Results of Multivariable Linear Regression

Multivariable linear regression analyses (Table 3-3) verified that number of morbidity is a significant predictor of creatinine concentration independent of age, sex and ethnicity ($p<0.001$). The presence of 2 or more morbidities resulted in a 2.37 μmol/L increase in creatinine compared to the presence of no morbidity ($p<0.001$).

Table 3-3: Results of Multivariable Linear Regression

Independent Variable	Model 1	Model 2	Model 3
Constant	95.05	96.10	101.27
Age	0.27	0.24	0.10
Sex			
Male (reference)	-	-	-
Female	-19.77	-19.96	-19.95
Ethnicity			
Non-hispanic white (reference)	-	-	-
Non-hispanic black	6.81	6.75	7.13
Mexican American	-9.09	-8.88	-8.73
Other Hispanic	-5.85	-5.74	-5.45
Other	-3.03	-2.95	-2.89
# of Morbidity			
0 (reference)		-	-
1		-0.54^{NS}	-5.57
≥2		2.37	-14.05
Interaction Terms			
Age X 1 morbidity			0.13
Age X 2 morbidities			0.32
Sig. F Change	0.001	0.001	0.001
R^2	0.406	0.409	0.420

*All β co-efficients are listed and statistically significant ($p<0.01$) unless noted as NS (Not Significant).

The distribution of morbidity between age groups 20-59 and ages ≥60 years was shown to be quite different (Figure 3-4). This difference was expected given the high prevalence of morbidity among older age groups. As such, the interaction between

number of morbidity and age was tested in another linear regression model. The interaction terms were found to be significant ($p<0.001$) (Table 3-3).

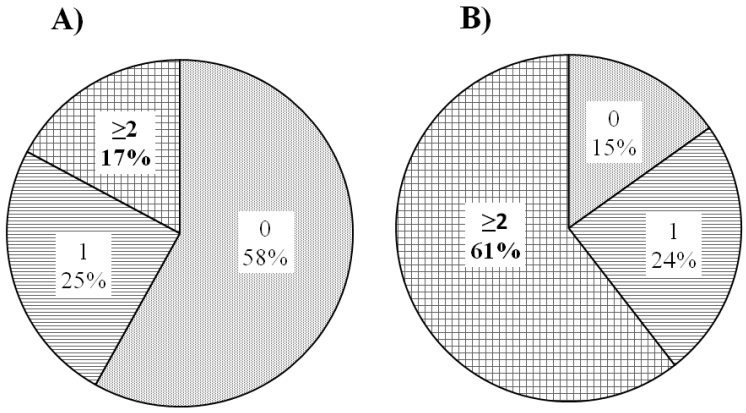


Figure 3-4: Distribution of Morbidity in Younger (A) and Older (B) Age Groups

These results suggest that the effect of age on creatinine concentration varies as a function of morbidity. To illustrate this; creatinine concentrations were plotted as a function of age and grouped by number of morbidity (Figure 3-5).

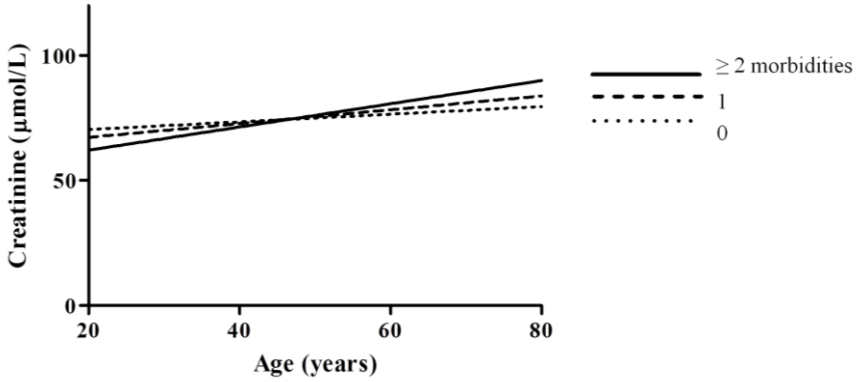


Figure 3-5: Average Creatinine Concentration as a Function of Age and Morbidity

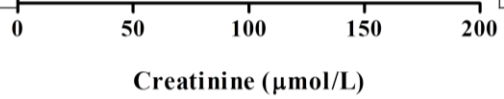
3.4.3 Reporting of Reference Intervals

The increase in creatinine concentration significantly affects the upper limit of creatinine RIs only. Lower limits for creatinine were relatively the same across all strata, with a significant amount of CI overlap (Figure 3-6). The increase in the upper limit for creatinine occurs with increasing number of morbidity in older age groups for both males and females. For instance, the upper limit for males aged 70-80 with ≥2 morbidities (162.7 (142.3-198.0)) is considered to be significantly different from males aged 70-80 with 0 morbidities (133.7 (114.6-139.5)). Contrastingly the upper limit for males aged 20-39 with ≥2 morbidities (121.4 (108.4-130.6)) is not significantly different from males aged 20-39 with 0 morbidity (115.8 (111.4-118.5)). Similar results were found in females, although a RI for females aged 70-80 years with no morbidity was unable to be calculated due to insufficient sample size.

Evaluation of the CIs for the upper and lower limits indicated that precision of the upper limit decreases with increasing age and number of morbidity. This can most clearly be seen by evaluating the strata with the largest sample sizes which should have adequate precision. Despite there being 228 individuals in the age range 70-80 years with >2 morbidities the CI is still the widest, even wider than strata with sample sizes as small as 87. This lack of precision was less obvious when evaluating precision estimates of the lower limits.

A)

Age	Morbidity(ies)	n	Lower Limit (90%CI)	Upper Limit (90%CI)
70-80 yrs	>2	228	68.1 (63.7-69.8)	162.7 (142.3-198.0)
	1	82	65.1 (62.1-67.8)	148.8 (136.1-158.4)
	0	41	58.5 (54.5-61.7)	133.7 (114.6-139.5)
60-69 yrs	>2	151	61.9 (56.6-65.4)	137.9 (126.4-148.5)
	1	95	62.9 (60.2-65.9)	121.2 (115.9-126.3)
	0	87	60.5 (58.9-62.3)	112.7 (109.0-117.6)
40-59 yrs	>2	194	61.0 (57.5-64.5)	122.0 (116.7-130.8)
	1	218	60.1 (56.6-64.5)	121.1 (108.7-125.5)
	0	434	59.2 (55.7-61.9)	114.9 (109.6-130.8)
20-39 yrs	>2	42	62.6 (60.4-65.3)	121.4 (108.4-130.6)
	1	177	61.0 (49.5-64.5)	114.0 (104.3-137.9)
	0	640	60.1 (58.3-61.9)	115.8 (114.4-118.5)



B)

Age	Morbidity(ies)	n	Lower Limit (90%CI)	Upper Limit (90%CI)
70-80 yrs	>2	289	47.7 (45.1-52.2)	165.3 (138.8-179.5)
	1	76	50.2 (47.1-52.5)	125.6 (106.6-141.6)
	0	20	N/A	N/A
60-69 yrs	>2	178	50.4 (38.0-52.2)	113.2 (92.8-125.5)
	1	88	43.7 (40.7-46.3)	104.6 (92.3-116.2)
	0	65	42.3 (37.7-46.2)	91.2 (84.7-99.3)
40-59 yrs	>2	259	45.9 (39.8-47.7)	105.2 (94.6-108.7)
	1	251	41.6 (39.8-44.2)	97.2 (88.4-101.7)
	0	350	44.2 (41.6-45.1)	89.2 (85.8-91.1)
20-39 yrs	>2	113	42.1 (39.7-44.4)	87.1 (83.1-89.7)
	1	229	41.6 (33.6-43.3)	83.1 (80.4-101.7)
	0	644	41.6 (38.0-42.4)	84.0 (83.1-85.8)

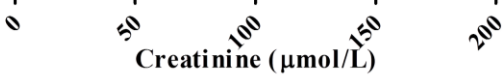


Figure 3-6: Male (A) and Female (B) Reference Intervals for Creatinine Stratified by Age and Number of Morbidity

Creatinine results were stratified by age and number of morbidity. Reference intervals were calculated for sample sizes larger than 30 and the upper and lower limits were represented by dots with the associated 90% confidence interval as bands. The non-parametric calculation method was used when sample sizes were ≥120. The robust approach was used for sample sizes <120 but ≥30.

3. 5 Discussion

One of the main difficulties in determining reference intervals in older populations is selecting a healthy reference sample. The analysis performed here suggests that selecting a typical 'healthy' reference sample may not necessary if morbidity is treated as a type of pre-analytical variation that you can control for by partitioning. Doing so may provide a new way of calculating geriatric RIs that allows the use of lenient exclusion criteria making it easier to obtain adequate sample sizes. Some of the limitations of this study and the proposed method for dealing with morbidity in elderly populations are presented below.

3.5.1 Benefits and Limitations to Using NHANES Data

One benefit of using NHANES data for this research is that it provided large enough samples that were representative of the population for all age groups. It is often very difficult to obtain large sample sizes in studies with the sole purpose of determining reference intervals, especially for geriatrics (5). Acquiring the amount of samples required for this analysis would have been very time-consuming and costly if participants were to have been newly recruited separately and analyzed in house (4). It is common to use laboratory test results that have already been collected although typically they are acquired from laboratory databases (4). This can also be difficult since statistical methods must be applied to laboratory data to exclude any potential unhealthy individuals, also decreasing sample size (5). Using large epidemiological studies, like NHANES, that collect biological samples provides a way of obtaining large sample sizes of community dwelling, relatively healthy populations that are representative of the total population

(13). The systematic review presented in Chapter 2 also demonstrated that the use of large epidemiological studies is becoming a more common approach to determining RIs in elderly populations but so far not included in guidelines for RI determination.

One limitation to using previously collected data is the impact of information bias. Often times, because this approach is retrospective in nature, it is impossible to obtain desired data about participants unless it was previously collected by the primary source. Although NHANES did not provide information regarding all morbidities that are possible to contract, it does provide data on the morbidities that affected the analyte of interest. In addition the morbidities that were included in the MCQ were those that are most prevalent in the general population (13). This included diseases that are quite prevalent in elderly patients including diabetes, cardiovascular disease, and arthritis (11).

The 2009-2010 cohort was chosen for this analysis as it was the most recent cohort that had all data available necessary to perform this study. The sample size was sufficient for the partitioning and did not necessarily need to be combined with other years of study. Sample sizes were not adequate however to determine the effect of all levels of morbidity, and had to be categorized as 0, 1 or ≥2 morbidity. In future, if the effect of having more morbidity is of interest it is recommended to combine multiple years of NHANES data.

NHANES data comes from interviews, examinations and laboratory data that undergo extensive quality control and assurance (14). Analytical variation should have had very little effect on the results presented since standardized procedures were used in creatinine measurement that have been subject to quality control and validation since they

were first measured in NHANES (17). Pre-analytical variables such as sample processing and obtainment are reasonably well controlled through standardized procedures, another benefit of using large epidemiological studies for RI determination studies.

With NHANES questionnaires being answered through a self-reported interview the results may be subjected to some bias in reporting (18). To limit this bias secondary information was considered when selecting the reference sample. Any persons that may have reported 'no' to the presence of diabetes or kidney disease but reported 'yes' to being on insulin or being treated with dialysis were excluded. In addition, if any person reported "Don't Know" to the question of whether any of the exclusion criteria were present, they were excluded from the study.

3.5.2 Difficulties Faced During Selection of 'Healthy' Reference Sample

When selecting a healthy reference sample in geriatrics it is important to be as inclusive as possible to keep sample size requirements acceptable. It is also important for the population to be representative of the population to which it will be applied. Thus, we did not want to employ very strict exclusion criteria. However it was necessary to exclude individuals with conditions known to affect creatinine levels so as not to bias the results. It was then decided to only exclude individuals that reported to have morbidities known to affect creatinine levels directly. Common exclusion criteria of other studies included the presence of kidney disease, the use of dialysis and diabetes (14, 15). RI determination guidelines also suggest using examination and laboratory data to exclude subjects based on the suspect of undiagnosed disease (5). However given that the aim of this study was to examine the effect of morbidity, the suggestion of using examination or laboratory data

to exclude individuals with suspected disease was disregarded. The presence of persons with undiagnosed disease within the population is possible but if they are present in the population the effect of morbidity on creatinine levels that we saw would be an underestimate of the true effect.

3.5.3 Discussion of Results

Reference curves (Figure 3-1) demonstrated the well documented increase in serum creatinine with age (7-10) however they also demonstrated increased variability in creatinine levels with age. Other examinations of creatinine concentration variability confirm between-subject variation of up to 18% (CV) in elderly populations but suggest that this is comparable to that of younger age groups (19). Given that there are some pre-analytical factors that affect geriatric blood tests and not adult (refer to Section 1.3) and that there are extreme physiological changes that occur with age, this variability is suspected to come from the heterogeneity of health states in the population. This was controlled for by partitioning the population by number of morbidity. It is possible that the between-subject variation is higher in this study than previously reported due to the limited exclusion criteria used in selecting the reference sample.

Evaluation of the reference curves also indicated a slight increase of creatinine concentration starting at approximately 50 years of age, however a large increase was apparent for ages 60 years and up. Based on the substantial increase seen by visual inspection it was chosen to use 60 years as the cut-off between young and old, this age is also closer to the widely used definition of elderly at 65 years of age (20). Statistical methods such as change-point analysis are typically for inflection point detection but are

often a cumbersome task requiring Bayesian statistics (21, 22). The visual inspection performed here was deemed valid given that subjective methods often yield similar results to those found by change-point analysis and change-point detection (21,22). Comparison using the Mann-Whitney U test also confirmed a significant difference between the medians of the 20-59 age group and the ≥60 age group.

To quantify the change in creatinine concentration with age, piecewise regression was used to examine the differences in slope between young and old age groups. These differences do not account for any other factor influencing biological variation, for example ethnicity. Ethnicity however was controlled for in the multivariable linear regression analyses demonstrating the effect morbidity has on creatinine levels after adjusting for known pre-analytical factors that affect creatinine. When partitioning for RI calculation ethnicity was considered as a factor to stratify by but it was decided to only stratify by age, sex, and number of morbidity. This was primarily because stratification by all four factors would have decreased the sample size in each partition. In addition having larger numbers of RIs makes interpretation of their results much harder (19). This is also why it was also decided to partition by number of morbidities rather than type of morbidities.

The known increase in morbidity among elderly was evidence to support that there may be an interacting effect between morbidity and age (18). Testing this interaction proved that the effect of morbidity is indeed different in older versus younger age groups. Figure 3-5 visually depicts this interacting effect however reasons for the opposite effect of morbidity seen above and below fifty years of age needs to be

investigated further. To illustrate the effect of morbidity with age and determine significant differences between RIs the difference Forrest plots were used to depict RI limits and their associated 95% CIs. Evaluating Figure 3-6 it is apparent that there are strong differences between the presence of morbidity in older age groups and few differences in younger age groups. This difference was present regardless of the fact that the CIs are significantly wider in older age groups. The widening of CIs in older age groups also demonstrated that there is more variability in creatinine concentration with increasing age and number of morbidity. In populations with large sample sizes confidence bands tend to be quite narrow however this is not the case for older individuals like it was for younger age groups. Though noticeable differences in the RIs for each partition were found, additional reference interval determinations using this method will need to be validated using other datasets.

Other considerations taken into account during analysis were the effect of weight and lifestyle factors. Weight and lifestyle factors were not included in the analysis as there was not a sufficient sample size to allow for the analysis performed (i.e. including morbidity, sex, ethnicity and age) in addition to these factors. The relationship with weight and creatinine is also confounded by the fact that weight is comprised of muscle mass and fat mass, and the production of creatinine is only a function of muscle mass (23). This is complicated further by the decrease in ratio of muscle mass to fat mass with age and differences in this ratio between males and females (23).

These results may change the way RI data is interpreted (24). For instance, using these results, consider a male aged 70 presenting with gout and no other known

morbidity. A creatinine concentration of 150 μmol/L in this individual would be flagged as high considering the RI presented (61.9 – 137.9). Of course these results are subject to the use of only the morbidities investigated. Though fairly comprehensive it should be examined if the morbidities present in the individual being compared are ones that were included in this examination.

3.6 Conclusion

Serum creatinine is known to increase with age due to known physiological changes. Up until this point it was not known whether the increasing prevalence of morbidity played a role in the increase of creatinine and what effect this would have on RIs. This study demonstrated that the presence of morbidity does not significantly affect the RIs for creatinine in persons aged 20-60 however it does significantly affect the RIs of persons aged 60 years and older. This study also demonstrated potential benefits in using data from large epidemiological studies to calculate geriatric RIs.

3.7 References

(1)Saylor, C. (2004). The Circle of Health: A Health Definition Model. *Journal of Holistic Nursing, 22*(2), 97-115.

(2)Breslow, L. (1972). A Quantitative Approach To The World Health Organization Definition Of Health: Physical, Mental And Social Well-being. *International Journal of Epidemiology, 1*(4), 347-355.

(3)Hansen-Kyle, L. (2005). A Concept Analysis Of Healthy Aging. *Nursing Forum, 40*(2), 45-57.

(4)Horn, P. S., & Pesce, A. J. (2003). Reference Intervals: An Update. *Clinica Chimica Acta, 334*(1-2), 5-23.

(5)Clinical and Laboratory Standards Institute (CLSI). (2008). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline. CLSI document C28-A3. 3rd ed. Wayne P.A.: Clinical and Laboratory Standards Institute.

(6)Tietz, N. W., Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2006). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (5th ed.). St. Louis, Mo.: Elsevier Saunders. Section I & Section III – Kidney Function Tests.

(7)Kane, R. L. (2009). *Essentials of Clinical Geriatrics* (6th ed.). New York: McGraw-Hill Medical. Chapters 1-3.

(8)Pottel, H., Vrydags, N., Mahieu, B., Vandewynckele, E., Croes, K., and Martens, F. (2008) Establishing Age/Sex Related Serum Creatinine Reference Intervals from Hospital Laboratory Data Based on Different Statistical Methods. *Clinica Chimica Acta, 1-2,* 49-55.

