ASSOCIATION BETWEEN SERUM C-REACTIVE PROTEIN AND SERUM GAMMA-GLUTAMYL TRANSFERASE WITH TYPE 2 DIABETES IN THE KUOPIO ISCHAEMIC HEART DISEASE RISK FACTOR STUDY

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Raised levels of serum C-Reactive Protein (CRP) is associated with the increased risk of Type 2 Diabetes (T2D). However, the influence of serum Gamma-Glutamyl Transferase (GGT) on the association between serum CRP and the risk of T2D is unclear.

The aim of this thesis was to investigate the association between serum GGT and serum CRP, the association of serum CRP with T2D incidence, and the influence of serum GGT concentration on the association between serum CRP and T2D in the middle-aged eastern Finnish men and women using the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD).

This study included 1541 men and women who took part in the 11-year re-examination round of KIHD. Plasma glucose was measured by glucose dehydrogenase method. Serum CRP was measured by immunometric assay and serum GGT activity was measured by spectrophotometric method. T2D was defined based on a self-reported physician diagnosis of T2D and/or fasting plasma glucose ≥7.0 mmol/L or 2-h oral glucose tolerance test plasma glucose ≥11.1 mmol/L at 4, 11 and 20 years’ examination rounds. T2D diagnosis was also ascertained from the hospital discharge records and from the register of Social Insurance Institution of Finland. The association between serum GGT and serum CRP was investigated by linear regression analysis. Cox regression analysis was used to estimate hazard ratios (HRs) for T2D incidence. The influence of serum GGT on the association between serum CRP and T2D incidence was assessed in Cox regression models.

The mean age of the participant was 62.7 years (SD = 6.5), the mean CRP concentration was 2.8 mg/L (SD = 4.9), and the mean GGT concentration was 27.2 U/L (SD = 34.1). During the average follow-up of 7.3-year, a total of 206 out of 1541 subjects developed T2D. When subjects were divided based on the median value of GGT (20U/L), 125 subjects in high GGT group and 81 subjects in the low GGT group developed T2D. In multivariable adjusted linear regression model, a direct association was observed between serum CRP and serum GGT concentrations (β=0.30, 95%CI=0.20-0.39, P= <0.001). In multivariable adjusted Cox regression analysis, after adjustment for known risk factors of T2D, subjects in the highest serum CRP group had 17% higher risk of developing T2D when compared with subjects in the lowest group (HR 1.17, 95% CI=0.73-1.89, P for trend 0.485). The association between serum CRP and T2D was significant and stronger in the subjects having serum GGT concentrations above the median value (>20 U/L) compared with subjects who had serum GGT concentrations below the median value in multivariable adjusted models (HR 2.02, 95% CI=1.12-3.66, P for trend 0.004).

In conclusion, a positive association was found between baseline serum CRP and T2D incidence which was dependent on BMI and serum GGT.
ACKNOWLEDGEMENT

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I am grateful to my parents, siblings and friends for their support and encouragement throughout my study period. My deepest appreciation to my dear husband Tulashi, who provided love and moral support for the completion of this work.

Thank you.

Radha Karki
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CARDIA</td>
<td>Coronary Artery Risk Development in Young Adults</td>
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<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
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<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
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<tr>
<td>FG</td>
<td>Fasting Glucose</td>
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<tr>
<td>FPG</td>
<td>Fasting Plasma Glucose</td>
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<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma-Glutamyl Transferase</td>
</tr>
<tr>
<td>GSH</td>
<td>gamma-L-glutamyl-L-cysteinylglycine</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated Haemoglobin</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostatic Model Assessment- Insulin Resistance</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
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<tr>
<td>ICAM</td>
<td>Intercellular Adhesion Molecule</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
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<tr>
<td>IKK</td>
<td>IkB kinase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin Receptor Substrate 1</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinase</td>
</tr>
<tr>
<td>KIHD</td>
<td>Kuopio Ischemic Heart Disease Risk Factor</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic Units</td>
</tr>
<tr>
<td>MiRs</td>
<td>Micro-Ribonucleic acids</td>
</tr>
<tr>
<td>MPs</td>
<td>Endothelial Micro Particles</td>
</tr>
<tr>
<td>NHANES III</td>
<td>Third National Health and Nutrition Survey</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>NHS</td>
<td>The Nurses’ Health Study</td>
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<td>OGGT</td>
<td>Oral Glucose Tolerance Test</td>
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<tr>
<td>ox LDL</td>
<td>Oxidized Low Density Lipoprotein</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphoryl Choline</td>
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<tr>
<td>PG</td>
<td>Plasma Glucose</td>
</tr>
<tr>
<td>POS</td>
<td>Polycystic Ovary Syndrome</td>
</tr>
<tr>
<td>Q</td>
<td>Quartile</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic Lupus Erythematosus</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Science</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 Diabetes</td>
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<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-α</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular Cell Adhesion Molecule</td>
</tr>
<tr>
<td>vWF</td>
<td>von Will Brand Factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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CONTENTS
1 INTRODUCTION........................................................................................................................10
2 LITERATURE REVIEW..................................................................................................................12
  2.1 Diabetes ..................................................................................................................................12
    2.1.1 Type 1 diabetes ..................................................................................................................12
    2.1.2 Type 2 diabetes ..................................................................................................................12
  2.2 Epidemiology ..........................................................................................................................12
  2.3 Risk factors .............................................................................................................................13
  2.4 Biomarkers of diabetes mellitus .............................................................................................15
  2.5 C-reactive protein ....................................................................................................................16
    2.5.1 Structure ...........................................................................................................................17
    2.5.2 Biological functions ..........................................................................................................17
    2.5.3 Clinical significance .........................................................................................................17
  2.6 Gamma-glutamyl transferase ...............................................................................................17
    2.6.1 Structure ...........................................................................................................................18
    2.6.2 Biological functions ..........................................................................................................19
    2.6.3 Clinical significance .........................................................................................................21
  2.7 Inflammation and type 2 diabetes .........................................................................................22
  2.8 CRP and type 2 diabetes .......................................................................................................23
    2.8.1 Prospective studies ............................................................................................................24
  2.9 Gammaglutamyl transferase and type 2 diabetes .................................................................27
    2.9.1 Prospective studies ............................................................................................................27
3 AIMS OF THE STUDY..................................................................................................................30
4 METHODOLOGY..........................................................................................................................31
  4.1 Study design ...........................................................................................................................31
  4.2 Ethical consideration ...............................................................................................................32
  4.3 Data collection .........................................................................................................................32
  4.4 Measurements ........................................................................................................................32
    4.4.1 Blood specimen collection and laboratory assays ............................................................32
  4.5 Diagnostic criteria for type 2 diabetes ...................................................................................33
  4.6 Statistical analysis ....................................................................................................................33
5 RESULTS.......................................................................................................................................37
  5.1 Baseline clinical characteristics ............................................................................................37
5.2 Association between serum GGT and serum CRP concentrations .........................40
5.3 Association between serum CRP concentration and incident type 2 diabetes ..........41
5.4 The influence of serum GGT concentration on the association between serum CRP concentration and type 2 diabetes incidence .................................................................43

6 DISCUSSION .................................................................................................................46
6.1 Summary of the main findings ..................................................................................46
6.2 Strengths of the study ..............................................................................................48
6.3 Limitations of the study ...........................................................................................48

7 CONCLUSION ...............................................................................................................49

8 RECOMMENDATIONS .................................................................................................49

9 REFERENCES ...............................................................................................................50
LIST OF TABLES

Table 1: Prevalence of diabetes in adults (18+ years) across WHO regions in 1980 and 2014 ......................................................... 13

Table 2: Prospective studies on the association between serum CRP and type 2 diabetes (2005-2016)........................................................................................................................................................................... 25

Table 3: Prospective studies on the association between serum GGT and type 2 diabetes (2010-2016)..................................................................................................................................................................................................... 28

Table 4: List of dependent variables, outcome variable, possible confounders and risk factors of type 2 diabetes ........................................................................................................................................................................................................... 36

Table 5: Baseline characteristics by the quartiles of serum CRP in 1541 eastern Finnish men and women in the Kuopio Ischemic Heart Disease Risk Factor Study in 1998-2001 ........................................... 38

Table 6: Baseline characteristics by the quartiles of serum GGT in 1541 eastern Finnish men and women in the Kuopio Ischemic Heart Disease Risk Factor Study in 1998-2001 ........................................... 39

Table 7: Regression coefficients of serum GGT (10 U/L) predicting serum CRP in multivariate models. ........................................................................................................................................................................................................... 40

Table 8: The risk of type 2 diabetes during the average follow-up of 7.3 years according to the quartiles of serum CRP concentration ...................................................................................................................................................................................................... 42

Table 9: The influence of serum GGT concentration on the association between serum CRP concentration and incident type 2 diabetes. ...................................................................................................................................................................................................... 44

Table 10: Association between serum CRP concentration and incident type 2 diabetes by median value of serum GGT concentration. ...................................................................................................................................................................................................... 45
LIST OF FIGURES

Figure 1: Reaction catalyzed by GGT (Hanigan & Pitot 1985) .....................................................18
Figure 2: Structure of Glutathione (Castellano and Merlino 2013) ..............................................20
Figure 3: Gamma-glutamyl cycle (Castellano and Merlino 2013) ...............................................21
Figure 4: Phenomena of Inflammation (Wellen & Hotamisligil 2005). ........................................23
Figure 5: Time frame of the KIHD study (Virtanen et al. 2015). ..................................................31
Figure 6: The schematic representation of the total number of participants included in the final analysis. ...........................................................................................................35
1 INTRODUCTION

According to World Health Organization (WHO), diabetes directly caused 1.5 million deaths in the year 2012. By 2030, diabetes will be the seventh leading cause of death (WHO 2016a). In Finland, the prevalence of diabetes is 7.7%, and 1% of deaths is due to diabetes (WHO 2016b). Most of the diabetes can be grouped into Type 1 diabetes (T1D) and Type 2 diabetes (T2D). T1D is characterized by absolute scarcity of insulin secretion and T2D occur due to relative insulin deficit in combination with peripheral insulin resistance (American Diabetes Association 2010).