(9)Kroll, J. and Saxtrup, O. (1998) On the Use of Patient Data for the Definition of Reference Intervals in Clinical Chemistry. *Scandinavian Journal of Clinical Laboratory Investigation, 6,* 469-473.

(10) F. Boulat, O., Krieg, M. A., Janin, B., Burckhardt, P., Francioli, P., and Bachmann, C. (1998) Clinical Chemistry Variables in Normal Elderly and Healthy Ambulatory Populations: Comparison with Reference Values. *Clinica Chimica Acta, 2,* 127-135.

(11) National Health and Nutrition Examination Survey. (2014, May 29). *Centers for Disease Control and Prevention*. Retrieved June 22, 2014, from http://www.cdc.gov/nchs/nhanes.htm

(12) Coresh, J. Calibration of Serum Creatinine in the National Health and Nutrition Examination Surveys (NHANES) 1988-1994, 1999-2004. *American journal of kidney diseases*, 918-926.

(13) Sirken, M., Hirsch, R., Mosher, W., Sonnenfeld, N., & Moriarity, C. Changing Methods of NCHS surveys: 1960-2010 and Beyond. (2011). *MMWR Surveillance Summary, 7*, 42-48.

(14) Junge, W., Wilke, B., Halabi, A., & Klein, G. (2004). Determination of Reference Intervals for Serum Creatinine, Creatinine Excretion and Creatinine Clearance with an Enzymatic and a Modified Jaffe Method. *Clinica Chimica Acta*, *344*(1-2), 137-148.

(15) Erlandsen, E. J., Randers, E., & Kristensen, J. H. (1998). Reference Intervals for Serum Cystatin C and Serum Creatinine in Adults. *Clinical Chemistry and Laboratory Medicine*, *36*(6), 393-397.

(16) Lacher, D., Curtin, L., & Hughes, J. (2004). Why Large Design Effects Can Occur in Complex Sample Designs: Examples from the NHANES 1999-2000 Survey.. *American Statistical Association Proceedings*, *1*, 3841-3846.

(17) Schenker, N., Raghunathan, T. E., & Bondarenko, I. (2009). Improving on Analyses of Self-reported Data in a Large-scale Health Survey by using i=Information from an Examination-based Survey. *Statistics in Medicine, 29*(5), 533-545.

(18) Faulkner, W. R., & Meites, S. (1994). *Geriatric Clinical Chemistry: Reference Values*. Washington, DC: AACC Press. Chapters 1-5.

(19) World Health Organization. Constitution in Basic Documents. Geneva: WHO, 1948.

(20) Li, H. (1999). A Piecewise Regression Analysis with Automatic Change-point Detection. *Intelligent Data Analysis*, *3*(1), 75-85.

(21) Barry, D., & Hartigan, J. A. (1993). A Bayesian Analysis for Change Point Problems. *Journal of the American Statistical Association, 88*(421), 309.

(22) Marks, V. (1985) *Clinical Biochemistry Nearer the Patient*. Edinburgh: Churchill Livingstone. Print.

(23) Kuan, Y., Hossain, M., Surman, J., Nahas, M.E., & Haylor, J. (2005) GFR Prediction Using the MDRD and Cockcroft and Gault equations in Patients

with end-stage Renal Disease. *Nephrology Dialysis Transplantation, 20*, 2394-2401.

(24) Sikaris, K.A. (2014). Physiology and its Importance for Reference Intervals. *Clinical Biochemistry Reviews, 35*(1), 3-14.

4 CONCLUSIONS AND FUTURE DIRECTIONS

This Master's thesis examined the field of geriatric RIs in two ways. A systematic review was first presented to identify what literature about RIs is currently available. From this review it was apparent that selecting a 'healthy' reference sample tends to be an area of concern for geriatric RI determination. For this reason, an investigation was performed to determine whether or not selecting a not so 'healthy' reference sample would greatly impact RI calculations. A summary of the results of these studies are described below in addition to possible future avenues of this research.

4.1 Results Summary for the Systematic Review of Geriatric Reference Intervals

A total of 62 articles over the last 25 years (1989 – present) have provided at least one RI of a clinical chemistry analyte for a population sub-group greater than 65 years of age. All geriatric RIs published within these articles were extracted and reported in Appendix D. The 1082 reference intervals listed captured data for 93 unique analytes.

Despite the volume of geriatric RIs available in literature it was very difficult to compare them due to lack of harmonization in methodology. This first became apparent during the data extraction phase. Three forms were built to evaluate 1) how the reference population was selected, 2) what pre-analytical and analytical characteristics were reported and 3) what RI calculation methods were used and how they were reported. In all areas there were significant differences in what information was reported by studies and how it was presented.

The selection of a healthy reference sample not only differed by paper but also according to which analyte was being measured. Some articles used very strict exclusion criteria where others used no exclusion criteria at all to try and be more representative of the geriatric population where disease is more prevalent. The source of sampling was also quite variable ranging from recruitment from clinics to recruitment hospital laboratories. A common method of sample recruitment was to sample from previously conducted epidemiological studies, occurring in 37.1% of cases.

Pre-analytical treatment of samples and analytical measurement of analytes was well reported within the papers selected for review but the analytical methods for a given analyte differed significantly across studies. It is expected that some of these differences arose from the fact that technology has significantly changed over the course of 25 years affecting how various analytes are measured.

Calculation of RIs differed between studies however each study was able to be classified into one of three calculation methods; parametric, non-parametric or robust. Despite using similar methods of calculations each study used a number of different partitions; ranging in age cut-offs, sex separations and other partitioning criteria. This variability and the extreme lack of confidence interval (CI) reporting made it even more impossible to compare RIs across studies.

Although there were so many sources of variation, the papers selected for review were fairly consistent in reporting trends in analyte concentration with age. Results from any study that performed a statistical test for differences in analyte concentration with age were captured and examined. A list of analytes that are reported to increase, decrease or

not change with age was generated along with the percentage of studies that were concordant. Specifically; alkaline phosphatase, creatinine, follicle-stimulating hormone, glucose, ϒ-glutamyltransferase, lactate dehydrogenase, low-density lipoprotein cholesterol, luteinizing hormone, magnesium, sex hormone binding globulin, thyroid-stimulating hormone and urea were agreed to increase with age. Multiple studies reported decreases in albumin, total bilirubin, total calcium, estradiol, insulin-like growth factor-I, iron, free and total testosterone and vitamin B_{12}. Lastly many studies reported no significant change in aspartate aminotransferase, chloride, high-density lipoprotein cholesterol, potassium, total protein, sodium and triglycerides. The actual RIs of young and old age groups can be found in Appendix C.

4.2 Summary of Results for the Effect of Morbidity on Creatinine Reference Intervals

Selecting a `healthy` geriatric reference sample has been proven to be a difficult process using typical exclusion criteria of RI studies (1). To determine the effect of including 'unhealthy' individuals in the reference sample the effect of morbidity on creatinine RIs was examined. Data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) provided adequate sample sizes for determining this effect and provided data on a number of common morbidities.

The well documented increase in creatinine concentration (2-3) was confirmed in this study. A significant increase in creatinine concentration at 60 years of age was determined through visual expectation and confirmed using piecewise linear regression

and a Mann-Whitney U test comparison of medians. Reference curves indicated that precision around upper limits for creatinine begins to decrease around 60 years of age.

Multivariable regression analyses demonstrated that number of morbidity is a significant predictor of creatinine concentration ($p<0.001$). Furthermore it was demonstrated that the effect age has on creatinine concentration is different according to how many morbidities are present. Specifically, upper limits for creatinine significantly increase with increasing morbidity in older age groups (70-80 years of age) compared to younger age groups (20-39 years of age). Forest plots of the reference limits also confirmed that precision for the upper limits of creatinine decreases with increasing age and increasing number of morbidity.

4.3 Future Directions

The wide variability in methods for calculating geriatric RIs makes it very difficult to consolidate what is available in literature. Because of this, standardization of protocols and harmonization of methods for calculating geriatric RIs are needed in the future.

A large source of variability in determining geriatric RIs are the methods used to select the ‘healthy’ reference sample. Part of this is because health is an ambiguous term and it is hard to determine what ‘normal’ is in the aging population is given extreme heterogeneity (4). It was determined in this thesis that it may be possible to use more lenient exclusion criteria when selecting an elderly reference sample if the presence of morbidity is considered. This method of RI calculation proved feasible, promotes the use

of large epidemiological data sets and may provide a way to examine the concept of biological aging.

Validation of this method is needed, using other analytes and other data sources. A possible source of data for future research is the Canadian Longitudinal Study on Aging (CLSA). In addition to helping validate these findings data from the CLSA will be used to produce Canadian based RIs which were identified as lacking in the systematic review. This Canada wide study follows 30, 000 people between the ages of 45-85 for 20 years and collects blood and urine samples from these individuals (5). Comprehensive data on the participant's health status is collected and a physical examination is performed (5). All data necessary for calculating geriatric RIs will be available and the study would provide a large sampling platform.

It is the hope that using large epidemiological datasets, like NHANES and CLSA, may provide a platform for geriatric RI determination. Given that these type of studies typically aim to be representative of the general population and they provide large sample sizes, they would be ideal for the development of a RI determination method.

4.4 Closing Remarks

A number of geriatric RIs are available in literature but no standardized method exists for their determination. Current methods of RI determination vary in the way they select healthy reference populations, the pre-analytical and analytical characteristics of analyte measurement and in the way RIs are calculated and reported. Despite this lack of standardization and extreme variability many analytes have been documented to increase

or decrease with age. When standardizing methods of geriatric RIs it is worthwhile investigating morbidity as a source of biological variation. Morbidity can be controlled for using partitioning and may provide a way of obtaining RIs that are easily applied to the geriatric population. Increasing morbidity was shown to be associated with increased creatinine concentrations in older age groups and similar results are expected to be seen when investigating other analytes.

4.5 References

(1)Horn, P. S., & Pesce, A. J. (2003). Reference Intervals: An Update. Clinica Chimica Acta, 334(1-2), 5-23.

(2)Kroll, J. and Saxtrup, O. (1998) On the Use of Patient Data for the Definition of Reference Intervals in Clinical Chemistry. *Scandinavian Journal of Clinical Laboratory Investigation, 6,* 469-473.

(3)F. Boulat, O., Krieg, M. A., Janin, B., Burckhardt, P., Francioli, P., and Bachmann, C. (1998) Clinical Chemistry Variables in Normal Elderly and Healthy Ambulatory Populations: Comparison with Reference Values. *Clinica Chimica Acta, 2,* 127-135.

(4)Faulkner, W. R., & Meites, S. (1994). Geriatric Clinical Chemistry: Reference Values. Washington, DC: AACC Press.

(5)Canadian Longitudinal Study on Aging. (n.d.). CLSA. Retrieved June 27, 2014, from https://www.clsa-elcv.ca/

APPENDIX A: LIBRARY SEARCH STRATEGY

Geriatric Reference Values
OVID-Medline
Dec 2 2011
1. Reference Values/
2. *Reference Values/
3. (reference adj2 (values or range? or intervals)).ti.
4. (lab* adj2 (values or results or parameters)).ti.
5. biochemical values.ti.
6. (clinical chem* or Blood Chem*).ti.
7. clinical chemistry variables.tw.
8. Clinical Chemistry Tests/st [Standards]
9. or/2-8
10. Clinical Laboratory Techniques/
11. "Laboratory Techniques and Procedures"/
12. 10 or 11
13. 1 and 12
14. 9 or 13
15. exp Aged/
16. Aging/
17. lifespan.ti.
18. (elderly or old* adults or seniors or Octogenarians or Mature Adults or centenarians).ti.
19. geriatric.ti.
20. or/15-19
21. 14 and 20
22. limit 21 to english language
23. animals/ not humans/
24. 22 not 23
25. limit 24 to yr="1989 -Current"

APPENDIX B: DATA EXTRACTION FORMS

Level 1 Title and Abstract Screening Form

1. Does this citation pertain to the measurement of a biochemical test, performed on any fluid biospecimen in older adults?
• Yes
• No
• Unsure

2. Comments:

Level 2 Full Text Screening Form

1. Is this study provided in English?
• Yes
• No

2. Is this a primary study?
• Yes
• No

3. Is this a study of living humans?
• Yes
• No

4. Is the specimen being investigated blood or a type of blood fraction?
• Yes
• No

5. Does this study measure a clinical chemistry analyte?
• Yes
• No

6. Does the study population contain persons aged 65 and older?
• Yes
• No

7. Comments:

Level 3 Full Text Screening Form (2)

1. Does this paper provide a reference interval for the clinical chemistry analyte?
• Yes
• No

2. Is there a reference interval subgroup for a reference population above the age of 65 years?
• Yes
• No

3. Is there indication that a reference interval was not necessary for a subgroup above the age of 65?
• Yes
• No

4. Comments:

Level 5 Data Extraction – Population

1. What country did this study take place in?
• Canada
• United State
• Sweden
• Germany
• Japan
• Denmark
• Norway
• Netherland
• China
• Australia
• Multiple
• Other
If other or multiple, please specify:_____

2. Please state the number of individuals that were recruited for the study:

3. Please state the number of individuals that participated in the study:

4. Please state the total number of individuals that were INCLUDED in the study:

5. Please state the total number of individuals that were EXCLUDED from the study:

6. Please report the inclusion/exclusion criteria:

Included If...	Excluded if...	n Included	% Included	n excluded	% excluded
...	...	...	...	...	...

If there were no exclusion criteria, please specify the reason:

7. Please state whether the following information was recorded for the included population:
• Medications
• BMI
• Height
• Weight
• Blood Pressure
• Other 1 (specify) _____
• Other 2 (specify) _____
• None of the above

8. Comment on the included population:

9. Specify the number of people included in the study, as outlined by the authors (Click all that apply)**:**

Total Population (n) _____
Total Included Population (n) _____
Group 1 (specify) _____
Group 1 (n) _____
Group 1 (% total) _____
Group 1 (% included) _____
Group 2 (specify) _____
Group 2 (n) _____
Group 2 (% total) _____
Group 2 (% included) _____
Group 3 (specify) _____
Group 3 (n) _____
Group 3 (% total) _____
Group 3 (% included) _____
Group 4 (specify) _____
Group 4 (n) _____
Group 4 (% total) _____
Group 4 (% included) _____

10. What are the COMORBIDITIES of the individuals in the study?

Comments:

Comorbidity	n	% of Included/Total	Further Sub-grouped?
None		% of Included_____ % of Total _____	Yes No
Alzheimer's		% of Included_____ % of Total _____	Yes No
Cancer		% of Included_____ % of Total _____	Yes No
Cardiovascular Disease (CVD)		% of Included_____ % of Total _____	Yes No
COPD		% of Included_____ % of Total _____	Yes No
Coronary Artery Disease (CAD)		% of Included_____ % of Total _____	Yes No

Dementia		% of Included_____ % of Total _____	Yes No
Diabetes		% of Included_____ % of Total _____	Yes No
Glycemia		% of Included_____ % of Total _____	Yes No
Hypertension		% of Included_____ % of Total _____	Yes No
Liver Disease		% of Included_____ % of Total _____	Yes No
Renal Disease		% of Included_____ % of Total _____	Yes No
Stroke		% of Included_____ % of Total _____	Yes No
Other 1 _____		% of Included_____ % of Total _____	Yes No
Other 2 _____		% of Included_____ % of Total _____	Yes No
Other 3 _____		% of Included_____ % of Total _____	Yes No

11. Are AGE CHARACTERISTICS reported (if yes, please specify)**:**
• Yes
• No
Comments on the age characteristics:

Characteristics	Included Population	Group 1	Group 2	Group 3	Group 4
Mean	...	...	...	...	...
Standard Deviation	...	...	...	...	...
Median	...	...	...	...	...
Minimum	...	...	...	...	...
Maximum	...	...	...	...	...

12. Are separate AGE GROUPS reported (if yes, please specify)**:**
• Yes
• No
Comments on the age groups reported:

Age Group	n	% of Included
...	...	...

13. Is GENDER reported, (if yes, please specify)**:**
• Yes
• No
Comments on gender:

	n	% of Included
Male	...	...
Female	...	...

14. Is RACE/ETHNICITY reported (if yes, please specify)**:**
• Yes
• No
Comments on race/ethnicity:

	n	% of Included
White	...	...
African	...	...
American	...	...
Hispanic	...	...
Aboriginal	...	...
Asian	...	...
Other_____	...	...

15. Is there a cross-reference study?
• Yes _____
• No

Level 5 Data Extraction - Analyte (2)

1. Does this paper investigate one analyte or multiple analytes?
• Single Analyte
• Multiple Analytes

2. If this paper looks at one analyte, does it compare different analyzers/methods?
• Yes
• No
• N/A

3. If this paper looks at multiple analytes, how many analytes were analyzed?

4. Please report the PRE-ANALYTICAL/COLLECTION characteristics:
Comments on pre-analytical/collection characteristics:

	Analyte	Patient Fasting	Patient Position	Patient Restrictions	Sample Type	Tube Type	Time on Bench
1							
...							

5. Please report the ANALYTICAL/PROCESSING characteristics:
Comments on analytical/processing characteristics:

	Analyte	Treatment of Sample	Reported Units	Analyzer (Name, Company, etc.)	Kit Method (if different from analyzer)	Fraction Type	Principle
1							
...							

6. Please report the POST-ANALYTICAL/STORAGE characteristics:
Comments on post-analytical/storage characteristics:

	Analyte	Storage	Temperature	Storage Time	Thaw Parametres
1					
...					