Modification in lifestyle factors and dietary patterns have increased the global prevalence of T2D. Modern western diets consisting of higher amount of total and saturated fats with low intake of fiber are linked with higher risk of T2D (Wikström et al. 2010). Despite the decrease in cardiovascular morbidity and mortality, the prevalence of T2D has been increasing in Finland. Obesity is one of the risk factor for T2D and the prevalence of obesity shows increasing pattern in Finland. T2D prevalence follows the trend of obesity pattern. In 2007, 57% of women and 70% of men were overweight and obese. Similarly, in 2008, more than half a million of Finnish population had T2D and 50% of them were still unaware that they suffered from the disease (Lindström et al. 2010). In between the years 2006-2011, there had been a sharp rise in the incidence of drug-treated T2D in Finland due to effective screening and prevention activities. After the highest incidence in 2011, there had been a decline in the incidence of T2D (Lindström et al. 2016).

C-reactive protein (CRP) is indefinite sensitive biomarker that is produced in the liver under the provocation of interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α) (Castell et al. 1990). Several prospective studies showed CRP as a hallmark of future diabetes risk (Pradhan et al. 2001, Freeman et al. 2002, Nakanishi et al. 2003). Similarly, several other evidences presented serum gamma-glutamyl transferase (GGT) as a predictor of T2D (Perry et al. 1998, Lee et al. 2003a, b, Lee et al. 2004a). Moreover, serum concentrations of GGT within its normal levels were strongly linked with CRP in a dose response manner regardless of confounding factors like ethnicity, smoking status, sex, Body mass index (BMI) and alcohol consumption (Lee et al. 2005). In addition, study conducted in the Chinese population showed positive association between CRP, GGT and prevalent T2D (Wen et al. 2010). Positive linkage of GGT was also seen with inflammatory markers such as fibrinogen, CRP and F2-isoprostanes in patients with hypertension (Franzini et al. 2010).
Hence, increase in serum GGT levels can predict inflammatory condition and oxidative stress (Turgut et al. 2009).

One of the evidence presented that inflammation plays a vital role in the pathogenesis of T2D, thus linking diabetes to various inflammatory mechanisms (Donath & Shoelson 2011). Inflammation increases blood glucose levels and causes insulin resistance resulting diabetes (Wang et al. 2013a). The mechanism by which increased level of GGT is connected to the pathophysiology of T2D include oxidative stress, inflammation, and underlying fatty liver, which are linked with impaired insulin secretion and insulin resistance (Hotamisligil 2003). Cellular GGT catalyzes the antioxidant glutathione, which produces reactive oxygen species (ROS). ROS is associated with the inflammatory process and the development of T2D (Ali et al. 2016). Thus, mechanism associated with oxidative stress and systemic inflammation is responsible for linking serum CRP and serum GGT with T2D (Wen et al. 2010). Earlier studies have shown associations between GGT and CRP or other inflammatory markers, relating GGT with sub-clinical inflammation (Nakanishi et al. 2004, Kerner et al. 2005, Lee & Jacobs 2005, Wen et al. 2010). It has also been assumed that rise in GGT might occur prior to the rise in CRP level, and oxidative stress may play a vital role in subsequent inflammatory response (Lee & Jacobs 2005).

In Finland, positive association of CRP with metabolic syndrome and diabetes in the middle-aged men was reported in the Kuopio Ischemic Heart Disease Risk Factor (KIHD) Study (Laaksonen et al. 2004). Similarly, relationship of GGT with T2D was reported in the middle-aged Finnish men and women from different parts of Finland (Lee et al. 2004a). However, the influence of serum GGT on the association between serum CRP and the risk of T2D is unclear. Hence, this thesis will investigate the association between serum GGT and serum CRP, the association of serum CRP with T2D incidence, and the influence of serum GGT concentration on the association between serum CRP and T2D in the middle-aged eastern Finnish men and women in the KIHD Study.
2 LITERATURE REVIEW

2.1 Diabetes

Diabetes is a cluster of metabolic diseases marked by hyperglycemia, which occurs due to impaired
insulin secretion, insulin action, or both. The disease is linked with symptoms like polyuria,
polydipsia and weight loss. Chronic hyperglycemia results major complications like impairment,
malfunction and deterioration of various organs (American Diabetes Association 2014). Diabetes
can be categorized as T1D, T2D, Gestational diabetes mellitus (GDM) and diabetes due to other
causes, like monogenic diabetes syndromes comprising of neonatal diabetes and maturity-onset
diabetes of the young (American Diabetes Association 2015).

2.1.1 Type 1 diabetes

T1D was previously recognized as insulin-dependent or childhood-onset diabetes outlined by the
deficiency of insulin production. It occurs because of cellular mediated autoimmune disruption of
the β-cells of the pancreas. 5-10 % of people with diabetes are suffering from this form of the
disease. Generally, it occurs in children and elder, but can occur at any age (American Diabetes
Association 2014).

2.1.2 Type 2 diabetes

Generally, 90–95% of diabetes is of T2D. Earlier it was identified as non-insulin-dependent
diabetes or adult-onset diabetes, occurs because of ineffective use of insulin by body. Thus, in T2D
patients’ insulin secretion is impaired and inadequate to overcome insulin resistance. Most patients
are overweight and obese in T2D, and obesity itself is the causative agent for insulin resistance.
The risk of developing T2D rises with age, obesity and physical inactivity. It is often present in
female with earlier GDM history and in persons with hypertension or dyslipidemia, and its
prevalence differs in varied racial/ethnic subgroups (Alberti & Zimmet 1998).

2.2 Epidemiology

In 2014, 422 million people over 18 years had diabetes according to WHO. The world-wide
prevalence of diabetes has raised from 4.7% in 1980 to 8.5% in 2014 and count of people has
grown from 108 million to 422 million that are approximately four-folds higher. The prevalence
of diabetes across WHO regions between 1980 and 2014 are illustrated in Table 1. Eastern
Mediterranean Region shows the highest prevalence (13.7%) in 2014 (Global Report on Diabetes,
WHO 2016c). This increase is due to urbanization, population ageing and life style modification
The WHO South-East Asia and Western Pacific Regions comprise around 50% of diabetes cases in the world (Global Report on Diabetes, WHO 2016c). Globally, diabetes and its complications are the seventh leading cause of disability and cause 2 million deaths each year (NCD-RisC 2016). The prevalence of diabetes mellitus is higher in low and middle-income countries than in high-income countries. Almost 80% of diabetic patients live in developing countries (Shaw et al. 2010). Asia has risen as the epicenter for diabetes in the globe (Chan et al. 2009). It is estimated that by 2030, five countries from Asia (China, India, Pakistan, Indonesia and Bangladesh) will have highest numbers of diabetic cases (Chen et al. 2012). In Finland, 250,000 people are suffering from T2D (Finnish Diabetes Association 2015).

Table 1: Prevalence of diabetes in adults (18+years) across WHO regions in 1980 and 2014

<table>
<thead>
<tr>
<th>WHO Region</th>
<th>Prevalence (%)</th>
<th>Number (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Region</td>
<td>3.1</td>
<td>7.1</td>
</tr>
<tr>
<td>American Region</td>
<td>5.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Eastern Mediterranean Region</td>
<td>5.9</td>
<td>13.7</td>
</tr>
<tr>
<td>European Region</td>
<td>5.3</td>
<td>7.3</td>
</tr>
<tr>
<td>South-East Asia Region</td>
<td>4.1</td>
<td>8.6</td>
</tr>
<tr>
<td>Western Pacific Region</td>
<td>4.4</td>
<td>8.4</td>
</tr>
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</table>


2.3 Risk factors

Risk factors for the development of T2D can be classified into four different groups: Genetic, Environmental, Life Style and Metabolic Risk Factors.

Life style factors consist of diet, overweight, obesity, smoking, sleeping patterns and physical inactivity. Various studies have shown different dietary factors as T2D risk. The study conducted on The Nurses’ Health Study (NHS) showed higher risk of T2D in the cohorts regularly consuming processed red meats, particularly bacon, sausages, and hot dogs (Pan et al. 2011). Similarly, higher consumption of potatoes (particularly French fries), trans fatty acids and starch are also linked with increased risk of T2D (Ley et al. 2016). Abdominal obesity is the major risk factor for the incidence of T2D at an earlier age (Haffner 2000). The risk for T2D increases significantly when obesity is complexed by physical inactivity (Fletcher et al. 2002). In the NHS cohorts, sedentary life style,
both short (≤ 5 hours per day), long (≥ 9 hours per day) sleep durations, and night shift work were linked with the increased risk of T2D. Moreover, a person’s T2D risk was increased by 14 % with each two additional years of being obese (Ley et al. 2016). One of the meta-analysis showed that smokers have 45% increased risk of T2D in comparison to non-smokers (Hu 2011). Life style intervention markedly decreased the risk of T2D among high-risk groups in the US Diabetes Prevention Program (Florez et al.2006) and Finnish Diabetes Prevention Study (Wang et al. 2007a) as determined by genetic polymorphisms.

Hyperinsulinemia, Impaired glucose tolerance (IGT), Insulin resistance, Hypertension and Polycystic Ovary syndrome (POS) are major metabolic risk factors for T2D. Obesity, age, physical inactivity and genetics cause insulin resistance and it is associated with the development of T2D (Fletcher et al. 2002). Hyperinsulinemia, hyperuricemia, prothrombotic state, atherogenic dyslipidemia, hypertension, glucose intolerance and POS are major metabolic problems that usually occur in individuals with insulin resistance (Reaven 1995). In addition, women with GDM are prone to 7 times higher risk of developing T2D in future compared with women experiencing a normal pregnancy (Bellamy et al. 2009).

Birth weight also predicted type 2 diabetes. In the NHS, birth weights less than 2500 grams and greater than 4000 grams were linked with the risk of T2D. Fetal undernutrition results lower birth weights leading to the development of the disease later in life. Similarly, over nutrition results higher birth weights leading to glucose intolerance later in life (Li et al. 2015). Moreover, fetal undernutrition is estimated to predispose people to insulin resistance (Dabelea et al. 1999), reduced βcell mass, and function, which then lead to their increased vulnerability to the development of T2D in later life (Fowden & Hill 2001). One of the meta-analysis comprising 28 populations from diverse ethnicities, showed consistent association of low birth weight with raised risk of T2D in future. It also showed that 1 kg increment in birth weight was linked with a 20 % decrease in risk of developing T2D (Whincup et al. 2008).

Genetic risk factors comprise family history, race/ethnicity and age. The Framingham Offspring Study found that the risk for T2D was 3.5 folds greater among the children with an only one parent with diabetes and it was 6 times higher for those with both parents having diabetic history when comparison was done with the children without parental diabetes (Meigs et al.2000).
Significant alterations occur in the locus of risk allele and consistency of incidence of specific risk alleles across various ethnic groups (Chan et al. 2009). The prevalence rate of diabetes was 1.7-fold greater for non-Hispanic blacks compared with the whites of same age groups in the study conducted on the Third National Health and Nutrition Survey-1988-1994 (NHANES III) (Harris et al. 1998). According to Fletcher and his colleagues, the Pima Indians in Arizona showed the greatest incidence of diabetes in the globe with nearly one of each two adults having T2D (Fletcher et al. 2002).

According to Steven and his colleagues, 68 loci have shown association with T2D. Several “diabetes-genies” are recognized related with the development of T2D (Elbein et al. 2012). Researchers have also found that variants present on the regulatory elements may contribute for the development of T2D. Around 50 single nucleotide polymorphisms or genetic variants situated inside or near to non-promoter regulatory elements are linked with islet-related diseases. Six such regulatory elements consist of the variants associated with T2D. Regulatory elements are responsible to enhance gene activity (National Human Genome Research Institute 2010).

Apart from these life style, environmental and genetic risk factors, some other risk factors are independently associated with the risk of T2D, for example sleeping disorders, depression, use of antidepressant medication, etc. (Kivimäki et al. 2010). A meta-analysis showed that depressed people have 37% increased risk for T2D (Knol et al. 2006). Usage of antidepressant drugs was associated with the increased risk of T2D. Risk of T2D also increased with increased symptoms of posttraumatic stress disorder (Roberts et al. 2015). Some studies have also showed that environmental toxins such as bisphenol A (Magdalena et al. 2011) and particulate matter in air pollution are also linked with the development of T2D (Brook et al. 2010, Krämer et al. 2010).

### 2.4 Biomarkers of diabetes mellitus

Pathological expressions of different biomarkers occur in diabetic patients. Inflammatory biomarkers, stress-associated biomarkers, coagulation associated biomarkers, and few currently emphasized markers like micro-ribonucleic acids (MiRs) and endothelial micro particles (MPs) are related biomarkers of diabetes mellitus. CRP, TNF-α, IL-6 and IL-18 are pro-inflammatory biomarkers (Hu et al. 2004) and vascular cell adhesion molecule (VCAM)-1 and von Willbrand factor (vWF) are the markers of endothelial function (Tousoulis et al. 2013). IL-6 and TNF-α are vital cytokines contributing in atherogenesis and are linked with the risk of T2D. In addition, IL-6
influences insulin sensitivity via adenosine monophosphate stimulated protein kinase and affects glucose homeostasis and metabolism (Ansar & Ghosh 2016). Higher concentration of CRP, IL-6, IL-1, fibrinogen and TNF-α control different pro coagulant event. White blood cells adhesion to the endothelium via chief intermediaries like VCAM-1 and intercellular adhesion molecule ICAM-1 cause variations in vaso- regulatory responses. Alteration in these responses later increase the risk of complications in diabetes mellitus (Meigs et al. 2004).

F2 isoprostanes and antibodies against Oxidized low-density lipoprotein (ox LDL) are circulating markers of oxidative stress, which are raised in individuals with obesity, insulin resistance and diabetes. MPs and MiRs are the novel biomarkers evolving as a promising competitor of the traditional diabetic biomarkers (Meigs et al. 2007). MiRs is a group of approximately 22 noncoding nucleotide ribonucleic acids which act as modulators in endothelial dysfunction and have important role in atherogenesis (Bartel 2004). MiRs also influence pancreatic β cells, insulin-target tissues and insulin secretion (Tousoulis et al. 2013). MPs measuring less than 1 mm in diameter are released into the circulation from different blood cells (thrombocytes, leucocytes, erythrocytes) and endothelial cells. Formation of MP controls apoptosis, mechanical damage, markers of endothelial impairment, platelet activation and cellular stimulation through cytokines. MPs are distributed in blood circulation of normal people, but their quantity is raised up in several cardiovascular diseases (Shantsila et al. 2010).

2.5 C-reactive protein

CRP is a plasma protein associated with the family of “pentraxin,” which is produced in the hepatocyte and secreted in the plasma. It has a major responsibility in the innate immune system and has a capacity of recognizing microorganisms by confining to their external cell surface called phosphoryl choline (PC). It then activates the complement system resulting phagocytosis. An inflammatory biomarker whose serum levels rises quickly to 1000-folds above the reference ranges during tissue injury, infection or other inflammatory conditions (Volanakis 2001). CRP resembles several characteristics shown by the immunoglobulins, such as the capability to enhance agglutination, precipitation of cationic and anionic compounds, complement fixation, bacterial capsular swelling and phagocytosis (Marnell et al 1995).
2.5.1 Structure
CRP has a conserved pentameric arrangement known as “Pentraxin.” Electron microscopic view has revealed that non-covalent bonds showing cyclic pentameric symmetry attach the five-similar polypeptide protomers with each other. Each subunit consists of a single, intramolecular disulphide bond. It has a distinctive calcium-dependent binding sites for poly cations and specific ligands like LDL cholesterol, PC, Sphingomyelin, etc. (Ansar & Ghosh 2016).

2.5.2 Biological functions
CRP prevents adhesion of neutrophils to the endothelial cell and acts as the mediator in innate host defense against microbes by activating the classical complement pathway. It acts as the modulator of inflammation and enhances attachment and involvement of monocytes and lymphocytes thereby resulting increased vascular wall inflammation. It also facilitates in the non-inflammatory removal of nuclear host substance like histones, chromatin and cellular host material as apoptotic cells by activating classical complement pathway (Ablij and Meinders 2002).

2.5.3 Clinical significance
Though CRP does not reveal exclusive diagnostic accuracy, it is extremely important in medical practice because variations in serum concentrations of CRP in a wide spectrum indicate the existence of varied inflammatory conditions. Hence, CRP is recognized as a significant biomarker of acute-phase reactions. Plasma/ Serum concentration of CRP is raised in several diseases and pathological conditions like neonatal septicemia and meningitis, postoperative infection, bacterial infection, systemic lupus erythematosus (SLE), leukemia, acute pancreatitis, acute appendicitis, acute osteomyelitis, acute rheumatic fever, rheumatoid arthritis, myocardial infarction, visceral leishmaniosis and insulin resistance (Ansar & Ghosh 2013).

In a normal individual, CRP level is generally less than 10 mg/L. In bacterial infection and burn, it rises to 200 mg/L and in viral infection its level is in between 10-40 mg/L. In trauma, inflammation, parasitic infection, tissue necrosis and malignant neoplasia serum concentration of CRP rises quickly to about 1,000-fold within 48 h of acute episode (Clyne & Olshaker 1999).

2.6 Gamma-glutamyl transferase
GGT is an enzyme capable of using a broad range of gamma-glutamyl compounds as substrates. It catalyzes the transfer of the gamma-glutamyl group to several peptide and amino acid acceptors.
The reaction catalyzed by GGT is shown in figure 1. Its enzymatic activity was demonstrated initially in the catalytic transfer of the gamma-glutamyl group from glutathione to acceptor amino acids. Cellular GGT is the only typical protease identified that can breakdown intact glutathione. Hence, it has a significant role in balancing glutamate cycle and glutathione metabolism (Hanigan & Pitot 1985). GGT is produced by a variety of animals ranging from bacteria to mammals. GGT is found in the cell membranes of the various organs such as liver, bile ducts, kidney, heart, lung, pancreas, small intestine, bone marrow, thymus, spleen and brain. The maximum activity of GGT has been observed in the kidney (Kuntz & Kuntz 2006). At present, eleven isoenzymes of it are recognized (Levine & Morgan 1996).

![Reaction catalyzed by GGT](image)

**Figure 1**: Reaction catalyzed by GGT (Hanigan & Pitot 1985)

### 2.6.1 Structure

GGT is a membrane-bound glycoprotein having two subunits, present on the external covering of the cell membrane (Hanigan & Pitot 1985). Mammalian GGTs are inserted in the plasma membrane by a N-terminal trans-membrane peptide and are heterologously glycosylated. All GGTs are programmed by the distinct gene and are transformed as a distinctive polypeptide, which then go through an auto-proteolytic cleavage into a large and a small subunit before it is inserted in to the cell membrane. Even after the cleavage, the two subunits remain connected by a non-covalent bonding (Chevalier et al. 1999). Both subunits are essential for enzymatic activity (Gardell & Tate 1981).
2.6.2 Biological functions

The main biologic functions of GGT are its involvement in glutathione metabolism and transportation of amino acids (Vroon & Israili 1990). Various factors like alcohol ingestion, body fat, plasma lipid and blood sugar levels alter serum levels of GGT (Castellano & Merlino 2013). Raised serum GGT levels are usually observed in pancreatitis, cardiovascular disease, stroke, hypertension and T2D (Lee et al. 2003a). GGT also acts as an important compound in the manufacture of free radicles through its collaboration with iron (Lee et al. 2004b). GGT works as a proatherogenic factor (Emdin et al. 2006), and its estimation in obesity is linked with insulin resistance resulting metabolic syndrome (Marchesini et al 2005).