Level 5 Data Extraction - Reference Intervals and Statistics

1. Please state the type of reference interval calculations performed:
• Parametric
• Non-Parametric
• Unknown/Not provided
Detail:

2. Please provide the percentiles AND/OR the confidence intervals used for reference interval calculations:
• Percentiles _____
• Confidence Intervals _____

3. Did the paper describe associations with any of the characteristics listed below?
• Yes
• No
• Age _____
• Sex _____
• Another Analyte _____
• Disease Outcome _____
• Co-morbidity _____
• Medication _____
• Other 1 _____
• Other 2 _____
• Other 3 _____

4. Was anyone excluded from the reference interval that were different from the study inclusion/exclusion criteria? (If yes, specify)
• Yes_____
• No

Included If...	**Excluded if...**	**n Included**	**% Included**	**n excluded**	**% excluded**
...	**...**	**...**	**...**	**...**	**...**

5. Was an outlier test performed?
• Yes
• No

6. If an outlier test was performed, which one?

7. Was a statistical test performed to test whether the grouping of the population was statistically significant?
• Yes
• No

If yes, what statistical tests were performed?

	Group Tested	Type of Test Performed	Result of Test
1			
...			

8. Was the sample distribution described for any of the analytes? (If yes, specify)
• Yes
• No
Comments:

	Analyte	Description of Distribution
1		• Not applicable • Gaussian • Log-Normal • Skewed • Other (Specify)_____
...		• Not applicable • Gaussian • Log-Normal • Skewed • Other (Specify)_____

9. How many applicable reference intervals were provided?

10. Please report the REFERENCE INTERVALS
Comments:

	Analyte	Age Group	Sex	Other Classification/Group	Reference Interval	Units
1			• Male • Female • Both			
...						

Level 4 - Removal of PSA

1. Does this paper investigate analytes other than PSA?
• Yes
• No

APPENDIX C: REFERENCE INTERVALS FOR YOUNG AND OLD AGE GROUPS

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Adrenocorticotropic hormone	**pmol/L**	Y	B	<60	0 - 22	60-90	NP	>90	2 - 23	57	
Alanine aminotransferase	**µkat/L**	N	M	21-25	<1.05	61-65	<0.73	66-70	<0.66	40	
			F	21-25	<0.68	61-65	<0.60	66-70	<0.56		
		Y	M	N/A	N/A	<64	0.15 - 0.99	>65	0.10 - 0.70	48	
			F	N/A	N/A	<64	0.10 - 0.66	>65	0.09 - 0.65		
		Y	B	N/A	N/A	<75	0.15 - 1.02	>75	0.15 - 0.65	49	
		Y	B	N/A	N/A	20-33	0.07 - 0.48	65-74	0.10 - 0.32	56	
		Y	M	<60	0.17 - 0.68	60-90	NP	>90	0.10 - 0.65	57	
			F	<60	0.12 - 0.60	60-90	NP	>90	0.09 - 0.41		
		Y	B	N/A	N/A	N/A	N/A	65-99	5.70 - 21.0	58	
Albumin	**g/L**	N	M	15-55	38 - 49	56-75	35 - 48	76-95	32 - 46	47	
		Y	M	N/A	N/A	<64	45 - 55	>65	36 - 48	48	
			F	N/A	N/A	<64	43 - 53	>65	36 - 49		
		Y	B	N/A	N/A	<75	37 - 49	>75	35 - 46	49	
		N	B	N/A	N/A	N/A	N/A	65-74	37 - 43	53	*
		Y	B	<60	34 - 48	60-90	32 - 46	>90	29 - 45	57	
		Y	B	N/A	N/A	N/A	N/A	65-99	34 - 59	58	*
		Y	B	N/A	N/A	<74	40 - 49	>75	36 - 48	75	
Alkaline phosphatase	**µkat/L**	N	M	21-25	0.88 - 2.19	61-65	0.83 - 2.21	66-70	0.83 - 2.18	40	
			F	21-25	0.66 - 2.02	61-65	0.78 - 2.28	66-70	0.77 - 2.21		
		Y	M	N/A	N/A	<64	0.71 - 1.80	>65	0.80 - 2.72 (M+F)	48	
			F	N/A	N/A	<64	0.54 - 1.62	>65	N/A		
		Y	B	N/A	N/A	<75	0.66 - 1.97	>75	0.70 - 2.26	49	
		Y	B	N/A	N/A	20-33	0.95 - 2.53	65-74	1.09 - 3.26	56	
		Y	M	<60	0.90 - 2.18	60-90	0.95 - 2.02	>90	0.95 - 2.64	57	
			F	<60	0.71 - 1.67	60-90	0.90 - 2.40	>90	0.73 - 2.72		
Amylase	**µkat/L**	Y	B	N/A	N/A	<64	0.58 - 2.28	>65	0.31 - 2.52	48	
		Y	B	<60	0.34 - 1.77	60-90	NP	>90	0.43 - 2.50	57	
Androstenedione	**nmol/L**	N	F	20-29	1.6 - 7.5	60-69	0.5 - 3.0	70-80	0.5 - 2.5	69	
		Y	M	<60	2.6 - 7.2	60-90	NP	>90	0.9 - 4.1	57	
			F	<60	3.0 - 9.6	60-90	NP	>90	0.1 - 7.9		

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Apolipoprotein A-1	**g/L**	Y	B	N/A	N/A	20-33	1.00 - 2.50	65-74	0.20 - 2.90	56	
		N	M	20-29	1.02 - 1.67	60-69	1.04 - 1.78	70-79	1.02 - 1.77	60	
			F	20-29	1.12 - 1.89	60-69	1.17 - 1.99	70-79	1.16 - 1.36		
Apolipoprotein B	**g/L**	N	M	<30	0.54 - 1.09	60-69	0.69 - 1.45	70-79	0.74 - 1.44	52	
			F	<30	0.59 - 1.14	60-69	0.72 - 1.45	70-79	0.78 - 1.42		
		N	M	20-29	0.63 - 1.56	60-69	0.90 - 2.01	70-79	0.84 - 1.96	60	
			F	20-29	0.61 - 1.40	60-69	0.91 - 2.10	70-79	0.87 - 2.07		
Arginine	**µmol/L**	N	B	35-50	53 - 113	50-65	51 - 125	>65	62 - 118	27	
Aspartate aminotransferase	**µkat/L**	N	M	21-25	<0.58	61-65	<0.58	66-70	<0.58	40	
			F	21-25	<0.48	61-65	<0.54	66-70	<0.56		
		Y	M	N/A	N/A	<64	0.22 - 0.60	>65	0.17 - 0.68	48	
			F	N/A	N/A	<64	0.20 - 0.51	>65	0.15 - 0.56		
		Y	B	N/A	N/A	20-33	0.07 - 0.41	65-74	0.10 - 0.27	56	
		Y	M	<60	0.27 - 0.71	60-90	NP	>90	0.19 - 0.65	57	
			F	<60	0.24 - 0.49	60-90	NP	>90	0.31 - 0.51		
		Y	B	N/A	N/A	N/A	N/A	65-99	0.09 - 0.53	58	*
Asymmetric dimethyl	**µmol/L**	N	B	35-50	0.45 - 0.73	50-65	0.46 - 0.78	>65	0.54 - 0.79	27	
Bilirubin, conjugated	**µmol/L**	Y	B	N/A	N/A	20-33	1.0 - 5.4	65-74	1.1 - 5.8	56	
		Y	B	<60	2.0 - 5.0	60-90	<2.0	>90	<2.0 - 3.0	57	
Bilirubin, total	**µmol/L**	N	M	21-25	<27	61-65	<21	66-70	<21	40	
			F	21-25	<17	61-65	<14	66-70	<15		
		Y	B	N/A	N/A	20-33	4 - 32	65-74	6 - 32	56	
		Y	B	<60	3 - 12	60-90	2 - 11	>90	3 - 15	57	
B-type natriuretic peptide	**ng/L**	N	B	N/A	N/A	N/A	N/A	>65	4.5 - 96.5	28	*
N-terminal-pro-B-type natriuretic peptide	**ng/L**	N	B	N/A	N/A	N/A	N/A	>65	20 - 540	28	*
		Y	M	20-30	<84	61-70	<137	71-85	<158	34	
			F	20-30	<114	61-70	<187	71-85	<218		
Calcium, total	**mmol/L**	Y	M	N/A	N/A	<64	2.27 - 2.62	>65	2.12 - 2.46	48	
			F	N/A	N/A	<64	2.21 - 2.62	>65	2.13 - 2.54		
		Y	B	<60	2.15 - 2.50	60-90	2.20 - 2.55	>90	2.05 - 2.40	57	
Carbon dioxide, partial pressure	**kPa**	N	M	N/A	N/A	N/A	N/A	>70	<5.85	38	*
			F	N/A	N/A	N/A	N/A	>70	<6.08		
Carbon dioxide, total	**mmol/L**	Y	B	<60	23 - 29	60-90	23 - 31	>90	20 - 29	57	
Carcinoembryonic antigen	**µg/L**	N	B	18	<3	64	<4	65	<4	22	
		Y	B	<60	0 - 3	60-90	NP	>90	0.4 - 9.2	57	

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	> 65 Age Group	> 65 RI	Study Ref	
Chloride	**mmol/L**	Y	B	N/A	N/A	<64	NP	>65	94 - 110	48	
		Y	B	<60	98 - 106	60-90	98 - 107	>90	98 - 111	57	
Cholesterol	**mmol/L**	N	M	15-25	3.3 - 6.2	56-65	4.2 - 8.2	66-75	4.0 - 8.1	47	
		Y	B	N/A	N/A	<64	3.8 - 8.3	>65	3.3 - 8.3	48	
		Y	B	N/A	N/A	20-33	3.6 - 6.5	65-74	4.3 - 8.7	56	
		N	M	N/A	N/A	N/A	N/A	65-74	4.4 - 6.2	53	*
			F	N/A	N/A	N/A	N/A	65-74	4.9 - 6.8		
		Y	M	<60	3.3 - 7.2	60-90	4.3 - 8.7	>90	2.9 - 6.6	57	
			F	<60	3.3 - 6.8	60-90	4.3 - 9.0	>90	3.8 - 7.0		
		Y	B	N/A	N/A	N/A	N/A	65-99	4.0 - 5.8	58	*
		N	M	20-29	2.8 - 6.9	60-69	3.9 - 8.4	70-79	3.8 - 8.2	60	
			F	20-29	3.0 - 6.9	60-69	4.3 - 9.0	70-79	4.2 - 9.0		
Copper	**µmol/L**	N	M	25-44	14.1 - 27.1	45-64	13.6 - 26.9	65-80	17.4 - 29.5	41	
			F	25-44	13.6 - 34.9	45-64	17.8 - 33.9	65-80	21.2 - 28.1		
		Y	M	<60	11.0 - 22.0	60-90	13.5 - 27.2	>90	11.8 - 29.0	57	
			F	<60	12.6 - 24.4	60-90	16.8 - 29.7	>90	15.7 - 31.0		
Cortisol, total	**nmol/L**	Y	B	<60	138 - 635	60-90	215 - 621	>90	154 - 635	57	
C-peptide	**nmol/L**	Y	B	<60	1.4 - 4.3	60-90	1.5 - 4.9	>90	0.6 - 4.4	57	
C-reactive protein	**mg/L**	Y	M	N/A	N/A	40-49	<4.8	65-99	<6.8	44	
			F	N/A	N/A	40-49	<3.3	65-99	<6.6		
		Y	B	N/A	N/A	20-39	<6.7	>70	<19.7	45	
Creatine kinase	**µkat/L**	Y	M	N/A	N/A	<64	0.95 - 7.40	>65	0.37 - 3.50	48	
			F	N/A	N/A	<64	0.65 - 3.59	>65	0.31 - 3.13		
		Y	B	N/A	N/A	<75	0.61 - 4.96	>75	0.49 - 3.62	49	
		N	M	25-34	0.92 - 4.85	55-64	0.92 - 4.85	>65	0.92 - 4.85	54	
			F	25-34	0.65 - 2.69	55-64	0.65 - 2.69	>65	0.65 - 2.69		
		Y	M	<60	0.88 - 3.40	60-90	NP	>90	0.36 - 3.45	57	
			F	<60	0.60 - 2.81	60-90	NP	>90	0.37 - 1.68		

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Creatinine	**µmol/L**	N	M	20-24	57 - 103	60-64	56 - 116	65-69	56 - 126	25	
			F	20-24	40 - 83	60-64	44 - 91	65-69	43 - 97		
		N	M	51-60	100 - 150	61-70	100 - 180	71-85	90 - 160	46	
			F	51-60	80 - 150	61-70	70 - 180	71-85	90 - 150		
		N	M	15-55	77 - 122	56-75	80 - 136	76-95	81 - 151	47	
		Y	M	N/A	N/A	<64	81 - 118	>65	67 - 141	48	
			F	N/A	N/A	<64	66 - 101	>65	62 - 134		
		Y	B	N/A	N/A	<75	62 - 124	>75	62 - 150	49	
		Y	B	N/A	N/A	N/A	N/A	65-74	87 - 116	53	*
				N/A	N/A	N/A	N/A	65-74	64 - 92		
		N	F	40-49	52 - 84	60-69	48 - 96	70-79	48 - 86	55	
		Y	B	N/A	N/A	20-33	64 - 105	65-74	59 - 119	56	
		Y	M	<60	80 - 115	60-90	71 - 115	>90	88 - 150	57	
			F	<60	53 - 97	60-90	53 - 106	>90	53 - 115		
		Y	B	N/A	N/A	N/A	N/A	65-99	80 - 180	58	*
Dehydroepiandrosterone sulfate	**µmol/L**	N	M	20-24	0.41 - 1.65	60-64	0.18 - 0.92	65-69	0.12 - 0.84	23	
			M	<60	0.49 - 1.22	60-90	NP	>90	0.10 - 2.00	57	
			F	<60	0.33 - 0.85	60-90	NP	>90	1.00 - 1.60		
Dehydroepiandrosterone, unconjugated	**nmol/L**	Y	B	<60	5.5 - 27.8	60-90	NP	>90	0.6 - 5.9	57	
Dihydrotestosterone	**nmol/L**	Y	M	N/A	N/A	N/A	N/A	70-89	0.49 - 3.16	73	*
Estradiol	**pmol/L**	N	M	N/A	N/A	64-69	0.06 - 0.17	>75	0.05 - 0.18	30	
		Y	B	<60	0.11 - 0.37	60-90	NP	>90	<0.02 - 0.07	57	
		Y	M	N/A	N/A	N/A	N/A	70-89	0.03 - 0.14	73	*
Ferritin	**µg/L**	N	M	N/A	N/A	N/A	N/A	>65	15 - 342	43	*
			F	N/A	N/A	N/A	N/A	>65	16 - 238		
		N	M	20-29	16 - 218	60-69	NP	>70	NP	63	
			F	20-29	16 - 136	60-69	12 - 304	>70	15 - 387		
α-Fetoprotein	**g/L**	N	B	18	<3.69	64	<9.80	65	<9.88	22	
Fibrinogen	**µmol/L**	N	M	N/A	N/A	N/A	N/A	65-74	7.33 - 10.55	53	*
			F	N/A	N/A	N/A	N/A	65-74	7.48 - 10.87		
Folate	**nmol/L**	Y	B	N/A	N/A	19-55	5 - 16	65-88	4 - 18	50	
		Y	M	<60	7 - 28	60-90	4 - 24	>90	8 - 36	57	
			F	<60	6 - 45	60-90	4 - 28	>90	7 - 36		

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Follicle-stimulating hormone	**IU/L**	N	M	20-29	1.4 - 8.9	60-69	2.4 - 13.4	70-79	2.7 - 14.2	20	
		N	M	N/A	N/A	64-69	2.6 - 31.1	>75	3.2 - 36.9	30	
		Y	M	<60	2.2 - 14.0	60-90	NP	>90	8.0 - 11.2	57	
			F	<60	3.5 - 17.0	60-90	NP	>90	5.5 - 16.8		
Glucose	**mmol/L**	N	M	15-45	3.5 - 10.0	NP	NP	>80	3.7 - 6.8	47	
		N	M	N/A	N/A	N/A	N/A	65-74	5.0 - 6.1	53	*
			F	N/A	N/A	N/A	N/A	65-74	4.9 - 5.9		
		Y	B	N/A	N/A	20-33	4.0 - 6.0	65-74	4.2 - 7.0	56	
		Y	B	<60	4.3 - 5.8	60-90	4.6 - 6.4	>90	4.2 - 6.7	57	
		Y	B	N/A	N/A	N/A	N/A	65-99	4.0 - 5.4	58	*
Glucose, fasting	**mmol/L**	Y	B	N/A	N/A	<64	NP	>65	3.9 - 8.6	48	
ϒ-Glutamyltransferase	**µkat/L**	Y	M	N/A	N/A	<64	0.19 - 1.73	>65	0.19 - 2.11	48	
			F	N/A	N/A	<64	0.14 - 0.80	>65	0.17 - 2.52		
		Y	B	N/A	N/A	20-33	0.03 - 0.36	65-74	0.05 - 0.58	56	
		Y	M	<60	0.17 - 0.58	60-90	0.19 - 0.71	>90	0.05 - 0.80	57	
			F	<60	0.12 - 0.51	60-90	0.15 - 0.94	>90	0.07 - 0.75		
High-density lipoprotein cholesterol	**mmol/L**	B	M	N/A	N/A	N/A	N/A	65-74	0.96 - 1.56	53	*
			F	N/A	N/A	N/A	N/A	65-74	1.20 - 1.98		
		Y	B	N/A	N/A	20-33	0.90 - 2.00	65-74	0.80 - 2.10	56	
		Y	M	<60	0.52 - 1.78	60-90	0.72 - 2.53	>90	0.72 - 2.12	57	
			F	<60	0.70 - 2.20	60-90	0.72 - 2.69	>90	0.83 - 2.15		
Homoarginine	**µmol/L**	N	B	35-50	1.38 - 5.29	50-65	1.43 - 4.69	>65	1.11 - 4.68	27	
Homocysteine	**µmol/L**	Y	M	N/A	N/A	19-30	<7.7	>70	<11.3	32	
			F	N/A	N/A	19-30	<5.9	>70	<10.2		
		Y	B	N/A	N/A	19-55	5.0 - 13.9	65-88	6.8 - 21.0	50	
Insulin, fasting	**pmol/L**	N	M	N/A	N/A	N/A	N/A	65-74	45 - 136	53	*
			F	N/A	N/A	N/A	N/A	65-74	42 - 126		
		Y	B	<60	43 - 165	60-90	47 - 263	>90	17 - 136	57	
Insulin-like growth factor-I	**µg/L**	N	M	25-29	117 - 328	60-64	60 - 214	65-69	55 - 209	17	
			F	25-29	118 - 377	60-64	65 - 233	65-69	61 - 222		
		N	M	20-29	97 - 250	60-69	61 - 155	70-79	54 - 138	19	
			F	20-29	86 - 279	60-69	49 - 159	70-79	43 - 140		
		N	B	20	>153	64	>48	65	>47	21	
		N	M	20-24	156 - 385	60-64	55 - 211	65-69	33 - 237	26	
			F	20-24	111 - 423	60-64	61 - 237	65-69	34 - 237		
		N	B	20-24	140 - 493	60-64	43 - 257	65-69	36 - 237	39	
		N	M	18-24	119 - 485	60-64	41 - 323	65-69	36 - 306	70	
			F	18-24	72 - 468	60-64	31 - 297	65-69	29 - 289		

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Insulin-like growth factor binding protein - 3	**mg/L**	N	M	25-29	2.99 - 6.57	60-64	2.22 - 5.34	65-69	2.04 - 5.13	17	
			F	25-29	3.08 - 6.57	60-64	2.53 - 6.06	65-69	2.46 - 5.99		
		N	M	20-24	1.35 - 2.93	60-64	0.82 - 2.50	65-69	0.73 - 2.52	26	
				20-24	1.16 - 3.11	60-64	1.02 - 2.81	65-69	0.95 - 2.79		
Insulin tolerance test	**mEq/L**	N	B	20	>22.9	64	>3.8	65	>3.6	21	
Iron	**µmol/L**	N	M	N/A	N/A	N/A	N/A	>65	9 - 33	43	*
			F	N/A	N/A	N/A	N/A	>65	8 - 27		
		Y	M	N/A	N/A	<64	8 - 29	>65	22 - 42 (M+F)	48	
			F	N/A	N/A	<64	5 - 30	>65	N/A		
		Y	M	<60	11 - 30	60-90	NP	>90	7 - 23	57	
			F	<60	8 - 29	60-90	NP	>90	6 - 24		
Lactate dehydrogenase	**µkat/L**	Y	M	N/A	N/A	<64	4.35 - 7.43	>65	3.45 - 8.65 (M+F)	48	
			F	N/A	N/A	<64	4.10 - 7.85	>65	N/A		
		Y	B	N/A	N/A	<75	1.89 - 4.06	>75	1.99 - 4.25	49	
		N	B	25-34	4.05 - 7.51	55-64	4.27 - 7.96	>65	4.51 - 8.42	54	
Lactate dehydrogenase isoenzyme 1	**µkat/L**	N	B	25-34	0.85 - 1.96	55-64	0.90 - 2.07	>65	0.95 - 2.19	54	
	%	Y	B	<60	19 - 35	60-90	13 - 38	>90	20 - 38	57	
Lead	**µmol/L**	Y	B	<60	<1.21	60-90	NP	>90	<0.72	57	
Lipase	**µkat/L**	Y	M	N/A	N/A	<64	0.00 - 3.38	>65	0.00 - 3.43 (M+F)	48	
			F	N/A	N/A	<64	0.26 - 3.03	>65	N/A		
		Y	B	<60	0.53 - 3.16	60-90	NP	>90	0.44 - 4.54	57	
Low-density lipoprotein cholesterol	**mmol/L**	N	M	<30	1.40 - 2.82	60-69	1.79 - 3.76	70-79	1.92 - 3.73	52	
			F	<30	1.53 - 2.95	60-69	1.86 - 3.76	70-79	2.02 - 3.68		
		N	M	N/A	N/A	N/A	N/A	65-74	2.56 - 4.22	53	*
			F	N/A	N/A	N/A	N/A	65-74	2.66 - 4.54		
		Y	B	N/A	N/A	20-33	1.70 - 4.50	65-74	2.60 - 6.00	56	
		Y	B	<60	2.40 - 4.77	60-90	NP	>90	2.56 - 7.36	57	
Luteinizing hormone	**IU/L**	N	M	20-29	2.0 - 9.4	60-69	2.1 - 10.8	70-79	2.2 - 11.2	20	
		N	M	N/A	N/A	64-69	2.2 - 13.9	>75	2.3 - 19.1	30	
		Y	M	<60	2.5 - 19.7	60-90	NP	>90	1.9 - 11.5	57	
			F	<60	3.5 - 31.0	60-90	NP	>90	3.5 - 15.2		
Magnesium	**mmol/L**	Y	M	N/A	N/A	<64	0.70 - 0.96	>65	0.62 - 1.03 (M+F)	48	
			F	N/A	N/A	<64	0.70 - 0.94	>65	N/A		
		Y	B	<60	0.66 - 1.03	60-90	0.66 - 0.99	>90	0.70 - 0.95	57	
		N	M	20-29	0.63 - 0.96	60-69	0.66 - 0.99	70-79	0.68 - 1.00	64	
			F	20-29	0.65 - 0.91	60-69	0.64 - 0.97	70-79	0.60 - 1.03		

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Methlymalonic acid	**nmol/L**	Y	B	N/A	N/A	<70	60 - 360	>70	50 - 480	14	
		Y	B	N/A	N/A	19-55	62 - 247	65-88	72 - 476	50	
Oxygen, arterial partial pressure	**kPa**	N	M	N/A	N/A	N/A	N/A	>70	<5.37	38	*
			F	N/A	N/A	N/A	N/A	>70	<5.49		
Oxygen, partial pressure	**kPa**	N	M	N/A	N/A	N/A	N/A	>70	<8.24	38	*
			F	N/A	N/A	N/A	N/A	>70	<7.93		
Oxygen, saturation	**%**	N	M	N/A	N/A	N/A	N/A	>70	>93.0	38	*
			F	N/A	N/A	N/A	N/A	>70	>92.0		
Parathyroid hormone	**pmol/L**	Y	B	<60	36 - 86	60-90	NP	>90	49 - 118	57	
pH	**pH**	Y	B	<60	7.35 - 7.45	60-90	7.31 - 7.42	>90	7.26 - 7.43	57	
Phosphate	**mmol/L**	Y	M	N/A	N/A	<64	0.8 - 1.4 (M+F)	>65	0.7 - 1.2	48	
			F	N/A	N/A	<64	N/A	>65	0.8 - 1.4		
		Y	M	<60	0.9 - 1.3	60-90	0.7 - 1.2	>90	0.7 - 1.3	57	
			F	<60	0.8 - 1.3	60-90	0.9 - 1.3	>90	0.8 - 1.4		
Potassium	**mmol/L**	Y	B	N/A	N/A	<64	3.3 - 4.8	>65	3.2 - 5.0	48	
		N	M	N/A	N/A	N/A	N/A	65-74	4.0 - 4.6	53	*
			F	N/A	N/A	N/A	N/A	65-74	3.9 - 4.5		
		Y	B	<60	3.8 - 4.9	60-90	3.9 - 5.3	>90	3.6 - 5.5	57	
Progesterone	**nmol/L**	Y	M	<60	0.4 - 3.1	60-90	NP	>90	<0.6 - 1.5	57	
			F	<60	0.5 - 2.2	60-90	NP	>90	<0.6 - 1.7		
Prolactin	**µg/L**	Y	M	<60	3.9 - 22.1	60-90	NP	>90	8.0 - 25.0	57	
			F	<60	3.5 - 27.0	60-90	NP	>90	7.0 - 53.0		
Protein, total	**g/L**	Y	M	N/A	N/A	<64	69 - 84	>65	64 - 83	48	
			F	N/A	N/A	<64	67 - 83	>65	60 - 83		
		Y	B	N/A	N/A	20-33	66 - 82	65-74	65 - 82	56	
		Y	B	<60	63 - 78	60-90	62 - 77	>90	60 - 80	57	
		Y	B	N/A	N/A	N/A	N/A	65-99	65 - 77	58	*
Serum amyloid A	**mg/L**	Y	B	N/A	N/A	<50	4.9	>50	8.3	29	
Sex hormone binding globulin	**nmol/L**	N	M	20-29	13.1 - 53.2	60-69	22.6 - 87.6	70-79	27.8- 101.0	20	
		N	M	N/A	N/A	64-69	9.0 - 100.0	>75	18.3 - 106.0	30	
		Y	M	<60	12.1 - 35.7	60-90	NP	>90	38.2 - 156.2	57	
			F	<60	9.0 - 19.0	60-90	NP	>90	38.2 - 208.2		
Silicon	**µmol/L**	Y	M	N/A	N/A	30-44	4.3 - 19.5	>75	3.6 - 16.1	36	
			F	N/A	N/A	30-44	4.2 - 20.4	>75	4.20 - 20.5		
Sodium (Na)	**mmol/L**	Y	M	N/A	N/A	<64	140 - 148	>65	132 - 146 (M+F)	48	
			F	N/A	N/A	<64	139 - 147	>65	N/A		
		Y	B	<60	137 - 143	60-90	137 - 144	>90	132 - 145	57	

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Symmetric dimethyl arginine	**µmol/L**	N	B	35-50	0.27 - 0.88	50-65	0.30 - 0.84	>65	0.33 - 0.88	27	
Testosterone, free	**pmol/L**	Y	M	N/A	N/A	18-69	121.5- 537.9	>70	104.1 - 469.5	16	
			F	N/A	N/A	18-69	0.4 - 22.2	>70	0.7 - 12.8		
		N	M	N/A	N/A	64-69	118.0 - 471.0	>75	101.0 - 468.0	30	
		Y	B	<60	180.3 - 971.8	60-90	NP	>90	40.9 - 258.9		
		N	F	20-29	2.0 - 29.4	60-69	2.8 - 23.0	70-80	3.0 - 21.3	69	
Testosterone, total	**nmol/L**	Y	M	N/A	N/A	18-69	8.7 - 38.2	>70	3.1 - 30.9	16	
			F	N/A	N/A	18-69	0.1 - 1.6	>70	0.1 - 1.4		
		N	M	20-29	11.6 - 33.8	60-69	8.9 - 30.9	70-79	8.6 - 30.7	20	
		N	M	20-24	10.4 - 32.3	60-64	7.0 - 29.5	65-69	6.7 - 29.0	23	
		N	M	N/A	N/A	64-69	8.0 - 37.7	>75	8.9 - 35.9	30	
		Y	B	<60	12.1 - 35.7	60-90	NP	>90	7.5 - 23.3	57	
		N	F	20-29	0.4 - 2.1	60-69	0.3 - 1.9	70-80	0.3 - 1.8	69	
		Y	M	N/A	N/A	N/A	N/A	70-89	6.40 - 25.7	73	*
Thyroid-stimulating hormone	**mIU/L**	N	B	20-39	0.53 - 4.16	60-69	0.57 - 4.75	>70	0.75 - 5.37	18	
		Y	B	18-29	0.80 - 5.40	60-69	0.73 - 4.90	>70	0.66 - 6.30	24	
		N	M	N/A	N/A	NP	NP	65-100	0.47 - 5.40	33	
			F	N/A	N/A	NP	NP	65-100	0.51 - 7.20		
		Y	B	<60	0.70 - 7.00	60-90	NP	>90	0.40 - 7.20	57	
		N	M	20-24	0.35 - 7.78	60-69	0.42 - 7.57	70-79	0.17 - 4.53	68	
			F	20-24	0.67 - 4.18	60-69	0.48 - 5.34	70-79	0.63 - 5.56		
		N	B	18-30	0.52 - 4.15	61-70	0.48 - 4.59	71-80	0.40 - 4.96	71	
		N	M	20-25	0.53 - 3.76	61-65	0.57 - 4.09	66-70	0.54 - 4.23	72	
			F	20-25	0.48 - 3.69	61-65	0.51 - 4.34	66-70	0.47 - 4.50		
Thyroxine 3, total	**nmol/L**	Y	B	<60	1.6 - 3.1	60-90	NP	>90	1.1 - 2.5	57	
Thyroxine 4, free	**pmol/L**	N	M	20-39	14.29 - 21.88	60-69	13.77 - 16.60	>70	12.87 - 19.69	18	
			F	20-39	13.13 - 20.46	60-69	13.00 - 15.70	>70	13.51 - 18.79		
		N	B	N/A	N/A	NP	NP	65-100	9.70 - 17.60	33	
Thyroxine 4, total	**nmol/L**	Y	B	<60	71 - 142	60-90	NP	>90	68 - 129	57	
Transferrin	**g/L**	Y	M	N/A	N/A	<64	2.06 - 3.25	>65	1.75 - 3.33 (M+F)	48	
			F	N/A	N/A	<64	2.14 - 3.81	>65	N/A		
		Y	B	<60	2.57 - 4.29	60-90	1.91 - 3.75	>90	1.86 - 3.47	57	

Analyte	Units	Young / Old Defined	Male/ Female /Both	Youngest Age Group	Youngest Age Group RI	< 65 Age Group	< 65 RI	≥ 65 Age Group	≥ 65 RI	Study Ref	
Triglycerides	**mmol/L**	Y	B	N/A	N/A	<64	NP	>65	0.60 - 3.70	48	
		Y	B	N/A	N/A	20-33	0.60 - 2.10	65-74	0.60 - 3.50	56	
		N	M	N/A	N/A	N/A	N/A	65-74	0.77 - 2.10	53	*
			F	N/A	N/A	N/A	N/A	65-74	0.82 - 2.05		
		Y	B	N/A	N/A	N/A	N/A	65-99	0.72 - 1.66	58	*
		N	M	20-29	<2.99	60-69	<3.97	70-79	<3.81	60	
			F	20-29	<2.00	60-69	<4.88	70-79	<3.40		
		Y	M	<60	0.46 - 2.18	60-90	0.35 - 3.95	>90	0.87 - 1.25	57	
			F	<60	0.34 - 2.09	60-90	0.50 - 3.29	>90	0.49 - 1.63		
Urea	**mmol/L**	N	M	51-60	1.6 - 8.1	61-70	2.0 - 8.3	71-85	1.7 - 8.7	46	
			F	51-60	1.5 - 6.9	61-70	1.5 - 9.3	71-85	2.3 - 8.5		
		N	M	15-55	2.2 - 7.3	56-75	2.9 - 9.1	76-95	3.3 - 13.3	47	
		Y	M	N/A	N/A	<64	3.8 - 9.5	>65	3.3 - 11.9 (M+F)	48	
			F	N/A	N/A	<64	3.2 - 8.0	>65	NP		
		Y	B	N/A	N/A	<75	2.4 - 7.6	>75	8.0 - 30.0	49	
		Y	M	N/A	N/A	20-33	3.3 - 7.7	65-74	4.6 - 8.5	56	
			F	N/A	N/A	20-33	2.5 - 5.8	65-74	3.8 - 7.7		
		Y	B	<60	2.1 - 7.1	60-90	2.9 - 8.2	>90	3.6 - 11.1	57	
		Y	B	N/A	N/A	N/A	N/A	65-99	5.6 - 11.6	58	*
Uric acid	**mmol/L**	Y	M	N/A	N/A	<64	0.23 - 0.60	>65	0.22 - 0.70	48	
			F	N/A	N/A	<64	0.16 - 0.35	>65	0.20 - 0.62		
		N	M	N/A	N/A	N/A	N/A	65-74	0.28 - 0.42	53	*
			F	N/A	N/A	N/A	N/A	65-74	0.22 - 0.36		
		Y	M	<60	0.26 - 0.45	60-90	0.25 - 0.48	>90	0.21 - 0.49	57	
			F	<60	0.14 - 0.39	60-90	0.21 - 0.43	>90	0.13 - 0.46		
		Y	B	N/A	N/A	N/A	N/A	65-99	0.25 - 0.42	58	*
Vitamin B_{12}	**pmol/L**	Y	B	N/A	N/A	35-40	167 - 573	75-80	113 - 515	42	
		Y	B	N/A	N/A	19-55	103 - 406	65-88	91 - 480	50	
		Y	B	<60	92 - 528	60-90	81 - 567	>90	43 - 644	57	
Zinc	**µmol/L**	N	M	25-44	12.1 - 27.6	45-64	12.1 - 23.0	65-80	11.2 - 28.3	41	
			F	25-44	10.4 - 24.0	45-64	9.7 - 26.9	65-80	14.3 - 27.4		
		Y	B	<60	10.7 - 23.0	60-90	9.6 - 16.4	>90	8.0 - 15.1	57	

NOTE: A star indicates the study listed only investigated individuals aged 65 and older. Although trends in analyte concentration with age were investigated in these studies the effect may have been diminished by the narrow age range evaluated.