2.6.2.1 Glutathione (GSH, gamma-L-glutamyl-L-Cysteinylglycine)

Glutathione is a tripeptide found at the levels of 1-10 mM in all mammalian tissues. It is the most profuse antioxidant molecule found in cells and is responsible to conduct vital cellular functions like fibrogenesis, apoptosis, redox signaling, and detoxification of xenobiotic and articulation of cell proliferation. It is also responsible for storing and transferring nitric oxide, regulating sulfur assimilation and safeguarding cells against oxidative stress. As shown in figure 2, it consists of strange peptide bond connecting glutamate and cysteine through the γ-carboxyl group of glutamate unlike the traditional α-carboxyl group. Thus, GSH cannot be catalyzed by common peptidases (Foyer and Noctor 2005). In mammals, γ-glutamyl cycle facilitates the GSH metabolism that involves two Adenosine triphosphate (ATP) - dependent GSH synthesis steps catalyzed by γ-glutamyl Cys-synthetase and GSH synthetase. GSH is synthesized in the cytosol and transported out of the cells where it acts as a substrate for GGT (Castellano and Merlino 2012).
2.6.2.2 Gamma-glutamyl cycle

Mammalian GGT is a glycoprotein incorporated into the plasma membrane with its active site fronting the extracellular space. It splits the gamma-glutamyl amide bond of GSH to yield gamma-glutamyl amino acid and Cysteinylglycine as shown in figure 3. The released gamma-glutamyl amino acid group is carried back into the cell where it is broken down to release glutamate. This glutamate is reincorporated into GSH. Dipeptidase acts on Cysteinylglycine to release glycine and cysteine, which is again carried back into the cell. Most of the cysteine act as substrate for intracellular GSH and protein synthesis while remaining amount is cleaved into sulfate and taurine. GGT maintains cellular redox homeostasis by balancing the antioxidant molecule GSH at the cellular levels. Thus, GGT plays a key role for protein synthesis in neoplastic cells that are dividing quickly (Pompella et al. 2006).
2.6.3 Clinical significance

The sensitivity and specificity of GGT is 95% in hepatobiliary diseases. Elevations of serum GGT are observed in all forms of primary and secondary hepatobiliary disorders. Increase in GGT concentration occur in cholestasis, liver cirrhosis, pancreatitis and pancreatic cancer, viral hepatitis, toxic liver damage, fatty liver, obesity, diabetes mellitus, myocardial infarction, nephrotic syndrome and brain tumors (Kuntz & Kuntz 2006). Raised serum concentrations of GGT are also present in persons using medicines like phenytoin or phenobarbital and alcohol (Vroon & Israel 1990). A decrease in GGT values from its normal reference range is found after the first trimester of pregnancy and during estrogen therapy (Alpers 2001).

Reference range of GGT at (37°C) for Women is < 40 U/l and for Men < 60 U/l (Kuntz & Kuntz 2006). Slight increases of GGT (2-5 times normal range) is present in hepatic cell injury and higher elevations (5-30 times normal level) is found in Cholestasis (Vroon & Israel 1990).
2.7 Inflammation and type 2 diabetes

Inflammation is assumed to play a crucial role in the development of T2D. CRP has been identified as an inflammatory biomarker, which was increased markedly in diabetic people (Ansar & Ghosh 2016). Obesity is linked with a low-grade inflammation that affect insulin action leading to insulin resistance (Yin et al. 1998). Inflammatory signaling pathways can also be activated by intracellular or extracellular metabolic stresses. It is shown that obesity burdens the functional capability of the Endoplasmic Reticulum (ER) and this ER stress activates inflammatory signaling pathways resulting insulin resistance (Ozcan et al. 2004, Nakatani et al. 2005). Moreover, raised glucose metabolism leads to the higher production of mitochondrial ROS in obesity. ROS production boost activation of inflammatory pathways (Furukawa et al. 2004, Lin et al. 2005).

Inflammation or stressful stimuli activate serine/threonine kinases like c-Jun N-terminal kinase (JNK), IkB kinase (IKK) and inhibit insulin signaling resulting diabetes (Zick 2003). In obesity, JNK has a vital role in the development of insulin resistance. Obesity creates a condition that increases the demand on the ER resulting ER stress. In response to ER stress, JNK is activated. Then it phosphorylates Insulin receptor substrate 1 (IRS-1) on Ser307 resulting impaired insulin action (Aguirre et al. 2000, Ozcan et al. 2004). This phenomenon has been illustrated in Figure 4.

Evidence shows that in obese mouse models, elimination of inflammatory intermediaries or pathway constituents, such as TNF-α, JNK and IKK safeguard against insulin resistance and use of medicines such as salicylates increases insulin sensitivity (Uysal et al. 1997, Yuan et al. 2001). This indicates that T2D is an inflammatory disease and that inflammation is a principal root of obesity-linked insulin resistance, hyperglycemia and hyperlipidemia (Wellen & Hotamisligil 2005).

A second mechanism, which is significant for the commencement of inflammation in obesity, is oxidative stress. In hyperglycemic situations, endothelial cells uptake higher amount of sugar and result increased production of ROS in mitochondria, which causes oxidative damage and starts inflammatory signaling pathways inside endothelial cells (Brownlee 2001). In addition, hyperglycemia also initiates production of ROS in adipocytes, which finally results high production of pro-inflammatory cytokines (Lin et al. 2005).
2.8 **CRP and type 2 diabetes**

CRP is normally measured circulating marker for subclinical inflammation (Pearson et al. 2003). CRP is a normal plasma protein, which rises significantly in various tissue injury, infection and inflammation because of cytokine-mediated response and serum CRP levels are commonly assessed as an index of disease activity in medical practice (Pepys 1995). CRP exhibits pro-inflammatory and pro coagulant effects because of its capability to actuate complement and to stimulate production of tissue-factor (Thompson et al. 1999). Insulin resistance and impaired insulin secretion are main physiological anomalies that cause T2D. The specific underlying mechanism is unclear. One of the population-based study presented that inflammatory mechanisms plays a vital role in the development of T2D. It also showed CRP as a marker of subclinical systemic inflammation and its association with hyperglycemia, insulin resistance, and T2D (Frohlich et al. 2000). The cytokine-mediated acute-phase response to infection and other inflammatory events probably result prolonged low-grade inflammation in T2D. Thus, T2D may be considered as a disease of the innate immune system (Pickup & Crook 1998). According to the systematic review and meta-analysis conducted by (Wang et al. 2013b), T2D development was predicted by chronic inflammation and T2D was considered as the auto inflammatory disease. They also showed that increased concentration of IL-6 and CRP could assist in the life style and therapeutic interventions for T2D in high-risk populations. But, one of the study done in Chinese
population recommended serum CRP may not be self-determining risk factor for T2D since no association was observed between serum CRP and T2D prevalence when serum GGT concentrations were low (Wen et al. 2010).

### 2.8.1 Prospective studies

Earlier meta-analysis done by Lee et al. 2009 had presented prospective studies between serum CRP concentrations and T2D until the end of December 2007. Therefore, this thesis includes updated prospective studies from 2005 onwards. The largest prospective study (Table 2) was conducted by Hu et al. 2009 in 12,861 Finnish participants in which they assessed whether the association between serum CRP and risk of developing T2D was altered by sex. They found that high serum CRP level at baseline was linked with higher risk of developing T2D in both sexes, but this association was stronger in female than male.
Table 2: Prospective studies on the association between serum CRP and type 2 diabetes (2005-2016)

<table>
<thead>
<tr>
<th>Reference (Authors)</th>
<th>Population</th>
<th>Age &amp; Sex</th>
<th>Duration Years</th>
<th>Case subject ascertainment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effoe et al. 2015</td>
<td>3340 African American</td>
<td>21-94 Men &amp; Women</td>
<td>7.5</td>
<td>FG ≥ 126 mg/dl or HbA1C ≥ 6.5% (48 mmol/mol) or Physician diagnosis or use of diabetes drugs</td>
<td>Positive graded association between baseline CRP and T2D incidence</td>
</tr>
<tr>
<td>Wang et al. 2013a</td>
<td>4213 Japanese civil servants</td>
<td>35-66 Men &amp; Women</td>
<td>6.0</td>
<td>FG ≥ 126 mg/dl or Self-report</td>
<td>Positive association of elevated CRP concentration with increased T2D incidence</td>
</tr>
<tr>
<td>Rubio-Martin et al. 2013</td>
<td>1226 Spanish</td>
<td>Adult Men &amp; Women</td>
<td>11.0</td>
<td>FG ≥ 110 mg/dl or 2h post glucose load ≥ 180 mg/dl</td>
<td>CRP predicts the risk of developing T2D</td>
</tr>
<tr>
<td>Hu et al. 2009</td>
<td>12,861 Finnish</td>
<td>35-74 Men &amp; Women</td>
<td>7.0</td>
<td>National Hospital Discharge Register or Social Insurance Institution’s Drug Register or FPG ≥ 7.0 mmol/L or OGTT ≥ 11.1 mmol/L</td>
<td>Increased plasma concentration of CRP is associated with an increased risk of T2D among both sexes</td>
</tr>
<tr>
<td>Dehghan et al. 2007a</td>
<td>5,901 Dutch</td>
<td>≥ 55 Men &amp; Women</td>
<td>9.8</td>
<td>ADA and WHO criteria</td>
<td>CRP is independently associated with the risk of developing T2D</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Mean Fasting Glucose (mmol/L)</td>
<td>Diagnosis Criteria</td>
<td>Findings</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------</td>
<td>------------</td>
<td>------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dehghan et al. 2007 b</td>
<td>7,983 Dutch</td>
<td>≥ 55 Men &amp; Women</td>
<td>10.8</td>
<td>FPG ≥ 7.0 mmol/L or Random plasma glucose level ≥ 11.1 mmol/L or Use of oral antidiabetic medications or Use of insulin</td>
<td>CRP is the major contributing factor for the development of T2D</td>
</tr>
<tr>
<td>Thorand et al. 2007</td>
<td>2,225 German</td>
<td>35-74 Men &amp; Women</td>
<td>10.8</td>
<td>Self-report</td>
<td>Increased concentrations of CRP and IL-6 are associated with an increased risk of T2D in both sexes.</td>
</tr>
<tr>
<td>Wang &amp; Hoy 2007b</td>
<td>620 Aboriginal Australian</td>
<td>20-74 Men &amp; Women</td>
<td>11.0</td>
<td>Hospital and clinic records</td>
<td>Positive association between concentrations of CRP and T2D incidence</td>
</tr>
<tr>
<td>Doi et al. 2005</td>
<td>1,759 Japanese</td>
<td>40-79 Men &amp; Women</td>
<td>9.0</td>
<td>ADA criteria</td>
<td>Increased concentration of CRP is an independent predictor of T2D among both male and female</td>
</tr>
</tbody>
</table>

FG, fasting glucose; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; OGGT, oral glucose tolerance test; ADA, American diabetes association; WHO, world health organization; CRP, C-reactive protein; T2D, Type 2 Diabetes
2.9 Gammaglutamyl transferase and type 2 diabetes

Serum concentration of GGT had a strong association with the risk of developing impaired fasting glucose and T2D (Perry et al. 1998). According to (Nakanishi et al. 2004) GGT can be used as a signal of risk for the development of metabolic syndrome and T2D. In that study, age and obesity had shown strong association with diabetes in participants having elevated GGT values. The strong association between incidence of diabetes and GGT had also been detected in non-alcohol drinkers and subjects with normal concentrations of liver enzymes. Thus, this association was not influenced by alcohol or any other hepatic abnormalities (Lee et. al 2003a). The possible pathophysiology behind this association is that raised serum levels of liver enzymes (GGT) indicate inflammation, which harms insulin signaling in the liver (Hotamisligil 2003). Moreover, mechanisms associated with oxidative stress also play a significant role because glutathione homeostasis is maintained by cellular GGT. Extracellular glutathione, which is a vital antioxidant defense for the cell is broken down by GGT (Whitfield 2001). Oxidative stress increases serum GGT activity, resulting higher conveyance of glutathione into cells. Serum GGT levels projected future levels of inflammation and oxidative stress markers like fibrinogen, uric acid, C-reactive protein, and F2-isoprostanes, in a dose-response manner (Lee et al. 2003b).

2.9.1 Prospective studies

Earlier systematic review and dose-response meta-analysis done by Kunutsor et al. 2014 included prospective studies on GGT and risk of T2D till the end of June 2014. Therefore, this thesis includes updated prospective studies from 2010 onwards. Schneider et al. 2013 conducted the prospective study (Table 3) with the longest follow-up among American men and women of different race, which examined the associations of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase(AST), and GGT with the risk of diabetes and found out whether associations differed by gender and/or race. They reported that GGT was more strongly associated with the risk of T2D compared with ALT and AST over 12-year follow-up period. No evidence was observed for effect modification by gender or race.
<table>
<thead>
<tr>
<th>Reference (Authors)</th>
<th>Population</th>
<th>Age &amp; Sex</th>
<th>Duration Years</th>
<th>Case subject ascertainment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al. 2015</td>
<td>10764 Chinese</td>
<td>≥ 50 Men &amp; Women</td>
<td>4.0</td>
<td>Self-reported or Use of oral antidiabetic medications or Use of insulin or FG ≥ 7.0 mmol/L or 2 h OGTT glucose level ≥ 11.1 mmol/L</td>
<td>Higher concentrations of GGT and ALT are associated with T2D incidence</td>
</tr>
<tr>
<td>Ahn et al. 2014</td>
<td>6,926 Korean</td>
<td>45-74 Men &amp; Women</td>
<td>4.2</td>
<td>Intake of anti-diabetic agents or FPG ≥ 126 mg/dl or HbA1c levels ≥6.5%</td>
<td>Serum ALT and GGT are positively associated with an increased risk of T2D among both male and female</td>
</tr>
<tr>
<td>Ryoo et al. 2014</td>
<td>22931 Korean</td>
<td>22-89 Men</td>
<td>3.5</td>
<td>HOMA-IR ≥ 2.7</td>
<td>Baseline elevation of serum GGT is positively and significantly associated with the development of insulin resistance</td>
</tr>
<tr>
<td>Schneider et al. 2013</td>
<td>9,337 American Black and White participants</td>
<td>63 Men &amp; Women</td>
<td>12.0</td>
<td>Self-report or Physician diagnosis or Intake of diabetic medication</td>
<td>GGT is a stronger risk factor for diabetes</td>
</tr>
<tr>
<td>Marques-Vidal et al. 2012</td>
<td>3,842</td>
<td>35-75 Men &amp; Women</td>
<td>5.5</td>
<td>FPG ≥7.0 mmol/L or Use of oral antidiabetic medications</td>
<td>A strong positive association is found between GGT concentrations and risk of developing T2D</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Sample Size</td>
<td>Age Range</td>
<td>Follow-Up</td>
<td>Case Definition</td>
</tr>
<tr>
<td>------------------</td>
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<td>-----------</td>
<td>-----------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Abbasi et al.</td>
<td>Dutch</td>
<td>38,379</td>
<td>20-70</td>
<td>10.2</td>
<td>Self-report and hospital records or validated by general practitioner and pharmacist</td>
</tr>
<tr>
<td>Onat et al.</td>
<td>Turkish</td>
<td>1667</td>
<td>33-84</td>
<td>3.9</td>
<td>Self-report or FPG ≥7.0 mmol/L or 2-h postprandial glucose ≥ 11.1 mmol/L</td>
</tr>
<tr>
<td>Fujita et al.</td>
<td>Japanese</td>
<td>36,873</td>
<td>40-79</td>
<td>8.0</td>
<td>FPG ≥7.0 mmol/L or Non-Fasting PG ≥11.1 mmol/L or Diagnosis of physician</td>
</tr>
<tr>
<td>Hozawa et al.</td>
<td>Japanese</td>
<td>3,095</td>
<td>41.5</td>
<td>4.0</td>
<td>FPG ≥7.0 mmol/L or Non-Fasting PG ≥11.1 mmol/L or Treatment with diabetic medication</td>
</tr>
</tbody>
</table>

FG, fasting glucose; OGGT, oral glucose tolerance test; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic model assessment- Insulin resistance; PG, plasma glucose; T2D, type 2 diabetes; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; DM, diabetes mellitus
3  AIMS OF THE STUDY

The objective of this thesis was to examine the association between serum CRP and serum GGT with T2D among Finnish men and women from eastern Finland in the KIHD study.

The specific aims of the study were:

- To investigate the association between serum GGT and serum CRP.
- To assess the association between serum CRP with T2D incidence.
- To examine the influence of serum GGT on the association between serum CRP and T2D.
4 METHODOLOGY

4.1 Study design

This is a prospective study and it used data from KIHD study. The KIHD study is an ongoing prospective population-based cohort study conducted in randomly selected sample of middle-aged men and women from eastern Finland to examine risk factors for cardiovascular disease, atherosclerosis and related outcomes (Karppi et al. 2009). The baseline examinations were carried out between 1984 and 1989 in a random sample of middle-aged men residing in the municipality of Kuopio and its surrounding communities. A total of 2682 men, who were 42, 48, 54 or 60 years old at baseline (83.3% of those eligible), were recruited in to two cohorts. The first cohort comprised of 1166 men who were 54 years old, registered between 1984 and 1986, and the second cohort consisted of 1516 men who were 42, 48, 54 or 60 years old, registered between 1986 and 1989. This was followed by the four-year examination round between 1991 and 1993 in which 1038 men from the second cohort participated. 854 men from the second cohort and 920 post-menopausal women aged 53-73 years were requested to participate the 11-year examination round in between the years 1998-2001. All eligible subjects from the first and second cohort of men and the cohort of women were invited to the 20-year examination round in between 2005-2008. A total of 1875 men and women participated (Aregbesola 2016).

Figure 5: Time frame of the KIHD study (Virtanen et al. 2015).
4.2 Ethical consideration
The ethical committee of the University of Kuopio has approved KIHD study with approval number 143/97, and the study subjects gave written informed consent for health records use.

4.3 Data collection
Three different specified set of self-executed questionnaire were sent to the participant. The first questionnaire consisted the information concerning demographic features, main life episodes, family life, recreational activities, childhood situations, socioeconomic history and health habits such as use of tobacco, alcohol intake, physical exercise, health condition and medication practice. Remaining two set of questionnaires were associated with psychosocial well-being. Finally, participants were invited for health checkup and interviewed by the nurse.

Anthropometric measurements of participants (weight in kilograms and height in meters) were compiled at the study site. BMI was calculated as weight over height squared; waist circumference and waist-to-hip ratio were also measured (Aregbesola 2016). The quantity, frequency and duration of use of cigarettes and tobacco products were documented on a self-administered questionnaire (Tuomainen et al. 1998). The consumption of alcohol was assessed by using quantity and frequency questionnaire based on the Nordic Alcohol Consumption Inventory. Beer, wine, strong wine and spirits were considered as alcoholic drink (Aregbesola 2016). Spare time physical activity was evaluated from a modified 12-month questionnaire. The participants were requested to note the frequency (number of sessions per month), time (hours and minutes per sessions) and magnitude of physical activity (scored as 0 for recreational activity, 1 for conditioning activity, 2 for brisk conditioning activity, and 3 for competitive, strenuous exercise) for each activity. The extent of physical activity was expressed in metabolic units (MET) (Lakka et al. 1994). For the assessment of consumption of nutrient participants were advised for keeping their complete food records of 4 days. A nutritionist provided the advices and reviewed the dietary records. NUTRICA software (version 2.5; National Public Health Institute, Turku, Finland) was used for computing dietary intake of nutrients and foods (Rissanen et al. 2003).