APPENDIX D: ALL REFERENCE INTERVALS

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Adrenocorticotropic hormone	pmol/L	31	75	B		2.0	(0.4 - 2.2)	18.9	(14 - 23)	120
		57	>90	B		2.0		23.0		84
Alanine aminotransferase	µkat/L	15	70	M	All	0.15		0.74		438
			70	M	w/o CVD	0.15		0.72		350
			70	F	All	0.15		0.80		459
			70	F	w/o CVD	0.15		0.84		408
		40	66-70	M				0.66		3616
			66-70	F				0.56		4999
			71-75	M				0.63		3287
			71-75	F				0.51		4854
			76-80	M				0.56		2734
			76-80	F				0.51		4277
			>80	M				0.51		2368
			>80	F				0.46		4860
		48	>65	M		0.10		0.70		71
			>65	F		0.09		0.60		165
		49	>75	B		0.15	(0.12 - 0.17)	0.65	(0.61 - 0.85)	497
		51	70	F	Healthy reference group	0.03		0.65		47
			70	F	Total study population	0.05		0.94		206
			70	M	Healthy reference group	0.05		0.66		64
			70	M	Total study population	0.05		0.87		250
		56	65-74	B		0.10		0.32		31
		57	>90	M		0.10		0.65		18
			>90	F		0.09		0.41		40
		58	100-120	B	Centenarians	0.10		0.36		120
			65-85	B	Control	0.43		0.90		381
		61	65-74	B	Mexican American	0.07		0.43		185
			65-74	B	Cuban	0.12		0.31		96
			65-74	B	Puerto Rican	0.14		0.43		66
		67	>65	M		0.22		1.23		247
			>65	F		0.20		0.91		333
		74	75	M	All	0.21	(0.18 - 0.23)	0.75	(0.64 - 0.85)	354
			75	M	w/o CVD	0.20	(0.18 - 0.23)	0.76	(0.62 - 0.90)	274
			75	F	All	0.20	(0.18 - 0.20)	0.60	(0.51 - 0.69)	373
			75	F	w/o CVD	0.19	(0.18 - 0.20)	0.60	(0.52 - 0.67)	338

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Albumin	g/L	15	70	M	All males	35.4	(34.9 - 36.0)	47.5	(46.7 - 48.2)	438
			70	M	Males w/o CVD	35.6	(35.0 - 36.1)	46.9	(45.7 - 48.2)	350
			70	F	Females	35.8	(35.2 - 36.4)	46.2	(45.4 - 47.1)	459
			70	F	Females w/o CVD	35.8	(35.2 - 36.4)	46.3	(45.5 - 47.1)	408
		47	76-95	M		32.0		46.0		414
		48	>65	M		36.0		48.0		71
			>65	F		38.0		49.0		165
		49	>75	B		35.0	(34.0 - 36.0)	46.0	(46.0 - 47.0)	497
		51	70	F	Healthy reference group	31.0		49.0		51
			70	F	Total study population	30.0		48.0		206
			70	M	Healthy reference group	32.0		48.0		61
			70	M	Total study population	32.0		48.0		250
		53	65-74	F		37.2		42.8		775
			65-74	M		37.2		42.8		454
			> 75	F		36.4		41.6		239
			> 75	M		36.9		43.1		202
		58	100-120	B	Centenarians	33.5		43.3		120
			65-85	B	Control	28.6		41.6		381
		61	65-74	B	Mexican American	36.0		48.0		185
			65-74	B	Cuban	37.1		45.0		96
			65-74	B	Puerto Rican	35.0		45.1		66
		75	75	M	All	37.2	(36.3 - 38.1)	52.5	(51.7 - 53.2)	354
			75	M	w/o CVD	37.5	(36.7 - 38.2)	52.1	(51.1 - 53.2)	274
			75	F	All	38.2	(37.7 - 38.8)	51.1	(50.3 - 52.0)	373
			75	F	w/o CVD	38.2	(37.7 - 38.8)	51.1	(50.3 - 52.3)	338
		75	>75	B		36.0		48.0		222
Alkaline phosphatase	µkat/L	15	70	M	All Males	0.52	(0.48 - 0.56)	1.65	(1.49 - 1.81)	438
			70	M	Males w/o CVD	0.52	(0.48 - 0.57)	1.65	(1.46 - 4.83)	350
			70	F	All females	0.51	(0.49 - 0.55)	1.69	(1.61 - 1.77)	459
			70	F	Females w/o CVD	0.52	(0.49 - 0.55)	1.70	(1.62 - 1.78)	408
		40	66-70	M		0.83		2.18		3616
			66-70	F		0.77		2.21		4999
			71-75	M		0.83		2.19		3287
			71-75	F		0.77		2.26		4854
			76-80	M		0.82		2.28		2734
			76-80	F		0.78		2.31		4277
			>80	M		0.85		2.43		2368
			>80	F		0.80		2.40		4860

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Alkaline phosphatase	µkat/L	48	>65	B		0.80		2.72		236
cont'd		49	>75	B		0.70	(0.63 - 0.77)	2.26	(2.11 - 2.55)	497
		51	70	F	Healthy reference group	1.85		3.43		47
			70	F	Total study population	1.62		7.19		206
			70	M	Healthy reference group	1.75		5.17		65
			70	M	Total study population	1.53		6.68		250
		56	65-74	B		1.09		3.26		32
		57	>90	M		0.95		2.64		14
			>90	F		0.73		2.72		29
		61	65-74	B	Mexican American	0.61		1.77		185
			65-74	B	Cuban	0.96		1.00		96
			65-74	B	Puerto Rican	0.99		1.02		66
		66	65-74	M		0.97		2.06		888
			65-74	F		1.05		2.14		656
		74	75	M	All	0.86	(0.81 - 0.91)	2.19	(1.99 - 2.39)	354
			75	M	w/o CVD	0.86	(0.80 - 0.92)	2.09	(1.84 - 2.33)	274
			75	F	All	0.84	(0.78 - 0.90)	2.34	(2.04 - 2.65)	373
			75	F	w/o CVD	0.86	(0.81 - 0.91)	2.42	(2.11 - 2.74)	338
Amylase	µkat/L	15	70	M	All Males	0.16	(0.14 - 0.18)	0.83	(0.76 - 0.91)	438
			70	M	Males w/o CVD	0.17	(0.14 - 0.19)	0.84	(0.77 - 0.92)	350
			70	F	All females	0.17	(0.15 - 0.19)	0.95	(0.83 - 1.05)	459
			70	F	Females w/o CVD	0.17	(0.14 - 0.20)	0.94	(0.83 - 1.04)	408
		48	>65	B		0.31		2.52		236
		57	>90	B		0.43		2.50		55
Androstenedione	nmol/L	31	75	M		1.7	(1.6 - 2.3)	12.2	(9.8 - 14.0)	54
			75	F		1.0	(1.0 - 14.0)	7.7	(7.3 - 10.1)	64
		57	>90	M		0.9		4.1		14
			>90	F		0.1		7.9		30
		69	70-80	F		0.5		2.5		163
Apolipoprotein A-1	g/L	56	65-74	B		0.02		0.29		25
		60	70-79	M		1.02		1.77		3657
			70-79	F		1.16		1.97		5388
			> 80	M		1.02		1.75		614
			> 80	F		1.16		1.96		1524
		15	70	M	All Males	1.10	(1.06 - 1.14)	2.15	(2.01 - 2.29)	438
			70	M	Males w/o CVD	1.11	(1.07 - 1.15)	2.18	(2.04 - 2.32)	350
			70	F	All females	1.27	(1.21 - 1.32)	2.45	(2.37 - 2.54)	459
			70	F	Females w/o CVD	1.26	(1.19 - 1.32)	2.46	(2.36 - 2.55)	408

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Apolipoprotein A-1 Cont'd	g/L	74	75	M	All	1.10	(1.07 - 1.12)	2.22	(2.13 - 2.31)	354
			75	M	w/o CVD	1.11	(1.08 - 1.14)	2.26	(2.10 - 2.42)	274
			75	F	All	1.33	(1.28 - 1.38)	2.50	(2.30 - 2.71)	373
			75	F	w/o CVD	1.34	(1.29 - 1.40)	2.52	(2.32 - 2.72)	338
Apolipoprotein B	g/L	60	70-79	M		0.84		1.96		3657
			70-79	F		0.87		2.07		5388
			> 80	M		0.75		1.81		614
			> 80	F		0.82		1.96		1524
		15	70	M	All Males	0.62	(0.60 - 0.65)	1.54	(1.46 - 1.61)	438
			70	M	Males w/o CVD	0.64	(0.61 - 0.67)	1.55	(1.44 - 1.66)	350
			70	F	All females	0.70	(0.66 - 0.73)	1.57	(1.48 - 1.66)	459
			70	F	Females w/o CVD	0.70	(0.67 - 0.74)	1.59	(1.50 - 1.69)	408
		52	70-79	M		0.74		1.44		59
			70-79	F		0.78		1.42		48
		74	75	M	All	0.64	(0.59 - 0.68)	1.61	(1.55 - 1.67)	354
			75	M	w/o CVD	0.66	(0.61 - 0.71)	1.60	(1.54 - 1.66)	274
			75	F	All	0.74	(0.70 - 0.77)	1.71	(1.66 - 1.76)	373
			75	F	w/o CVD	0.73	(0.69 - 0.77)	1.70	(1.65 - 1.76)	338
Arginine	µmol/L	27	>65	B		62.3		118.3		39
Aspartate aminotransferase	µkat/L	31	75	B		0.18	(0.17 - 0.20)	0.68	(0.63 - 0.75)	120
		40	66-70	M				0.58		3616
			66-70	F				0.56		4999
			71-75	M				0.60		3287
			71-75	F				0.56		4854
			76-80	M				0.56		2734
			76-80	F				0.56		4277
			>80	M				0.60		2368
			>80	F				0.56		4860
		48	>65	M		0.17		0.68		71
			>65	F		0.15		0.56		165
		51	70	F	Healthy reference group	0.34		0.78		47
			70	F	Total study population	0.31		1.04		206
			70	M	Healthy reference group	0.31		0.77		64
			70	M	Total study population	0.27		0.94		250
		56	65-74	B		0.10		0.27		31
		57	>90	M		0.19		0.65		17
			>90	F		0.31		0.51		32
		58	100-120	B	Centenarians	0.28		0.53		120
			65-85	B	Control	0.20		0.51		381

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Aspartate aminotransferase	µkat/L	61	65-74	B	Mexican American	0.02		0.60		185
Cont'd			65-74	B	Cuban	0.24		0.48		96
			65-74	B	Puerto Rican	0.22		0.46		66
		67	>65	B		0.22		0.66		574
Asymmetric dimethyl arginine	µmol/L	27	>65	B		0.54		0.79		39
Bilirubin, conjugated	µmol/L	56	65-74	B		1.1		5.8		32
		57	>90	B		2		3		76
Bilirubin, total	µmol/L	15	70	M	All Males	4.9	(4.5 - 5.4)	23.9	(21.9 - 26.0)	438
			70	M	Males w/o CVD	5.0	(4.4 - 5.6)	23.1	(19.9 - 26.3)	350
			70	F	All females	3.8	(3.4 - 4.1)	16.4	(13.8 - 19.0)	459
			70	F	Females w/o CVD	3.9	(3.5 - 4.2)	16.4	(13.7 - 19.1)	408
		40	66-70	M				20.5		3616
			66-70	F				15.4		4999
			71-75	M				18.8		3287
			71-75	F				15.4		4854
			76-80	M				20.5		2734
			76-80	F				15.4		4277
			>80	M				20.5		2368
			>80	F				15.4		4860
		51	70	F	Healthy reference group	7.5		23.3		51
			70	F	Total study population	6.6		22.0		206
			70	M	Healthy reference group	8.0		28.0		61
			70	M	Total study population	6.3		27.0		250
		57	>90	B		3.0		15.0		80
		61	65-74	B	Mexican American	6.8		20.5		185
			65-74	B	Cuban	8.6		17.1		96
			65-74	B	Puerto Rican	6.8		15.4		66
		66	65-74	M		3.4		12.0		888
			65-74	F		3.4		10.3		656
		74	75	M	All	1.7	(1.6 - 1.9)	18.8	(16.8 - 20.8)	354
			75	M	w/o CVD	1.8	(1.6 - 2.0)	18.2	(16.3 - 20.1)	274
			75	F	All	1.7	(1.7 - 1.7)	11.8	(11.1 - 12.6)	373
			75	F	w/o CVD	1.7	(1.7 - 1.7)	11.6	(10.9 - 12.3)	338
B-type natriuretic peptide	ng/L	28	>65	B	Group 1 - Healthy elderly	4.5	(3.1 - 6.1)	96.5	(79.5 - 116.3)	215
N-terminal-pro-B-type	ng/L	34	71-85	M				158.2		15
natriuretic peptide			71-85	F				218.4		44
			> 75	B				207.6		NP
		28	>65	B		20	(15 - 27)	540.0	(398 - 785)	215
Calcium, ionized	mmol/L	57	>90	B		1.12		1.32		54

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Calcium, total	mmol/L	15	70	M	All Males	2.17	(2.15 - 2.19)	2.67	(2.60 - 2.74)	438
			70	M	Males w/o CVD	2.17	(2.15 - 2.19)	2.66	(2.58 - 274)	350
			70	F	All females	2.18	(2.16 - 2.20)	2.70	(2.64 - 2.77)	459
			70	F	Females w/o CVD	2.18	(2.15 - 2.20)	2.70	(2.63 - 2.76)	408
		31	75	B		2.20	(2.10 - 2.30)	2.70	(2.60 - 2.70)	120
		48	>65	M		2.12		2.46		71
			>65	F		2.13		2.54		165
		51	70	F	Healthy reference group	2.00		2.50		49
			70	F	Total study population	1.80		2.80		206
			70	M	Healthy reference group	2.00		2.60		56
			70	M	Total study population	1.90		2.60		250
		57	>90	B		2.05		2.40		77
		61	65-74	B	Mexican American	4.65		5.75		185
			65-74	B	Cuban	4.55		5.60		96
			65-74	B	Puerto Rican	4.55		5.45		66
		65	65-89	B		2.27		2.59		NP
		66	65-74	M		2.23		2.48		893
			65-74	F		2.28		2.53		658
		67	>65	B		2.10		2.50		548
		74	75	M	All	2.18	(2.16 - 2.20)	2.55	(2.52 - 2.59)	354
			75	M	w/o CVD	2.19	(2.18 - 2.20)	2.55	(2.52 - 2.58)	274
			75	F	All	2.22	(2.19 - 2.24)	2.57	(2.54 - 2.60)	373
			75	F	w/o CVD	2.22	(2.20 - 2.24)	2.57	(2.54 - 2.60)	338
Carbon dioxide, partial pressure	kPa	38	>70	M				5.85		79
			>70	F				6.08		67
Carbon dioxide, total	mmol/L	57	>90	B		20		29		76
		61	65-74	B	Mexican American	22		33		185
			65-74	B	Cuban	22		31		96
			65-74	B	Puerto Rican	24		32		66
		65	65-89	B		21		29		NP
Carcinoembryonic antigen	µg/L	22	65	B				3.99	(3.68 - 4.35)	NP
			66	B				4.02	(3.70 - 4.37)	NP
			67	B				4.04	(3.73 - 4.40)	NP
			68	B				4.07	(3.75 - 4.43)	NP
			69	B				4.09	(3.77 - 4.45)	NP
			70	B				4.12	(3.80 - 4.48)	NP
			71	B				4.14	(3.82 - 4.51)	NP
			72	B				4.17	(3.84 - 4.54)	NP
			73	B				4.19	(3.87 - 4.56)	NP

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Cholesterol	mmol/L	15	70	M	All Males	3.4	(3.2 - 3.6)	7.2	(7.0 - 7.5)	438
			70	M	Males w/o CVD	3.7	(3.5 - 3.9)	7.3	(7.0 - 7.6)	350
			70	F	All females	4.0	(3.8 - 4.2)	7.7	(7.5 - 7.9)	459
			70	F	Females w/o CVD	4.0	(3.8 - 4.2)	7.7	(7.5 - 7.9)	408
		31	75	B		4.0	(3.4 - 4.5)	8.7	(8.0 - 9.0)	119
		47	66-75	M		4.0		8.1		598
			76-85	M		3.8		7.7		282
			86-95	M		3.3		7.3		132
		48	>65	B		3.3		8.3		236
		51	70	F	Healthy reference group	4.4		7.7		51
			70	F	Total study population	4.3		8.9		206
			70	M	Healthy reference group	3.4		8.5		61
			70	M	Total study population	3.6		8.2		250
		53	65-74	F		4.9		6.8		777
			65-74	M		4.4		6.2		456
			> 75	F		4.8		6.7		239
			> 75	M		4.2		5.9		202
		56	65-74	B	Group 1 - Elderly	4.3		8.7		32
		57	>90	M		2.9		6.6		28
			>90	F		3.8		7.0		45
		58	100-120	B	Centenarians	4.0		5.8		120
			65-85	B	Control	4.6		6.6		381
		60	70-79	M		4.9		7.1		3657
			70-79	F		5.4		7.8		5388
			> 80	M		5.3		7.5		614
			> 80	F		5.2		7.5		1524
		66	65-74	M		4.8		7.6		907
			65-74	F		5.5		8.4		671
		67	>65	B		3.5		8.0		581
		74	75	M	All	3.2	(3.0 - 3.4)	7.4	(7.2 - 7.6)	354
			75	M	w/o CVD	3.5	(3.3 - 3.8)	7.4	(7.2 - 7.6)	274
			75	F	All	3.9	(3.6 - 4.2)	7.9	(7.7 - 8.2)	373
			75	F	w/o CVD	4.1	(3.8 - 4.4)	8.0	(7.7 - 8.3)	338
Copper	µmol/L	41	65-80	M		17.4		29.5		28
			65-80	F		21.2		28.1		21
		57	>90	M		11.8		29.0		15
			>90	F		15.7		31.0		32
Cortisol, total	nmol/L	31	75	B		180	(160 - 200)	720	(630 - 810)	118

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Carcinoembryonic antigen	µg/L		74	B				4.22	(3.89 - 4.59)	NP
Cont'd			75	B				4.24	(3.91 - 4.62)	NP
			76	B				4.27	(3.94 - 4.64)	NP
			77	B				4.29	(3.96 - 4.67)	NP
			78	B				4.32	(3.98 - 4.69)	NP
			79	B				4.34	(4.00 - 4.72)	NP
			80	B				4.36	(4.03 - 4.75)	NP
			81	B				4.39	(4.05 - 4.77)	NP
			82	B				4.41	(4.07 - 4.80)	NP
			83	B				4.44	(4.09 - 4.82)	NP
			84	B				4.46	(4.12 - 4.85)	NP
			85	B				4.49	(4.14 - 4.88)	NP
			86	B				4.51	(4.16 - 4.90)	NP
		57	>90	B		0.40		9.20		54
		57	>90	B		0.40		9.20		54
Chloride	mmol/L	15	70	M	All Males	103	(102 - 104)	122	(118 - 125)	438
			70	M	Males w/o CVD	103	(102 - 104)	119	(114 - 124)	350
			70	F	All females	103	(103 - 120)	102	(118 - 123)	459
			70	F	Females w/o CVD	103	(104 - 120)	102	(118 - 123)	408
		48	>65	B		94		110		236
		51	70	F	Healthy reference group	100		109		49
			70	F	Total study population	97		111		206
			70	M	Healthy reference group	100		113		56
			70	M	Total study population	99		111		250
		57	>90	B		98		111		73
		61	65-74	B	Mexican American	97		109		185
			65-74	B	Cuban	99		106		96
			65-74	B	Puerto Rican	96		104		66
		65	65-89	B		100		111		NP
		66	65-74	M		99		107		893
			65-74	F		98		107		657
		74	75	M	All	102	(101 - 102)	111	(110 - 112)	354
			75	M	w/o CVD	102	(101 - 103)	111	(110 - 111)	274
			75	F	All	99	(98 - 101)	111	(111 - 112)	373
			75	F	w/o CVD	99	(98 - 101)	111	(111 - 112)	338

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
C-peptide	nmol/L	57	>90	B		154		635		86
			>90	B		0.20		1.47		15
C-reactive protein	mg/L	15	70	M	All Males	0.33	(0.39 - 12.63)	12.63	(9.11 - 16.14)	438
			70	M	Males w/o CVD	0.34	(0.27 - 0.40)	14.24	(9.69 - 18.78)	350
			70	F	All females	0.34	(0.29 - 0.40)	12.42	(8.53 - 16.32)	459
			70	F	Females w/o CVD	0.35	(0.28 - 0.41)	11.57	(8.93 - 14.21)	408
		31	75	B		0.00		15.00	(13.00 - 19.00)	116
		35	71-80	B				10.00		NP
		44	65-99	M				6.80		47
			65-99	F				6.80		40
		45	>70	M	All races		(16.5 - 22.9)	19.70		NP
			>70	F	All races		(14.7 - 20.7)	17.70		NP
			>70	M	Non-Hispanic white		(16.3 - 23.1)	19.70		NP
			>70	F	Non-Hispanic white		(13.6 - 20.0)	16.80		NP
			>70	M	Non-hispanic black		(13.5 - 30.3)	21.90		NP
			>70	F	Non-hispanic black		(15.0 - 52.6)	15.00		NP
			>70	M	Mexican American			23.70	(16.90 - 30.50)	NP
			>70	F	Mexican American			18.50	(9.90. - 27.10)	NP
		74	75	M	All	0.32	(0.23 - 0.42)	24.11	(16.02 - 32.20)	354
			75	M	w/o CVD	0.36	(0.25 - 0.47)	23.50	(17.11 - 29.89)	274
			75	F	All	0.37	(0.29 - 0.45)	15.89	(12.46 - 19.32)	373
			75	F	w/o CVD	0.37	(0.29 - 0.44)	16.49	(11.89 - 21.10)	338
Creatine kinase	µkat/L	15	70	M	All Males	0.49	(0.39 - 0.58)	4.98	(4.24 - 5.72)	438
			70	M	Males w/o CVD	0.49	(0.40 - 0.58)	4.81	(4.11 - 5.50)	350
			70	F	All females	0.41	(0.35 - 0.46)	3.01	(2.78 - 3.30)	459
			70	F	Females w/o CVD	0.41	(0.36 - 0.46)	3.02	(2.73 - 3.32)	408
		48	>65	M		0.37		3.50		71
			>65	F		0.31		3.13		165
		49	>75	B		0.49	(0.41 - 0.54)	3.62	(3.03 - 4.27)	497
		51	70	F	Healthy reference group	0.54		6.83		47
			70	F	Total study population	0.53		3.86		206
			70	M	Healthy reference group	0.54		4.59		64
			70	M	Total study population	0.65		4.62		250
		54	>65	M	Group 1 - Healthy reference	0.92		4.85		NP
			>65	F	Group 1 - Healthy reference	0.65		2.69		NP
			65-74	M	Group 2 - Healthy patients	0.53		3.47		NP
			65-74	F	Group 2 - Healthy patients	0.43		3.40		NP
			>74	M	Group 2 - Healthy patients	0.49		3.26		NP
			>74	F	Group 2 - Healthy patients	0.43		3.40		NP

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90%CI	Upper Limit	90%CI	n
Cholesterol	mmol/L	15	70	M	All Males	3.4	(3.2 - 3.6)	7.2	(7.0 - 7.5)	438
			70	M	Males w/o CVD	3.7	(3.5 - 3.9)	7.3	(7.0 - 7.6)	350
			70	F	All females	4.0	(3.8 - 4.2)	7.7	(7.5 - 7.9)	459
			70	F	Females w/o CVD	4.0	(3.8 - 4.2)	7.7	(7.5 - 7.9)	408
		31	75	B		4.0	(3.4 - 4.5)	8.7	(8.0 - 9.0)	119
		47	66-75	M		4.0		8.1		598
			76-85	M		3.8		7.7		282
			86-95	M		3.3		7.3		132
		48	>65	B		3.3		8.3		236
		51	70	F	Healthy reference group	4.4		7.7		51
			70	F	Total study population	4.3		8.9		206
			70	M	Healthy reference group	3.4		8.5		61
			70	M	Total study population	3.6		8.2		250
		53	65-74	F		4.9		6.8		777
			65-74	M		4.4		6.2		456
			> 75	F		4.8		6.7		239
			> 75	M		4.2		5.9		202
		56	65-74	B	Group 1 - Elderly	4.3		8.7		32
		57	>90	M		2.9		6.6		28
			>90	F		3.8		7.0		45
		58	100-120	B	Centenarians	4.0		5.8		120
			65-85	B	Control	4.6		6.6		381
		60	70-79	M		4.9		7.1		3657
			70-79	F		5.4		7.8		5388
			> 80	M		5.3		7.5		614
			> 80	F		5.2		7.5		1524
		66	65-74	M		4.8		7.6		907
			65-74	F		5.5		8.4		671
		67	>65	B		3.5		8.0		581
		74	75	M	All	3.2	(3.0 - 3.4)	7.4	(7.2 - 7.6)	354
			75	M	w/o CVD	3.5	(3.3 - 3.8)	7.4	(7.2 - 7.6)	274
			75	F	All	3.9	(3.6 - 4.2)	7.9	(7.7 - 8.2)	373
			75	F	w/o CVD	4.1	(3.8 - 4.4)	8.0	(7.7 - 8.3)	338
Copper	µmol/L	41	65-80	M		17.4		29.5		28
			65-80	F		21.2		28.1		21
		57	>90	M		11.8		29.0		15
			>90	F		15.7		31.0		32
Cortisol, total	nmol/L	31	75	B		180	(160 - 200)	720	(630 - 810)	118

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Creatine Kinase Cont'd	µkat/L	57	>90	M		0.36		3.45		29
			>90	F		0.37		1.68		50
		74	75	M	All	0.45	(0.35 - 0.54)	4.90	(4.06 - 5.77)	354
			75	M	w/o CVD	0.44	(0.32 - 0.56)	5.19	(4.12 - 6.25)	274
			75	F	All	0.44	(0.36 - 0.52)	3.35	(2.91 - 3.80)	373
			75	F	w/o CVD	0.46	(0.37 - 0.55)	3.45	(2.96 - 3.94)	338
Creatinine	µmol/L	15	70	M	All Males	59	(57 - 60)	125	(116 - 133)	438
			70	M	Males w/o CVD	59	(57 - 60)	122	(113 - 130)	350
			70	F	All females	45	(44 - 47)	103	(99 - 107)	459
			70	F	Females w/o CVD	45	(43 - 48)	102	(97 - 106)	408
		25	67.5-72.5	F	Non-parametric	43		97		1507
			67.5-72.5	F	Bhattacharya procedure	41		88		1507
			67.5-72.5	F	Cumulative Gaussian dist.	41		87		1507
			72.5-77.5	F	Non-parametric	45		103		1621
			72.5-77.5	F	Bhattacharya procedure	42		89		1621
			72.5-77.5	F	Cumulative Gaussian dist.	42		86		1621
			77.5-82.5	F	Non-parametric	43		118		1542
			77.5-82.5	F	Bhattacharya procedure	38		97		1542
			77.5-82.5	F	Cumulative Gaussian dist.	40		92		1542
			82.5-87.5	F	Non-parametric	45		131		1259
			82.5-87.5	F	Bhattacharya procedure	37		101		1259
			82.5-87.5	F	Cumulative Gaussian dist.	38		101		1259
			87.5-92.5	F	Non-parametric	42		148		566
			87.5-92.5	F	Bhattacharya procedure	39		103		566
			87.5-92.5	F	Cumulative Gaussian dist.	38		103		566
			92.5-97.5	F	Non-parametric	47		142		311
			92.5-97.5	F	Bhattacharya procedure	36		107		311
			92.5-97.5	F	Cumulative Gaussian dist	38		110		311
			97.5-102.5	F	Non-parametric	45		164		66
			97.5-102.5	F	Bhattacharya procedure	42		112		66
			97.5-102.5	F	Cumulative Gaussian dist.	38		115		66
			67.5-72.5	M	Non-parametric	56		126		1347
			67.5-72.5	M	Bhattacharya procedure	53		110		1347
			67.5-72.5	M	Cumulative Gaussian dist.	51		111		1347
			72.5-77.5	M	Non-parametric	56		137		1372
			72.5-77.5	M	Bhattacharya procedure	53		113		1372
			72.5-77.5	M	Cumulative Gaussian dist.	52		115		1372

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90%CI	Upper Limit	90%CI	n
Creatinine Cont'd	µmol/L		77.5-82.5	M	Non-parametric	57		149		1070
			77.5-82.5	M	Bhattacharya procedure	52		125		1070
			77.5-82.5	M	Cumulative Gaussian dist.	54		118		1070
			82.5-87.5	M	Non-parametric	57		156		721
			82.5-87.5	M	Bhattacharya procedure	54		116		721
			82.5-87.5	M	Cumulative Gaussian dist.	51		121		721
			87.5-92.5	M	Non-parametric	59		171		262
			87.5-92.5	M	Bhattacharya procedure	55		126		262
			87.5-92.5	M	Cumulative Gaussian dist.	55		126		262
			92.5-97.5	M	Non-parametric	63		187		72
			92.5-97.5	M	Bhattacharya procedure	65		111		72
			92.5-97.5	M	Cumulative Gaussian dist.	57		127		72
		31	75	B		71	(62 - 72)	124	(123 - 141)	120
		46	71-85	M		90		160		45
			71-85	F		90		150		38
		47	76-95	M		81		151		414
		48	>65	M		67		141		71
			>65	F		62		134		165
		49	>75	B		62		150	(130 - 153)	497
		51	70	F	Healthy reference group	61		98		50
			70	F	Total study population	53		118		206
			70	M	Healthy reference group	68		152		57
			70	M	Total study population	70		220		250
		53	65-74	F		64		92		775
			65-74	M		84		116		454
			> 75	F		60		99		239
			> 75	M		88		120		202
		55	70-79	F		48		86		56
			> 80	F		44		90		6
		56	65-74	B	Group 1 - Elderly	59		119		32
		57	>90	M		88		150		19
			>90	F		53		115		37
		58	100-120	B	Centenarians	79		149		120
			65-85	B	Control	53		88		381
		61	65-74	B	Mexican American	50		150		185
			65-74	B	Cuban	70		130		96
			65-74	B	Puerto Rican	70		110		66

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Creatinine Cont'd	µmol/L	66	65-74	M		86		132		892
			65-74	F		68		114		658
		67	>65	M		80		160		243
			>65	F		62		133		330
		74	75	M	All	62	(61 - 64)	133	(123 - 143)	354
			75	M	w/o CVD	62	(60 - 64)	123	(111 - 136)	274
			75	F	All	53	(51 - 54)	101	(96 - 107)	373
			75	F	w/o CVD	53	(51 - 54)	98	(93 - 104)	338
Dehydroepiandroesterone sulfate	µmol/L	31	75	B		3.8	(3.0 - 4.6)	6.2	(5.1 - 7.4)	118
		57	>90	M		0.1		2.0		14
			>90	F		0.1		1.6		30
		23	65-69	M	Linear regression	0.9		9.5		91
			65-69	M	Quantile regression	1.2		8.4		91
			70-74	M	Linear regression	0.8		8.1		64
			70-74	M	Quantile regression	1.1		7.5		64
			>74	M	Linear regression	0.7		6.9		50
			>74	M	Quantile regression	1.0		6.7		50
Dehydroepiandroesterone, unconjugated	nmol/L	57	>90	B		0.6		5.9		40
Dihydrotestosterone	nmol/L	73	>70	M		0.49		3.16		394
Estradiol	pmol/L	30	70-74	M		50		170		119
			> 75	M		50		180		123
		31	75	M		7	(2 - 25)	140	(124 - 150)	55
			75	F		6	(1 - 11)	187	(140 - 240)	65
		57	>90	F		18		73		29
		73	>70	M		28		139		394
Ferritin	µg/L	31	75	M		36	(26 - 49)	870	(639 - 1184)	55
			75	F		15	(11 - 19)	352	(265 - 467)	65
		63	>70	F		15		387		NP
α-Fetoprotein	g/L	22	65	B				9.88	(9.07 - 10.84)	NP
			66	B				9.97	(9.14 - 10.93)	NP
			67	B				10.05	(9.22 - 11.02)	NP
			68	B				10.13	(9.29 - 11.11)	NP
			69	B				10.21	(9.37 - 11.20)	NP
			70	B				10.29	(9.45 - 11.28)	NP
			71	B				10.37	(9.51 - 11.36)	NP
			72	B				10.45	(9.59 - 11.45)	NP
			73	B				10.52	(9.59 - 11.45)	NP

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
α-Fetoprotein Cont'd	g/L		74	B				10.60	(9.73 - 11.61)	NP
			75	B				10.67	(9.79 - 11.70)	NP
			76	B				10.75	(9.87 - 11.77)	NP
			77	B				10.83	(9.93 - 11.85)	NP
			78	B				10.89	(10.00 - 11.93)	NP
			79	B				10.97	(10.07 - 12.01)	NP
			80	B				11.03	(10.14 - 12.09)	NP
			81	B				11.11	(10.21 - 12.16)	NP
			82	B				11.17	(10.27 - 12.24)	NP
			83	B				11.25	(10.34 - 12.32)	NP
			84	B				11.32	(10.39 - 12.38)	NP
			85	B				11.38	(10.46 - 12.46)	NP
			86	B				11.45	(10.52 - 12.53)	NP
Fibrinogen	µmol/L	31	75	B		7.64	(7.06 - 7.94)	16.17	(15.29 - 17.05)	115
		53	65-74	F		7.48		10.87		774
			65-74	M		7.33		10.55		453
			> 75	F		7.67		11.08		238
			> 75	M		7.20		11.09		199
Folate	nmol/L	50	65-88	M		5.0	(4.3 - 5.9)	14.0	(12.0 - 16.3)	20
			65-88	F		4.1	(3.6 - 5.0)	19.5	(16.5 - 22.9)	44
			65-88	B		4.3	(3.9 - 5.0)	17.7	(15.6 - 20.2)	64
		57	>90	M		8.0		36.0		19
			>90	F		7.0		36.0		32
Folic acid	nmol/L	31	75	B		6.6	(4.7 - 9.3)	54.0	(50.0 - 56.0)	120
		51	70	F	Healthy reference group	7.5		54.4		67
			70	F	Total study population	7.9		54.4		206
			70	M	Healthy reference group	6.3		54.4		86
			70	M	Total study population	7.3		54.4		250
Follicle-stimulating hormone	U/L	20	70-80	M		2.7		14.2		NP
			80-90	M		3.1		15.1		NP
		30	70-74	M		2.5		38.1		119
			> 75	M		3.2		36.9		123
		31	75	M		3.7	(3.1 - 4.3)	19.9	(16.8 - 23.5)	53
			75	F		13.1	(5.7 - 20.5)	94.7	(87.2 - 102.1)	62
		57	>90	M		8.0		112.0		26
			>90	F		55.0		168.0		48

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Glucose	mmol/L	74	75	M	All	3.8	(3.7 - 3.9)	6.6	(6.2 - 7)	354
			75	M	w/o CVD	3.8	(3.7 - 4.0)	6.5	(6.1 - 6.8)	274
			75	F	All	3.8	(3.6 - 4.0)	6.2	(6 - 6.3)	373
			75	F	w/o CVD	3.7	(3.6 - 4.0)	6.2	(6 - 6.4)	338
		48	>65	B		3.9		8.6		236
		61	65-74	B	Mexican American	4.3		13.6		185
			65-74	B	Cuban	4.7		7.9		96
			65-74	B	Puerto Rican	4.9		13.3		66
		62	>65	M	Healthy, 95% range	4.7	(4.2 - 4.8)	11.5	(10.7 - 12.3)	NP
			>65	M	Healthy, 90% range	4.8	(4.5 - 5.1)	10.7	(10.0 - 11.5)	NP
			>65	F	Healthy, 95% range	4.3	(4.0 - 4.6)	12.0	(11.2 - 12.9)	NP
			>65	F	Healthy, 90% range	4.7	(4.5 - 4.9)	11.0	(10.2 - 11.8)	NP
Glucose, fasting	mmol/L	47	>80	M		3.7		6.8		294
		51	70	F	Healthy reference group	3.6		8.2		50
			70	F	Total study population	3.9		12.7		206
			70	M	Healthy reference group	4.2		7.1		61
			70	M	Total study population	4.2		13.8		250
		53	65-74	F		4.9		5.9		775
			65-74	M		5.0		6.1		454
			> 75	F		4.9		5.8		239
			> 75	M		4.6		6.7		202
		56	65-74	B		4.2		7.0		32
		57	>90	B		4.2		6.7		59
		58	100-120	B	Centenarians	4.0		5.3		120
			65-85	B	Control	4.4		6.2		381
		67	>65	B		4.0		8.5		580
ϒ - Glutamyltransferase	µkat/L	15	70	M	Males w/o CVD	0.18	(0.16 - 0.20)	1.76	(1.45 - 2.08)	350
			70	F	All females	0.14	(0.13 - 0.15)	1.22	(1.04 - 1.42)	459
			70	F	Females w/o CVD	0.14	(0.13 - 0.15)	1.25	(1.04 - 1.47)	408
			70	M	All Males	0.18	(0.17 - 0.20)	1.95	(1.58 - 2.31)	438
		31	75	M		0.21	(0.19 - 0.27)	1.04	(0.89 - 1.20)	54
			75	F		0.16	(0.14 - 0.18	0.72	(0.63 - 0.83)	64
		48	>65	M		0.19		2.11		71
			>65	F		0.17		2.52		165
		51	70	F	Healthy reference group	0.12		0.82		47
			70	F	Total study population	0.12		2.70		206
			70	M	Healthy reference group	0.14		1.17		62
			70	M	Total study population	0.15		1.70		250