4.4 Measurements
4.4.1 Blood specimen collection and laboratory assays
Blood specimens were taken between eight and ten in the morning after having refrained from alcohol intake for 3 days, smoking and eating for 12 hours. The subject was allowed to rest in the
supine position for half an hour prior to the sample collection. Blood was collected in Terumo Venoject VT-100PZ vacuum tubes (Terumo Corp., Tokyo) without using tourniquet (Salonen et al. 1992). Plasma glucose was measured by using a kinetic method called glucose dehydrogenase method. Prior to the measurement trichloro acetic acid was used to precipitate proteins (Laaksonen et al. 2002). Immunometric assay (Immulite High Sensitivity CRP Assay, DPC, Los Angeles, CA, USA) was used for the measurement of serum CRP (Virtanen et al. 2012). GGT Activity was measured based on Scandinavian recommendation (Salonen 2003). Enzyme reaction was measured at the specified wavelength of 405-410 nm for the production of 4-nitro aniline by spectrophotometric method. γ-Glutamyl-4-nitroanilide and Glycylglycine were used as substrates in this method (Scand J Clin Lab Invest. 1976).

4.5 Diagnostic criteria for type 2 diabetes
T2D was defined based on a self-reported physician diagnosis of T2D and/or fasting plasma glucose ≥7.0 mmol/L or 2-h oral glucose tolerance test plasma glucose ≥11.1 mmol/L at 4, 11 and 20 years’ examination rounds. T2D was also ascertained from the hospital discharge records and from the register of Social Insurance Institution of Finland in which diabetes patients are registered for reimbursement of medicine expenses used for T2D.

4.6 Statistical analysis
The statistical test was done by using statistical package for social science (SPSS) version 23 for windows. This study comprised 1774 men and women who took part in the 11-year re-examination round of KIHD Study. After exclusion of participants with prevalent T2D cases at 11-year re-examination round (n=209) and those with missing serum CRP and serum GGT concentrations (n=24), 1541 subjects were included in the final analysis (Figure 6). Serum CRP and serum GGT concentrations were categorized separately into fourths, Q1, Q2, Q3 and Q4. The baseline characteristics were presented according to the quartiles of serum CRP (mg/L); 0.10 to 0.75, 0.76 to 1.49, 1.50 to 3.05, 3.06 to 76.90 and serum GGT (U/L); 3 to 14, 15 to 19, 20 to 29, 30 to 836. The descriptive data were presented in the form of mean and standard deviation for continuous variables.

Skewed serum CRP value and serum GGT value (10 U/L) were normalized by logarithmic transformation. Then they were used as continuous variables in the multiple linear regression models used to find the association between serum GGT and serum CRP, controlling for possible
confounders. Multivariable Cox regression model was used to investigate the hazard ratios (HRs) for T2D incidence in the subjects with raised serum CRP as compared to those with low serum CRP (lowest quartile as the reference group). The multivariable Cox regression models were adjusted for known T2D risk factors and confounding variables (Table 4). Logarithmic transformed serum GGT (10 U/L) was introduced in Cox regression model to examine the influence of serum GGT on the association between serum CRP and T2D incidence. In addition, multiplicative interaction test was performed between serum CRP and serum GGT, and the influence was tested with risk of T2D. The median value of serum GGT was used as the cut-off point to assess the effect of serum GGT based on the serum GGT concentrations above and below the median value (20 U/L). The same confounding variables as listed in Table 4 were adjusted in the Cox regression models. The two-sided P-values of < 0.05 were considered as statistically significant for the analysis.
11-year follow-up examination (1998 to 2001)

854 Men, Age:- 53-73 years

+ 

920 Women, Age:- 53-73 years

1774 Men and Women

Excluded data:

Prevalent T2D at 11 years follow-up examination 209

Missing serum CRP and serum GGT 24

Data in the final analysis 1541

Figure 6: The schematic representation of the total number of participants included in the final analysis.
Table 4: List of dependent variables, outcome variable, possible confounders and risk factors of type 2 diabetes

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome variable</th>
<th>Possible confounders and risk factors of type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRP</td>
<td>Type 2 Diabetes</td>
<td>Age (years)</td>
</tr>
<tr>
<td>Serum GGT</td>
<td></td>
<td>Sex (M, F)</td>
</tr>
<tr>
<td>Serum CRP*Serum GGT</td>
<td></td>
<td>Date of examination (year)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smoking (pack years)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alcohol intake (grams/week)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Physical activity (sessions/week)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Energy intake (kcal/day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum low-density lipoprotein (mmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum high-density lipoprotein (mmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum triglyceride (mmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family history of diabetes (n, %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>History of hypertension (n, %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug for high cholesterol (n, %)</td>
</tr>
</tbody>
</table>
5 RESULTS

5.1 Baseline clinical characteristics

The characteristics of the participants by serum CRP and serum GGT quartiles are presented in Table 5 and 6 respectively. The mean age of the participant was 62.7 years (SD = 6.5, range = 53.3-73.6 years), the mean CRP concentration was 2.8 mg/L (SD = 4.9, range = 0.1-76.9 mg/L), and the mean GGT concentration was 27.2 U/L (SD = 34.1, range = 3 - 836 U/L). The subjects in high serum CRP group had a higher BMI, waist circumference and waist to hip ratio than the subjects in the low serum CRP group. Those subjects with higher serum CRP concentrations were more likely to have lower daily energy intake, fewer sessions of leisure-time physical activity per week, higher blood pressure and dyslipidemia. They also had higher serum glucose, insulin, ferritin and blood leucocyte concentrations. The mean GGT concentration increased with increasing serum CRP quartiles [22.8 U/L (SD = 43.6), 24.0 U/L (SD = 17.3), 27.0 U/L (SD = 23.0), 34.9 U/L (SD = 42.7), respectively, P-trend <0.001] (Table 5).

By serum GGT quartiles, subjects in the high serum GGT quartile were younger, had higher BMI, waist to hip ratio and waist girth. Subjects with higher serum GGT concentrations were more likely to have higher daily energy intake, alcohol intake, diastolic blood pressure and fewer sessions of leisure-time physical activity per week. They had dyslipidemia and were more likely to smoke more cigarettes, had higher serum glucose, insulin, ferritin and blood leucocyte concentrations. The mean CRP concentration increased with increasing serum GGT quartiles [2.0 mg/L (SD = 3.6), 2.2 mg/L (SD = 3.1), 2.9 mg/L (SD = 4.2), 4.2 mg/L (SD = 7.3), respectively, P-trend <0.001] (Table 6).
Table 5: Baseline characteristics by the quartiles of serum CRP in 1541 eastern Finnish men and women in the Kuopio Ischemic Heart Disease Risk Factor Study in 1998-2001

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum CRP quartiles (mg/L)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=389)</td>
<td>2 (n=380)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.9±6.4</td>
<td>62.8±6.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2±3.1</td>
<td>26.9±3.4</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.88±0.08</td>
<td>0.90±0.08</td>
</tr>
<tr>
<td>Mean waist (cm)</td>
<td>85.7±10.6</td>
<td>90.3±10.7</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1885±583</td>
<td>1841±547</td>
</tr>
<tr>
<td>Physical activity (sessions/week)</td>
<td>3.7± 3.0</td>
<td>3.6± 3.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132±17</td>
<td>136±16</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80±9</td>
<td>81±9</td>
</tr>
<tr>
<td>Alcohol intake (grams/week)</td>
<td>38.8±63.7</td>
<td>47.2±78.6</td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>2.2±8.8</td>
<td>2.8±9.6</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/L)</td>
<td>3.49±0.86</td>
<td>3.64±0.89</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
<td>1.31±0.31</td>
<td>1.27±0.31</td>
</tr>
<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>1.07±0.54</td>
<td>1.17±0.55</td>
</tr>
<tr>
<td>Serum GGT (U/L)</td>
<td>22.8±43.6</td>
<td>24.0±17.3</td>
</tr>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>4.7±0.41</td>
<td>4.8±0.44</td>
</tr>
<tr>
<td>Serum insulin (mU/L)</td>
<td>7.3±15.2</td>
<td>7.8±5.5</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>89.3±97.9</td>
<td>96.8±90.4</td>
</tr>
<tr>
<td>Blood Leucocytes (g/L)</td>
<td>4.65±1.14</td>
<td>4.96±1.18</td>
</tr>
</tbody>
</table>

Values are means (standard deviation).

CRP, C-reactive protein; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein

The term n is the number of subjects in each quartiles of serum CRP.
Table 6: Baseline characteristics by the quartiles of serum GGT in 1541 eastern Finnish men and women in the Kuopio Ischemic Heart Disease Risk Factor Study in 1998-2001.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum GGT quartiles (U/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n=389)</td>
<td>2 (n=372)</td>
<td>3 (n=407)</td>
<td>4 (n=373)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-14</td>
<td>15-19</td>
<td>20-29</td>
<td>30-836</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.2±6.5</td>
<td>63.0±6.5</td>
<td>62.7±6.5</td>
<td>61.8±6.4</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.1±4.1</td>
<td>27.3±4.2</td>
<td>27.9±4.2</td>
<td>28.8±4.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.85±0.07</td>
<td>0.89±0.08</td>
<td>0.91±0.08</td>
<td>0.94±0.07</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mean waist (cm)</td>
<td>85.6±10.8</td>
<td>90.1±11.0</td>
<td>93.6±11.9</td>
<td>97.4±11.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1725±559</td>
<td>1794±580</td>
<td>1845±538</td>
<td>1878±576</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Physical activity (sessions/week)</td>
<td>3.7±3.1</td>
<td>3.6±3.2</td>
<td>3.3±2.9</td>
<td>3.0±3.1</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134±17</td>
<td>135±18</td>
<td>136±17</td>
<td>135±17</td>
<td>0.341</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79±8</td>
<td>80±9</td>
<td>81±9</td>
<td>82±8</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake (grams/week)</td>
<td>18.6±39.1</td>
<td>33.3±62.5</td>
<td>42.0±65.9</td>
<td>96.6±156.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>1.9±7.4</td>
<td>2.9±9.9</td>
<td>2.7±10.5</td>
<td>4.9±12.8</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/L)</td>
<td>3.50±0.93</td>
<td>3.64±0.87</td>
<td>3.68±0.93</td>
<td>3.67±0.91</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
<td>1.32±0.32</td>
<td>1.26±0.31</td>
<td>1.23±0.29</td>
<td>1.22±0.30</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>1.07±0.49</td>
<td>1.16±0.56</td>
<td>1.27±0.61</td>
<td>1.42±0.74</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Serum CRP (mg/L)</td>
<td>2.0±3.6</td>
<td>2.2±3.1</td>
<td>2.9±4.2</td>
<td>4.2±7.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Serum glucose (mmol/L)</td>
<td>4.6±0.4</td>
<td>4.7±0.4</td>
<td>4.7±0.4</td>
<td>4.9±0.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Serum insulin (mU/L)</td>
<td>6.2±2.9</td>
<td>7.2±3.7</td>
<td>7.9±4.0</td>
<td>10.7±17.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>60.3±57.5</td>
<td>83.3±70.1</td>
<td>101.8±85.2</td>
<td>143.5±140.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Blood Leucocytes (g/L)</td>
<td>4.87±1.25</td>
<td>5.00±1.24</td>
<td>5.14±1.33</td>
<td>5.44±1.45</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (standard deviation).

CRP, C-reactive protein; GGT, gamma-glutamyl transferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein

The term n is the number of subjects in each quartiles of serum GGT.
5.2  Association between serum GGT and serum CRP concentrations

In linear regression analysis, a direct association was observed between serum GGT (10 U/L, log transformed) and serum CRP, even after adjustment for possible confounders [β=0.30, 95% CI 0.20-0.39, P<0.001 (Table 7, Model 3^b)].

Table 7: Regression coefficients of serum GGT (10 U/L) predicting serum CRP in multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2^a</th>
<th>Model 2^b</th>
<th>Model 3^a</th>
<th>Model 3^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficient (β)</td>
<td>0.49 (95% CI= 0.40-0.57)</td>
<td>0.44 (95% CI= 0.34-0.53)</td>
<td>0.29 (95% CI= 0.20-0.38)</td>
<td>0.38 (95% CI= 0.29-0.48)</td>
<td>0.30 (95% CI= 0.20-0.39)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age, gender and date of examination
Model 2^a, adjusted for Model 1 plus smoking (pack/years), alcohol intake (grams/week), physical activity (sessions/ year) and energy intake
Model 2^b, adjusted for Model 2^a plus body mass index
Model 3^a, adjusted for Model 2^a plus serum low-density lipoprotein, serum high-density lipoprotein, serum triglyceride, drug for high cholesterol, family history of diabetes and history of hypertension
Model 3^b adjusted for Model 3^a plus body mass index
Serum GGT and serum CRP were log-transformed
CRP, C-reactive protein; GGT, gamma-glutamyl transferase
5.3 Association between serum CRP concentration and incident type 2 diabetes

During the average follow-up of 7.3-year, 206 out of 1541 subjects developed T2D. Out of which, 105 cases were female and 101 cases were male. Table 8 presents the adjusted HRs of incident T2D according to the quartiles of serum CRP in Cox-proportional hazard regression models. In model 1, subjects in the highest quartile of serum CRP had 2.1 times higher risk of developing T2D than those in lowest quartile (HR 2.12, 95% CI=1.42-3.17, P for trend <0.001). Although the association was attenuated with the introduction of other confounders into the models, the model still showed a significant association between highest quartile of serum CRP and incident T2D (HR 1.76, 95% CI=1.13-2.74, P for trend 0.008, Model 3a). Subjects in the fourth quartile of serum CRP showed 76% increased risk of T2D compared with those in the lowest quartile. Further adjustment with BMI (Model 3b) attenuated the risk of T2D in the fourth quartile of model 3a by approximately 78 % (HR 1.17, 95% CI=0.73-1.89, P for trend 0.485).
Table 8: The risk of type 2 diabetes during the average follow-up of 7.3 years according to the quartiles of serum CRP concentration.

<table>
<thead>
<tr>
<th>CRP quartiles (mg/L)</th>
<th>Model 1</th>
<th>Model 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0.10-0.75)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>2 (0.76-1.49)</td>
<td>1.22 (95% CI=0.78-1.90)</td>
<td>1.34 (95% CI=0.84-2.13)</td>
<td>1.11 (95% CI=0.70-1.78)</td>
<td>1.25 (95% CI=0.79-2.00)</td>
<td>1.10 (95% CI=0.69-1.76)</td>
</tr>
<tr>
<td>3 (1.50-3.05)</td>
<td>1.72 (95% CI=1.14-2.59)</td>
<td>1.89 (95% CI=1.22-2.93)</td>
<td>1.31 (95% CI=0.83-2.06)</td>
<td>1.51 (95% CI=0.97-2.35)</td>
<td>1.19 (95% CI=0.75-1.87)</td>
</tr>
<tr>
<td>4 (3.06-76.90)</td>
<td>2.12 (95% CI=1.42-3.17)</td>
<td>2.29 (95% CI=1.48-3.53)</td>
<td>1.27 (95% CI=0.79-2.04)</td>
<td>1.76 (95% CI=1.13-2.74)</td>
<td>1.17 (95% CI=0.73-1.89)</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age, gender and date of examination

Model 2<sup>a</sup>, adjusted for Model 1 plus smoking (pack/years), alcohol intake (grams/week), physical activity (sessions/ year) and energy intake

Model 2<sup>b</sup>, adjusted for Model 2<sup>a</sup> plus body mass index

Model 3<sup>a</sup>, adjusted for Model 2<sup>a</sup> plus serum low-density lipoprotein, serum high-density lipoprotein, serum triglyceride, drug for high cholesterol, family history of diabetes and history of hypertension

Model 3<sup>b</sup> adjusted for Model 3<sup>a</sup> plus body mass index

CRP, C-reactive protein
5.4 The influence of serum GGT concentration on the association between serum CRP concentration and type 2 diabetes incidence

In Cox-proportional hazard regression models to observe the influence of serum GGT concentration on the association between serum CRP and incident T2D, risk of T2D in the highest CRP quartile decreased with introduction of serum GGT (HR 1.14, 95% CI=0.71-1.84 and P for trend 0.535, Model 2\(^b\), Table 9). Likewise, the risk of T2D in the highest quartile of serum CRP in model 3\(^a\) was attenuated by approximately 70% with introduction of serum GGT when compared with the highest CRP group in model 3\(^b\) (HR 1.05, 95% CI= 0.65-1.70 and P for trend 0.829).

Moreover, when interaction test was performed between serum GGT and quartiles of serum CRP, a statistically significant interaction was observed between serum GGT and the highest quartile of serum CRP (HR 1.94, 95% CI= 1.03-3.65 and P for trend 0.038).

When serum GGT was divided into low serum GGT and high serum GGT groups based on the median value of GGT (20 U/L), 125 subjects in High serum GGT group and 81 subjects in the Low serum GGT group developed T2D. As shown in Table 10, the association between serum CRP and T2D risk was stronger among subjects in the highest group of CRP who had serum GGT concentrations above the median value of serum GGT (HR 2.02, 95% CI=1.12-3.66 and P for trend 0.004, Model 2\(^a\)).
Table 9: The influence of serum GGT concentration on the association between serum CRP concentration and incident type 2 diabetes.

<table>
<thead>
<tr>
<th>CRP quartiles (mg/L)</th>
<th>Model 1</th>
<th>Model 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 3&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0.10-0.75)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>2 (0.76-1.49)</td>
<td>1.22 (95% CI=0.78-1.90)</td>
<td>1.11 (95% CI=0.70-1.78)</td>
<td>1.08 (95% CI=0.68-1.73)</td>
<td>1.10 (95% CI=0.69-1.76)</td>
<td>1.06 (95% CI=0.66-1.70)</td>
</tr>
<tr>
<td>3 (1.50-3.05)</td>
<td>1.72 (95% CI=1.14-2.59)</td>
<td>1.31 (95% CI=0.83-2.06)</td>
<td>1.23 (95% CI=0.78-1.93)</td>
<td>1.19 (95% CI=0.75-1.87)</td>
<td>1.12 (95% CI=0.71-1.77)</td>
</tr>
<tr>
<td>4 (3.06-76.90)</td>
<td>2.12 (95% CI=1.42-3.17)</td>
<td>1.27 (95% CI=0.79-2.04)</td>
<td>1.14 (95% CI=0.71-1.84)</td>
<td>1.17 (95% CI=0.73-1.89)</td>
<td>1.05 (95% CI=0.65-1.70)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.001</td>
<td>0.266</td>
<td>0.535</td>
<td>0.485</td>
<td>0.829</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age, gender and date of examination

Model 2<sup>a</sup>, adjusted for Model 1 plus smoking (pack/years), alcohol intake (grams/week), physical activity (sessions/ year), energy intake and body mass index

Model 2<sup>b</sup>, adjusted for Model 2<sup>a</sup> plus gamma-glutamyl transferase

Model 3<sup>a</sup>, adjusted for Model 2<sup>a</sup> plus serum low-density lipoprotein, serum high-density lipoprotein, serum triglyceride, drug for high cholesterol,

family history of diabetes and history of hypertension

Model 3<sup>b</sup> adjusted for Model 3<sup>a</sup> plus gamma-glutamyl transferase

Serum GGT was log- transformed

CRP, C-reactive protein; GGT, gamma-glutamyl transferase
Table 10: Association between serum CRP concentration and incident type 2 diabetes by median value of serum GGT concentration.