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Ɣ - Glutamyltransferase Cont'd	µkat/L	56	65-74	B		0.05		0.58		31
		57	>90	M		0.05		0.80		26
			>90	F		0.07		0.75		46
		66	65-74	M		0.10		0.68		370
			65-74	F		0.09		0.44		529
		67	>65	B		0.10		1.46		566
		74	75	M	All	0.30	(0.29 - 0.32)	2.16	(1.91 - 2.43)	354
			75	M	w/o CVD	0.30	(0.28 - 0.33)	2.10	(1.83 - 2.38)	274
			75	F	All	0.25	(0.24 - 0.27)	1.81	(1.37 - 2.24)	373
			75	F	w/o CVD	0.25	(0.24 - 0.27)	1.65	(1.16 - 2.13)	338
Growth hormone releasing hormone	mEq/L	21	65	B		3.7				NP
			66	B		3.2				NP
			67	B		2.7				NP
			68	B		2.3				NP
			69	B		1.9				NP
			70	B		1.5				NP
High-density lipoprotein cholesterol cholesterol	mmol/L	15	70	M	All Males	0.80	(0.70 - 0.90)	2.20	(2.00 - 2.30)	438
			70	M	Males w/o CVD	0.80	(0.70 - 0.90)	2.20	(2.00 - 2.40)	350
			70	F	All females	1.00	(1.00 - 1.10)	2.80	(2.60 - 3.00)	459
			70	F	Females w/o CVD	1.00	(1.00 - 1.10)	2.80	(2.50 - 3.00)	408
		31	75	B		1.03	(0.87 - 1.08)	2.57	(2.21 - 2.75)	120
		53	65-74	F		1.20		1.98		776
			65-74	M		0.96		1.56		456
			> 75	F		1.22		2.05		237
			> 75	M		0.96		1.65		202
		56	65-74	B		0.80		2.10		32
		57	>90	M		0.72		2.12		27
			>90	F		0.83		2.15		46
		58	100-120	B	Centenarians	1.17		1.79		120
			65-85	B	Control	1.19		1.97		381
		66	65-74	M		0.88		1.61		894
			65-74	F		1.01		1.92		669
		67	>65	B		0.91		2.33		570
		74	75	M	All	0.77	(0.72 - 0.82)	2.29	(2.10 - 2.48)	354
			75	M	w/o CVD	0.80	(0.75 - 0.84)	2.35	(2.13 - 2.58)	274
			75	F	All	1.02	(0.98 - 1.06)	2.78	(2.57 - 30.00)	373
			75	F	w/o CVD	1.03	(0.98 - 1.09)	2.82	(2.60 - 3.04)	338

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Homoarginine	µmol/L	27	>65	B		1.11		4.68		39
Homocysteine	µmol/L	31	75	B		7.4	(7.0 - 8.1)	23.0	(20.8 - 27.8)	120
		32	>70	M		11.2		11.5		736
			>70	F		10.0		10.4		772
			>70	B	Non-Hispanic White	10.5		10.6		1025
			>70	B	Non-Hispanic Black	11.6		12.4		198
			>70	B	Mexican American or Hisp.	10.6		11.2		285
		50	65-88	M		6.6	(5.6 - 7.9)	11.5	(17.0 - 24.0)	20
			65-88	F		6.9	(6.2 - 7.8)	21.2	(18.8 - 23.9)	44
			65-88	B		6.8	(6.2 - 7.5)	21.0	(18.9 - 22.9)	64
Insulin, fasting	pmol/L	53	65-74	F		42		126		771
			65-74	M		45		136		451
			> 75	F		44		117		239
			> 75	M		44		121		202
		57	>90	B		17		136		14
Insulin-like growth factor I	µg/L	19	70-79	F		43		140		36
			80-91	F		37		121		25
			70-79	M		54		138		25
			80-91	M		48		122		7
		21	65	B		47				NP
			66	B		47				NP
			67	B		46				NP
			68	B		44				NP
			69	B		43				NP
			70	B		41				NP
		17	65-69	F	Without hormone medication	61		222		NP
			65-69	F	With hormone medication	55		218		NP
			65-69	M		55		209		72
			70-74	F	Without hormone medication	60		213		40
			70-74	M		50		207		61
			75-79	F	Without hormone medication	59		207		23
			75-79	M		46		208		34
			80-84	F	Without hormone medication	59		203		12
			80-84	M		42		212		23
			65-69	F	Without hormone medication	2456		5988		NP
		26	65-69	F	Linear regression	34		237		65
			70-74	F	Linear regression	30		228		45
			>74	F	Linear regression	25		216		65

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Insulin-like growth factor I	µg/L		65-69	M	Linear regression	33		237		105
Cont'd			70-74	M	Linear regression	30		230		85
			> 74	M	Linear regression	28		226		35
			65-69	F	Quantile regression	59		241		65
			70-74	F	Quantile regression	57		237		45
			>74	F	Quantile regression	55		219		65
			65-69	M	Quantile regression	49		209		105
			70-74	M	Quantile regression	46		207		85
			>74	F	Quantile regression	48		202		35
		37	66-70	B	Percentile	67		195		32
			66-70	B	+/- 2SD	66		198		32
			71-75	B	Percentile	62		184		56
			71-75	B	+/- 2SD	61		186		56
			76-80	B	Percentile	57		172		24
			76-80	B	+/- 2SD	57		174		24
			81-85	B	Percentile	53		162		13
			81-85	B	+/- 2SD	52		164		13
		39	65-69	B		36		237		98
			65-69	M		44		238		68
			65-69	F		27		223		30
			70-74	B		31		220		63
			70-74	M		38		223		40
			70-74	F		22		204		23
			75-80	B		27		209		42
			75-80	M		35		213		22
			75-80	F		21		199		20
		70	65-69	M		36		306		65
			65-69	F		29		289		55
			> 70	M		30		281		115
			> 70	F		27		282		92
Insulin-like growth factor binding protein-3	mg/L	26	65-69	F	Linear regression	0.95		2.79		65
			70-74	F	Linear regression	0.91		2.75		45
			>74	F	Linear regression	0.85		2.68		65
			65-69	M	Linear regression	0.73		2.54		105
			70-74	M	Linear regression	0.68		2.47		85
			>74	M	Linear regression	0.62		2.41		35

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Insulin-like growth factor binding protein-3 Cont'd	mg/L		>74	F	Quantile regression	0.97		2.70		65
			65-69	M	Quantile regression	0.76		2.45		105
			70-74	M	Quantile regression	0.71		2.40		85
			>74	M	Quantile regression	0.65		2.36		35
		17	65-69	F	Without hormone medication	5.99		5.99		NP
			65-69	F	With hormone medication	2.57		5.47		NP
			65-69	M		2.04		5.13		72
			70-74	F	Without hormone medication	2.38		5.92		40
			70-74	M		1.83		4.92		61
			75-79	F	Without hormone medication	2.30		5.84		23
			75-79	M		1.60		4.70		34
			80-84	F	Without hormone medication	2.22		5.77		12
			80-84	M		1.35		4.48		23
		37	66-70	B	Percentile	2.90		6.00		32
			66-70	B	+/- 2SD	2.90		6.00		32
			71-75	B	Percentile	2.60		5.50		56
			71-75	B	+/- 2SD	2.60		5.50		56
			76-80	B	Percentile	2.30		4.90		24
			76-80	B	+/- 2SD	2.30		4.90		24
			81-85	B	Percentile	2.00		4.20		13
			81-85	B	+/- 2SD	2.00		4.30		13
		31	75	B		2.20	(2.00 - 2.30)	6.30	(5.40 - 7.70)	120
			75	B		1.75	(1.44 - 2.35)	9.63	(8.47 - 11.59)	120
Insulin tolerance test	mEq/L	21	65	B		3.6				NP
			66	B		3.3				NP
			67	B		3.0				NP
			68	B		2.8				NP
			69	B		2.5				NP
			70	B		2.3				NP
Iron	µmol/L	15	70	M	All Males	10.3	(9.5 - 11.2)	32.8	(29.8 - 35.7)	438
			70	M	Males w/o CVD	10.6	(9.8 - 11.5)	33.6	(30.3 - 36.9)	350
			70	F	All females	10.3	(9.7 - 10.9)	30.0	(28.5 - 31.4)	459
			70	F	Females w/o CVD	10.5	(9.9 - 11.0)	30.0	(28.4 - 31.5)	408
		31	75	B		6.2	(4.1 - 10.4)	32.2	(27.4 - 34.6)	120
		43	>65	M		9.1	(8.3 - 9.9)	32.7	(29.2 - 36.2)	220
			>65	F		7.7	(6.8 - 8.6)	27.1	(26.1 - 28.1)	327
		48	>65	B		22.0		42.0		236
		51	70	F	Healthy reference group	5.5		24.8		66

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Iron Cont'd	µmol/		70	F	Total study population	5.0		27.3		206
			70	M	Healthy reference group	5.2		26.7		76
			70	M	Total study population	6.0		26.3		250
		57	>90	M		7.0		23.0		16
			>90	F		6.0		24.0		37
		74	75	M	All	8.3	(6.8 - 9.9)	34.3	(32.9 - 35.6)	354
			75	M	w/o CVD	8.4	(6.3 - 10.4)	34.8	(33.3 - 36.3)	274
			75	F	All	8.8	(8.0 - 9.7)	28.8	(27.5 - 30.1)	373
			75	F	w/o CVD	8.9	(7.9 - 9.8)	28.7	(27.5 - 30.0)	338
L-Lactate	mmol/L	31	75	B		6.19	(4.12 - 10.39)	32.24	(27.4 - 34.57)	120
Lactate dehydrogenase	µkat/L	15	70	M	All Males	1.64	(1.54 - 1.74)	3.89	(3.48 - 3.89)	438
			70	M	Males w/o CVD	1.64	(1.54 - 1.74)	3.89	(3.48 - 4.30)	350
			70	F	All females	1.54	(1.33 - 1.74)	3.99	(3.58 - 4.40)	459
			70	F	Females w/o CVD	1.43	(1.33 - 1.64)	3.89	(3.58 - 4.30)	408
		31	75	B		2.35	(2.24 - 2.48)	4.31	(4.17 - 4.42)	118
		48	>65	B		3.45		8.65		236
		49	>75	B		1.99	(1.51 - 2.13)	4.25	(4.05 - 4.80)	497
		51	70	F	Healthy reference group	2.86		7.99		47
			70	F	Total study population	3.84		8.98		206
			70	M	Healthy reference group	3.79		8.86		65
			70	M	Total study population	3.60		8.40		250
		54	>65	B	Group 1 - Healthy reference	4.51		8.42		NP
			65-74	B	Group 2 - Healthy patients	3.91		9.86		NP
			>74	B	Group 2 - Healthy patients	4.05		10.25		NP
		57	>90	B		1.68		4.83		74
		74	75	M	All	1.64	(1.54 - 1.74)	3.38	(3.07 - 3.69)	354
			75	M	w/o CVD	1.64	(1.54 - 1.74)	3.48	(3.07 - 3.90)	274
			75	F	All	1.64	(1.54 - 1.74)	3.48	(3.38 - 3.58)	373
			75	F	w/o CVD	1.64	(1.54 - 1.74)	3.38	(3.28 - 3.48)	338
		61	65-74	B	Mexican American	0.95		2.28		185
			65-74	B	Cuban	0.61		1.33		96
			65-74	B	Puerto Rican	0.78		1.33		66
Lactate dehydrogenase isozyme 1	µkat/L	54	65-74	B	Group 2 - Healthy patients	0.90		2.24		NP
			>65	B	Group 1 - Healthy reference	0.95		2.19		NP
			>74	B	Group 2 - Healthy patients	0.95		2.38		NP
	%	57	>90	B		20		38		71

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Lactate dehydrogenase isozyme 2	%	57	>90	B		31		47		71
Lactate dehydrogenase isozyme 3	%	57	>90	B		14		29		71
Lactate dehydrogenase isozyme 4	%	57	>90	B		4		10		70
Lactate dehydrogenase isozyme 5	%	57	>90	B		1		11		71
Lead	µmol/L	57	>90	B				0.72		58
Lipase	µkat/L	48	>65	B		0.00		3.43		236
		57	>90	B		0.44		4.54		58
		74	75	M	All	0.60	(0.52 - 0.68)	5.61	(4.88 - 6.34)	354
			75	M	w/o CVD	0.60	(0.48 - 0.73)	5.35	(4.68 - 6.03)	274
			75	F	All	0.74	(0.61 - 0.87)	5.84	(4.99 - 6.68)	373
			75	F	w/o CVD	0.70	(0.55 - 0.85)	5.66	(4.79 - 6.53)	338
Low-density lipoprotein cholesterol	mmol/L	15	70	M	All Males	1.8	(1.6 - 1.9)	5.1	(4.9 - 5.2)	438
			70	M	Males w/o CVD	1.9	(1.8 - 2.0)	5.1	(4.8 - 5.4)	350
			70	F	All females	2.1	(1.9 - 2.2)	5.3	(5.1 - 5.5)	459
			70	F	Females w/o CVD	2.1	(1.9 - 2.3)	5.3	(5.1 - 5.6)	408
		31	75	B		2.0	(1.2 - 2.5)	6.0	(5.8 - 6.7)	119
		52	70-79	M		1.9		5.1		56
			70-79	F		2.4		5.1		47
		53	65-74	F		2.7		4.5		775
			65-74	M		2.6		4.2		453
			> 75	F		2.6		4.4		237
			> 75	M		2.4		3.9		202
		56	65-74	B		2.6		6.0		32
		67	>65	B		1.9		5.5		567
		74	75	M	All	1.4	(1.2 - 1.7)	5.4	(5.1 - 5.7)	354
			75	M	w/o CVD	1.8	(1.6 - 1.9)	5.4	(5.0 - 5.7)	274
			75	F	All	1.8	(1.6 - 2.0)	5.4	(5.2 - 5.6)	373
			75	F	w/o CVD	1.8	(1.6 - 2.0)	5.4	(5.1 - 5.7)	338
Luteinizing hormone	U/L	20	70-80	M		2.2		11.2		NP
			80-90	M		2.4		11.7		NP
		30	70-74	M		2.0		14.2		119
			> 75	M		2.3		19.1		123
		31	75	M		1.5	(1.2 - 1.8)	13.0	(1.0 - 16.0)	53
			75	F		9.7	(8.4 - 11.4)	41.6	(34.0 - 48.0)	62
		57	>90	M		1.9		11.5		27
			>90	F		3.5		15.2		45

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Methylmalonic acid	nmol/L	14	>70	B	Group 2 (Database Samples)	50		480		2149
		50	65-88	M		76	(61 - 941)	296	(239 - 367)	20
			65-88	F		76	(61 - 931)	547	(445 - 674)	44
			65-88	B		72	(61 - 851)	476	(403 - 561)	64
Neopterin	nmol/L	56	65-74	B		3.7		21.9		32
Osmolality	mOsm/kg	57	>90	B		277		301		63
Oxygen, arterial partial pressure	kPa	38	>70	M				5.37		79
			>70	F				5.49		67
Oxygen, partial pressure	kPa	38	>70	M		8.25				79
			>70	F		7.93				67
Oxygen, saturation	%	38	>70	M		93				79
			>70	F		92				67
Parathyroid hormone	pmol/L	57	>90	B		49		118		56
pH	pH	57	>90	B		7.26		7.43		54
Phosphate	mmol/L	15	70	M	All Males	0.77	(0.74 - 0.80)	1.33	(1.30 - 1.36)	438
			70	M	Males w/o CVD	0.78	(0.74 - 0.81)	1.33	(1.31 - 1.36)	350
			70	F	All females	0.87	(0.83 - 0.91)	1.51	(1.48 - 1.54)	459
			70	F	Females w/o CVD	0.88	(0.85 - 0.92)	1.51	(1.48 - 1.54)	408
		31	75	B		0.70	(0.60 - 0.80)	1.40	(1.30 - 1.50)	120
		48	>65	M		0.70		1.20		71
			>65	F		0.80		1.40		165
		51	70	F	Healthy reference group	0.68		1.26		49
			70	F	Total study population	0.65		1.42		206
			70	M	Healthy reference group	0.47		1.31		56
			70	M	Total study population	0.66		1.29		250
		57	>90	M		0.71		1.26		27
			>90	F		0.81		1.36		48
		61	65-74	B	Mexican American	0.81		1.39		185
			65-74	B	Cuban	0.84		1.26		96
			65-74	B	Puerto Rican	0.87		1.29		66
		66	65-74	M		0.81		1.23		892
			65-74	F		0.94		1.29		658
		67	>65	B		0.70		1.30		547
		74	75	M	All	0.86	(0.82 - 0.9)	1.38	(1.33 - 1.42)	354
			75	M	w/o CVD	0.84	(0.77 - 0.9)	1.35	(1.32 - 1.38)	274
			75	F	All	0.98	(0.93 - 1.02)	1.47	(1.44 - 1.50)	373
			75	F	w/o CVD	0.97	(0.92 - 1.01)	1.47	(1.43 - 1.51)	338

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Potassium	mmol/L	15	70	M	All Males	3.3	(3.2 - 3.4)	4.5	(4.4 - 4.5)	438
			70	M	Males w/o CVD	3.2	(3.1 - 3.4)	4.5	(4.4 - 4.6)	350
			70	F	All females	3.3	(3.3 - 3.3)	4.5	(4.4 - 4.6)	459
			70	F	Females w/o CVD	3.3	(3.3 - 3.3)	4.5	(4.4 - 4.7)	408
		31	75	B		3.9	(3.8 - 4.0)	5.2	(5.1 - 5.3)	118
		48	>65	B		3.2		5		236
		51	70	F	Healthy reference group	3.6		5		49
			70	F	Total study population	3.5		6.8		206
			70	M	Healthy reference group	3.4		5.3		56
			70	M	Total study population	3.5		5.3		250
		53	65-74	F		3.9		4.48		775
			65-74	M		4.0		4.6		454
			> 75	F		3.9		4.51		239
			> 75	M		4.1		4.73		202
		57	>90	B		3.6		5.5		71
		61	65-74	B	Mexican American	3.5		5.01		185
			65-74	B	Cuban	4.2		4.28		96
			65-74	B	Puerto Rican	4.1		4.21		66
		62	>65	M	Healthy, 95% range	3.5	(3.1 - 3.9)	4.91	(4.4 - 5.4)	NP
			>65	M	Healthy, 90% range	3.6	(3.2 - 3.9)	4.8	(4.4 - 5.2)	NP
			>65	F	Healthy, 95% range	3.0	(2.8 - 3.2)	5.23	(5.0 - 5.5)	NP
			>65	F	Healthy, 90% range	3.2	(3.0 - 3.3)	5.05	(4.9 - 5.2)	NP
		65	65-89	B		4.0		5.3		NP
		66	65-74	M		3.9		5.5		892
			65-74	F		3.7		5.1		657
		74	75	M	All	3.7	(3.6 - 3.8)	4.9	(4.7 - 5)	354
			75	M	w/o CVD	3.7	(3.6 - 3.8)	4.9	(4.7 - 5)	274
			75	F	All	3.7	(3.6 - 3.8)	4.8	(4.7 - 4.8)	373
			75	F	w/o CVD	3.6	(3.5 - 3.7)	4.8	(4.7 - 4.8)	338
Progesterone	nmol/L	31	75	B		0.3	(0.3 - 0.3)	4.5	(3.2 - 9.5)	120
		57	>90	M		0.6		1.5		13
			>90	F		0.6		1.7		29
Prolactin	pmol/L	57	>90	M		8.0		25.0		17
			>90	F		7.0		53.0		34
		31	75	B		3.3	(2.9 - 3.8	26.4	(23.1 - 30.5)	113

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Protein, total	g/L	31	75	B		67	(64 - 68)	88	(82 - 96)	120
		48	>65	M		64		83		71
			>65	F		60		83		165
		51	70	F	Healthy reference group	63		82		51
			70	F	Total study population	60		86		206
			70	M	Healthy reference group	55		81		61
			70	M	Total study population	58		85		250
		56	65-74	B		65		82		31
		57	>90	B		60		80		79
		58	100-120	B	Centenarians	66		77		120
			65-85	B	Control	60		78		381
		61	65-74	B	Mexican American	61		78		185
			65-74	B	Cuban	65		79		96
			65-74	B	Puerto Rican	61		78		66
		66	65-74	M		63		74		893
			65-74	F		64		74		658
Serum amyloid A	mg/L	29	>70	F		1.3		6.1		11
			>70	M		0.9		5.1		13
		31	75	B		1.9	(1.6 - 2.2)	20.0	(17 - 23)	116
Sex hormone binding globulin	nmol/L	20	70-80	M		27.8		101.0		NP
			80-90	M		33.8		115.4		NP
		57	>90	M		38.2		156.2		14
			>90	F		38.2		208.2		27
		30	70-74	M		17.4		97.5		119
			> 75	M		18.3		106.0		123
Silicon	µmol/L	36	>75	M		3.63		16.06		NP
			>75	F		3.84		16.63		NP
Sodium	mmol/L	15	70	M	All Males	136	(136 - 137)	157	(152 - 162)	438
			70	M	Males w/o CVD	136	(136 - 137)	156	(150 - 161)	350
			70	F	All females	137	(136 - 137)	158	(155 - 161)	459
			70	F	Females w/o CVD	137	(136 - 138)	158	(155 - 161)	408
		31	75	B		136	(135 - 137)	145	(144 - 145)	120
		48	>65	B		132		146		236
		51	70	F	Healthy reference group	135		146		49
			70	F	Total study population	132		140		206
			70	M	Healthy reference group	135		148		56
			70	M	Total study population	133		147		250

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Sodium Cont'd	mmol/L	57	>90	B		132		145		76
		61	65-74	B	Mexican American	134		145		185
			65-74	B	Cuban	135		144		96
			65-74	B	Puerto Rican	136		142		66
		62	>65	M	Healthy, 95% range	132	(129 - 135)	143	(140 - 145)	NP
			>65	M	Healthy, 90% range	133	(131 - 135)	142	(140 - 144)	NP
			>65	F	Healthy, 95% range	131	(129 - 133)	144	(143 - 146)	NP
			>65	F	Healthy, 90% range	132	(130 - 134)	143	(142 - 145)	NP
		65	65-89	B		137		147		NP
		66	65-74	M		139		145		893
			65-74	F		139		145		658
		74	75	M	All	137	(137 - 138)	146	(145 - 147)	354
			75	M	w/o CVD	137	(136 - 138)	145	(145 - 146)	274
			75	F	All	135	(134 - 137)	145	(145 - 146)	373
			75	F	w/o CVD	135	(134 - 137)	145	(145 - 146)	338
Symmetric dimethyl arginine	µmol/L	27	>65	B		0.33		0.88		39
Testosterone, free	pmol/L	16	70-79	M		135.3		513.6		NP
			80-89	M		69.4		440.7		NP
			70-89	M		104.1		468.5		NP
			70-79	F		0.3		13.5		NP
			80-89	F		1.4		12.1		NP
			70-89	F		0.7		12.8		NP
		30	70-74	M		125.0		458.0		119
			> 75	M		101.0		468.0		123
		57	>90	M		40.9		258.9		13
		69	70-80	F		3.0		21.3		163
Testosterone, total	nmol/L	16	70-79	M		4.16		29.15		NP
			80-89	M		2.26		24.98		NP
			70-89	M		3.12		30.88		NP
			70-79	F		0.07		1.32		NP
			80-89	F		0.07		1.32		NP
			70-89	F		0.09		1.39		NP
		20	70-80	M		8.60		30.70		NP
			80-90	M		8.40		30.40		NP
		23	65-69	M	Linear regression	5.20		27.80		91
			65-69	M	Quantile regression	6.70		29.00		91
			70-74	M	Linear regression	5.30		27.90		64

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Testosterone, total	nmol/L	30	70-74	M		7.10		39.50		119
			> 75	M		8.90		35.90		123
		31	75	M		7.60	(6.60 - 8.70)	30.00	(27.10 - 31.90)	55
			75	F		0.03	(0.03 - 0.04)	2.60	(2.30 - 2.90)	65
		57	>90	M		7.50		23.30		13
		69	70-80	F		0.29		1.82		163
		73	>70	M		6.40		25.65		394
Thyroid-stimulating hormone	mIU/L	18	>70	B		0.8		5.4		36
		24	>70	B	Without selection	0.8	(0.8 - 1.0)	5.4	(4.1 - 9.4)	80
			>70	B	Antibody Selection	0.6	(0.5 - 0.9)	5.1	(4.6 - 5.9)	70
		31	75	B		0.1	(0.0 - 0.3)	4.2	(3.5 - 6.0)	119
		33	65-100	M	Outliers excluded (3SD)	0.5	(0.4 - 0.6)	5.4	(4.7 - 6.1)	496
			65-100	M	↑ antibodies + (3SD) exc	0.5	(0.4 - 0.6)	5.2	(4.6 - 5.9)	419
			65-100	M	Mode method + 3SD	0.5	(0.4 - 0.7)	5.4	(4.6 - 6.7)	283
			65-100	F	Outliers excluded (3SD)	0.5	(0.4 - 0.6)	7.2	(6.0 - 8.4)	580
			65-100	F	↑ antibodies + (3SD) exc	0.5	(0.4 - 0.6)	5.8	(5.2 - 7.7)	384
			65-100	F	Mode method + 3SD	0.5	(0.4 - 0.6)	5.7	(5.2 - 6.8)	246
			65-100	B	Clinical purpose	0.5	(0.4 - 0.5)	5.6	(5.2 - 6.7)	808
		51	70	F	Healthy reference group	0.8		7.1		80
			70	F	Total study population	0.3		12.7		206
			70	M	Healthy reference group	0.7		9.6		106
			70	M	Total study population	0.4		8.1		250
		57	>90	B		0.4		7.2		43
		59	>65	B		0.2		3.0		2430
		67	>65	B		0.3		5.8		558
		68	70-79	M		0.2		4.5		46
			70-79	F		0.6		5.6		24
			80-85	M		0.5		2.5		7
			80-85	F		0.5		4.3		6
		71	71-80	B		0.4		5.0		20934
			81-90	B		0.4		5.5		12233
			>90	B		0.3		5.9		2265
		72	66-70	B		0.5		4.4		10285
			71-75	B		0.5		4.4		8155
			76-80	B		0.5		4.7		6192
			81-85	B		0.5		4.8		4715
			86-90	B		0.5		5.0		2478
			>90	B		0.5		5.6		1224

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Thyroxine 3, total	nmol/L	57	>90	B		1.1		2.5		70
		59	>65	B		1.2		3.5		2430
Thryoxine 4, free	pmol/L	31	75	B		11.6	(10.3 - 11.7)	21.9	(20.6 - 22.3)	73
		18	>70	M		12.9		19.7		23
			>70	F		13.5		18.7		13
		33	65-100	B	Outliers excluded (3SD)	9.7	(9.6 - 9.9)	17.6	(17.4 - 18.2)	1074
			65-100	B	Elevated antibodies excluded (	9.8	(9.6 - 10.1)	17.6	(17.3 - 18.3)	804
			65-100	B	Elevated antibodies excluded (	9.9	(9.7 - 10.3)	17.9	(17.3 - 18.9)	528
Thyroxine 4, total	nmol/L	51	70	F	Healthy reference group	63.2		140.6		77
			70	F	Total study population	63.2		147.1		206
			70	M	Healthy reference group	63.2		132.9		104
			70	M	Total study population	64.5		139.3		250
		57	>90	B		68.0		129.0		80
		59	>65	B		64.5		180.6		2430
Transferrin	g/L	15	70	M	All Males	1.72	(1.65 - 1.78)	3.08	(2.97 - 3.19)	438
			70	M	Males w/o CVD	1.70	(1.63 - 1.76)	3.00	(2.92 - 3.09)	350
			70	F	All females	1.74	(1.64 - 1.85)	3.12	(3.00 - 3.23)	459
			70	F	Females w/o CVD	1.74	(1.65 - 1.83)	3.04	(2.90 - 3.18)	408
		31	75	B		1.40	(1.29 - 1.45)	2.46	(2.18 - 3.24)	120
		43	>65	M		1.61	(1.45 - 1.77)	3.39	(3.11 - 3.66)	220
			>65	F		1.61	(1.45 - 1.77)	3.39	(3.11 - 3.66)	327
		48	>65	B		1.23		2.35		236
		74	75	M	All	1.79	(1.70 - 1.88)	3.27	(3.06 - 3.48)	354
			75	M	w/o CVD	1.77	(1.67 - 1.87)	3.06	(2.85 - 3.27)	274
			75	F	All	1.80	(1.70 - 1.89)	3.27	(3.06 - 3.25)	373
			75	F	w/o CVD	1.79	(1.68 - 1.89)	3.16	(3.06 - 3.25)	338
Triglycerides	mmol/L	51	70	F	Healthy reference group	0.60		3.90		51
			70	F	Total study population	0.60		3.90		206
			70	M	Healthy reference group	0.40		4.90		61
			70	M	Total study population	0.50		4.10		250
		53	65-74	F		0.82		2.05		777
			65-74	M		0.77		2.10		456
			> 75	F		0.73		2.00		239
			> 75	M		0.65		2.06		202
		31	75	B		0.50	(0.47 - 0.68)	2.80	(2.20 - 3.10)	119
		48	>65	B		0.80		3.70		236
		56	65-74	B		0.60		3.50		32
		57	>90	M		0.87		1.25		6

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Triglycerides Cont'd	mmol/L		>90	F		0.49		1.63		6
		58	100-120	B	Centenarians	0.73		1.66		120
			65-85	B	Control	0.41		1.92		381
		60	70-79	M		0.57		2.73		3657
			70-79	F		0.50		2.34		5388
			> 80	M		0.64		2.48		614
			> 80	F		0.57		2.41		1524
		67	>65	B		0.70		4.00		580
		74	75	M	All	0.64	(0.58 - 0.69)	3.14	(2.63 - 3.66)	354
			75	M	w/o CVD	0.63	(0.58 - 0.69)	2.92	(2.54 - 3.30)	274
			75	F	All	0.60	(0.51 - 0.69)	3.06	(2.48 - 3.63)	373
			75	F	w/o CVD	0.59	(0.50 - 0.67)	3.18	(2.57 - 3.78)	338
		15	70	M	All Males	0.52	(0.50 - 0.54)	2.72	(2.32 - 3.12)	438
			70	M	Males w/o CVD	0.51	(0.47 - 0.55)	2.80	(2.23 - 3.36)	350
			70	F	All females	0.52	(0.47 - 0.57)	2.63	(2.48 - 2.78)	459
			70	F	Females w/o CVD	0.52	(0.48 - 0.57)	2.60	(2.44 - 2.75)	408
Urea	mmol/L	15	70	M	All Males	3.7	(3.4 - 3.9)	9.3	(8.5 - 10.0)	438
			70	M	Males w/o CVD	3.7	(3.4 - 3.9)	8.8	(8.2 - 9.3)	350
			70	F	All females	3.4	(3.3 - 3.5)	8.3	(8.0 - 8.5)	459
			70	F	Females w/o CVD	3.4	(3.3 - 3.6)	8.2	(8.0 - 8.4)	408
		46	71-85	M		1.7		8.7		45
			71-85	F		2.3		8.5		38
		47	76-95	M		3.3		13.3		414
		48	>65	B		3.3		11.9		236
		49	>75	B		2.8	(2.4 - 3.1)	10.4	(10.1 - 13.2)	497
		51	70	F	Healthy reference group	3.4		10.3		50
			70	F	Total study population	3.6		11.2		206
			70	M	Healthy reference group	3.9		11.0		57
			70	M	Total study population	4.4		15.8		250
		56	65-74	M		4.6		8.5		17
			65-74	F		3.8		7.7		15
		57	>90	B		3.6		11.1		45
		58	100-120	B	Centenarians	5.6		11.6		120
			65-85	B	Control	4.6		7.6		381
		61	65-74	B	Mexican American	1.8		6.0		185
			65-74	B	Cuban	2.0		4.7		96
			65-74	B	Puerto Rican	2.2		3.8		66

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Urea Cont'd	mmol/L	66	65-74	M		4.2		8.6		892
			65-74	F		4.2		8.3		658
		67	>65	B		4.2		14.8		579
		74	75	M	All	4.0	(3.7 - 4.3)	11.2	(9.8 - 12.7)	354
			75	M	w/o CVD	3.9	(3.6 - 4.3)	10.5	(9.1 - 11.9)	274
			75	F	All	3.6	(3.5 - 4.3)	9.6	(9.1 - 10.1)	373
			75	F	w/o CVD	3.6	(3.5 - 3.7)	9.5	(8.7 - 10.2)	338
Uric acid	mmol/L	15	70	M	All Males	0.24	(0.22 - 0.25)	0.55	(0.53 - 0.58)	438
			70	M	Males w/o CVD	0.23	(0.22 - 0.25)	0.53	(0.51 - 0.55)	350
			70	F	All females	0.19	(0.18 - 0.20)	0.47	(0.45 - 0.49)	459
			70	F	Females w/o CVD	0.19	(0.18 - 0.20)	0.46	(0.45 - 0.48)	408
		48	>65	M		0.22		0.70		71
			>65	F		0.20		0.62		165
		51	70	F	Healthy reference group	0.13		0.34		50
			70	F	Total study population	0.12		0.45		206
			70	M	Healthy reference group	0.17		0.52		50
			70	M	Total study population	0.17		0.51		250
		53	65-74	F		0.22		0.36		775
			65-74	M		0.28		0.42		454
			> 75	F		0.22		0.35		239
			> 75	M		0.27		0.41		202
		57	>90	M		0.21		0.49		27
			>90	F		0.13		0.46		36
		58	100-120	B	Centenarians	0.25		0.42		120
			65-85	B	Control	0.20		0.34		381
		61	65-74	B	Mexican American	0.20		0.51		185
			65-74	B	Cuban	0.21		0.48		96
			65-74	B	Puerto Rican	0.24		0.45		66
		66	65-74	M		0.27		0.48		890
			65-74	F		0.24		0.44		657
		67	>65	M		0.22		0.58		245
			>65	F		0.17		0.47		245
		74	75	M	All	0.22	(0.20 - 0.24)	0.50	(0.47 - 0.52)	354
			75	M	w/o CVD	0.22	(0.20 - 0.24)	0.48	(0.46 - 0.51)	274
			75	F	All	0.18	(0.17 - 0.19)	0.43	(0.40 - 0.47)	373
			75	F	w/o CVD	0.18	(0.17 - 0.19)	0.42	(0.40 - 0.47)	338

Analyte	Units	Paper Ref.	Age Group	Sex	Other Strata (if applicable)	Lower Limit	90% CI	Upper Limit	90% CI	n
Vitamin B_{12}	pmol/L	31	75	B		109	(95 - 151)	1440	(817 - 1766)	119
		42	65 or 70	B	2.5th and 97.5th	118		579		193
			65 or 70	B	5th and 95th	151		533		193
			65 or 70	B	2.5th and 97.5th	113		515		183
			65 or 70	B	5th and 95th	134		457		183
		50	65-88	M		102	(80 - 1301)	474	(373 - 604)	20
			65-88	F		89	(75 - 106)	190	(406 - 581)	44
			65-88	B		91	(81 - 108)	480	(417 - 555)	64
		51	70	F	Healthy reference group	111		1333		67
			70	F	Total study population	116		1053		206
			70	M	Healthy reference group	63		615		86
			70	M	Total study population	75		689		250
		57	>90	B		43		644		48
Zinc	µmol/L	41	65-80	M		11.2		28.3		28
			65-80	F		14.3		27.4		21
		57	>90	B		8.0		15.1		47