<table>
<thead>
<tr>
<th>Serum CRP quartiles (mg/L)</th>
<th>HR</th>
<th>HR</th>
<th>HR</th>
<th>HR</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0.10-0.75)</td>
<td>1.00 (reference)</td>
<td>1.40 (95% CI=0.76-2.60)</td>
<td>2.00 (95% CI=1.10-3.64)</td>
<td>1.45 (95% CI=0.72-2.92)</td>
<td>0.101</td>
</tr>
<tr>
<td>2 (0.76-1.49)</td>
<td>1.00 (reference)</td>
<td>1.55 (95% CI=0.82-2.93)</td>
<td>2.23 (95% CI=1.20-4.15)</td>
<td>1.63 (95% CI=0.79-3.33)</td>
<td>0.057</td>
</tr>
<tr>
<td>3 (1.50-3.05)</td>
<td>1.00 (reference)</td>
<td>1.31 (95% CI=0.68-2.50)</td>
<td>1.63 (95% CI=0.85-3.14)</td>
<td>1.07 (95% CI=0.49-2.31)</td>
<td>0.644</td>
</tr>
<tr>
<td>4 (3.06-76.90)</td>
<td>1.00 (reference)</td>
<td>1.50 (95% CI=0.79-2.86)</td>
<td>1.98 (95% CI=1.06-3.72)</td>
<td>1.56 (95% CI=0.75-3.23)</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>1.00 (reference)</td>
<td>1.33 (95% CI=0.70-2.55)</td>
<td>1.61 (95% CI=0.84-3.10)</td>
<td>1.12 (95% CI=0.51-2.45)</td>
<td>0.558</td>
</tr>
</tbody>
</table>

**Low serum GGT, n=817**

- Model 1
- Model 2\(^a\)
- Model 2\(^b\)
- Model 3\(^a\)
- Model 3\(^b\)

**High serum GGT, n=724**

- Model 1
- Model 2\(^a\)
- Model 2\(^b\)
- Model 3\(^a\)
- Model 3\(^b\)

Low serum GGT value \(\leq 20\) U/L, High serum GGT value >20 U/L

Model 1, adjusted for age, gender and date of examination
Model 2\(^a\), adjusted for Model 1 plus smoking (pack/years), alcohol intake (grams/week), physical activity (sessions/year) and energy intake
Model 2\(^b\), adjusted for Model 2\(^a\) plus body mass index
Model 3\(^a\), adjusted for Model 2\(^a\) plus serum low-density lipoprotein, serum high-density lipoprotein, serum triglyceride, drug for high cholesterol, family history of diabetes and history of hypertension
Model 3\(^b\), adjusted for Model 3\(^a\) plus body mass index
CRP, C-reactive protein; GGT, gamma-glutamyl transferase

The term n is the number of subjects in each group of serum GGT.
6 DISCUSSION

6.1 Summary of the main findings
In this prospective study, a direct association was observed between serum GGT concentrations and serum CRP concentrations even after adjustment for possible confounders. A positive association was found between baseline serum CRP and T2D incidence. This association to some extent was explained by BMI and serum GGT. After further adjustment for known risk factors of T2D, subjects in the highest serum CRP group had 17% higher risk of developing T2D compared with subjects in the lowest group. When interaction test was performed, the effect of serum GGT was significant only for the highest serum CRP group, further suggesting that serum GGT contributed to the association observed between serum CRP and T2D risk. Furthermore, the association between serum CRP and incident T2D was stronger among subjects with serum GGT concentrations above the median for the cohort compared with subjects who had serum GGT concentrations below the median value for the cohort. These results suggest that BMI and serum GGT to some extent explain the association between serum CRP and T2D incidence.

Cross-sectional studies by (Lee & Jacobs 2005, Wen et al. 2010) showed positive association between serum GGT and serum CRP. Prospective Coronary Artery Risk Development in Young Adults (CARDIA) study (Lee et al. 2003b) showed that serum GGT levels predicted the concentrations of serum CRP. Our findings are consistent with these earlier studies. These observations suggest that there is some linkage between serum GGT, CRP and T2D. Previous studies that investigated the associations between serum CRP and T2D among Finnish population (Laaksonen et al. 2004, Hu et al. 2009) showed CRP as a significant predictor of T2D incidence after adjusting for obesity indexes like BMI and WHR. Although, the association was attenuated as also observed in this study. In several other studies (Duncan et al. 2003, Lee et al. 2009, Bertoni et al. 2010, Effoe et al. 2015), CRP was not a significant predictor of T2D incidence after adjustment for obesity indexes. Our findings are consistent with these studies. BMI is a known predictor of T2D risk, and in this study, it explained about 78% risk reduction among subjects in the highest group of serum CRP in model adjusted for BMI. The association between serum CRP and T2D incidence was statistically insignificant after additional adjustment for serum GGT in the model that is in line with previous studies (Lee et al. 2009, Wen et al. 2010) which showed similar result. Furthermore, this study also showed the influence of serum GGT on the association between serum CRP and T2D risk. About a half of the association between serum CRP and T2D risk among
Subjects in the highest quartile of serum CRP was due to the contribution of serum GGT. This suggests that serum GGT plays an important role in the association between serum CRP concentrations and T2D incidence.

Previous studies have shown that raised levels of serum CRP are associated with obesity and insulin resistance (Festa et al. 2000, Kahn et al. 2006). One of the studies demonstrated that reduction in weight lowered serum CRP levels (Heilbronn et al. 2001). Thus, obesity-triggered inflammation could play an important role in the pathogenesis of T2D. Low grade inflammation may stimulate insulin resistance through several mechanisms, including activation of signaling cascades (Zick 2003), increased production of ROS (Brownlee 2001) and decreased expression of endothelial nitric oxide synthase (Venugopal et al. 2002). Inflammation activates serine/threonine kinases like JNK, IKK and inhibit insulin signaling resulting diabetes (Zick 2003). High dose aspirin, a non-steroidal anti-inflammatory drug has been shown to improve glucose tolerance and insulin sensitivity in patients with T2D (Hundal et al. 2002), probably by inhibiting the serine kinase and IkB kinase (Yuan et al. 2001).

White adipose tissues are active endocrine tissues that produces a variety of proteins known as adipokines. Adipokines consist of leptin, adiponectin, IL-6, TNF-α, IL-1β, monocyte chemotactic protein-1, vascular endothelial growth factor, nerve growth factor, plasminogen activator inhibitor 1 and haptoglobin, which are associated with inflammation and the inflammatory response (Trayhurn & Wood 2005). Obesity is regarded as a low-grade inflammatory disease (Fantuzzi 2005, Greenberg & Obin 2006) with an increased circulation of numerous inflammatory markers: CRP, TNF-α, IL-6, IL-18 and haptoglobin (Yudkin 2003, Trayhurn & Wood 2004). The increased production of inflammatory adipokines is highly associated with the progression of diseases linked to obesity, particularly T2D (Hotamisligil 2003, Yudkin 2003). Thus, it is expected that overweight or obesity attenuates the association between serum CRP and T2D, since adipose tissue is a main source of pro-inflammatory cytokines such as IL-6 and TNF-α.

Serum GGT is regarded as a marker of alcohol abuse and hepatobiliary disorder. However, neither alcohol consumption nor liver damage explains the association of serum GGT with T2D incidence in (Perry et al. 1998, Lee et al. 2003a and Lee et al. 2003b) studies. Several possible mechanisms explain how serum GGT concentration is linked with the development of T2D. First, high serum GGT levels may indicate fatty liver which causes hepatic insulin resistance and further systemic
insulin resistance (Marchesini et al. 2001, Chitturi et al. 2002). Second, serum GGT plays an important role in the defensive response to oxidative stress. Oxidative stress and systemic inflammation lead to impaired insulin secretion and insulin resistance (Hotamisligil 2003, Ceriello & Motz 2004). It is considered that oxidative stress initiates inflammatory response through chromatin modelling and activation of transcription factors leading to gene expression of pro-inflammatory mediators. It is also assumed that an increase in serum GGT might occur prior to the rise in serum CRP concentrations (Lee & Jacobs 2005).

GGT maintains cellular redox homeostasis by balancing the antioxidant molecule GSH at the cellular levels (Pompella et al. 2006). GGT activity is increased in response to oxidative stress consequently increasing transportation of glutathione into cells. GGT is also involved in the production of ROS in the presence of iron or other transition metals (Drozdz1 et al. 1998). ROS is associated with the inflammatory process and the development of T2D (Ali et al. 2016). Therefore, in addition to elevated serum GGT level, high body iron may be associated with the pathogenesis of T2D. Moreover, serum GGT concentrations predicted the concentration of oxidative stress and inflammation markers such as fibrinogen, uric acid, CRP, and F2-isoprostanes in the CARDIA study (Lee et al. 2003b). These explanations suggest that serum GGT may be considered as an important marker of oxidative stress and inflammation associated with the development of T2D.

6.2 Strengths of the study
This study was carried out in a relatively homogenous population. The study was relatively large which enhanced the power of the analysis. Also, this study adjusted for many of the possible confounders of T2D, thus reducing the confounding effect of these factors on the association. Non-differential misclassification of cases might have been decreased due to extensive ascertainment of T2D diagnosis. The diagnosis of T2D in this study was not only based on blood glucose measurements or self-reported previous physician diagnosis of T2D, but were further confirmed from hospital discharge records and from the medication register of Social Insurance Institution of Finland through the Finnish personal identity code.

6.3 Limitations of the study
Single measurement of serum CRP and serum GGT might not reflect the actual concentrations of these biomarkers overtime, which may influence on the strength of the association observed. The complete exclusion of the effects of residual confounding was impossible. Other pro-inflammatory
adipocytokines (IL-6 and TNF-α) which contribute to serum CRP concentrations were not investigated which may have affected the strength of the association between serum CRP and T2D risk.

7 CONCLUSION
In this study serum GGT concentration was directly associated with serum CRP concentration. Raised concentration of serum CRP was associated with an increased risk of T2D in the middle-aged men and women, but this association was largely dependent on BMI and serum GGT. This association was insignificant particularly after adjustment for BMI and serum GGT. The association between serum CRP and T2D incidence was stronger among subjects with higher concentration of serum GGT compared with those with lower serum GGT concentration. These observations suggest that the association between serum CRP and T2D incidence is modified by BMI and serum GGT in the middle-aged and aging population. Further prospective studies in other populations are warranted to confirm these findings.

8 RECOMMENDATIONS
These findings may have important implications for the prevention and treatment of T2D. High risk populations for development of T2D may be recognized by measuring serum CRP and serum GGT concentrations in overweight healthy subjects. Lifestyle interventions such as weight loss and exercise are effective in reducing inflammation (Nicklas 2005). As shown in this study, the subjects in high serum CRP group had a higher BMI, waist circumference and WHR than the subjects in the low serum CRP group. Hence, lifestyle changes such as increasing physical activity and decreasing energy intake may lower serum CRP concentrations, thus reducing the risk of developing T2D later in life. Other inflammatory biomarkers which are known to contribute to serum CRP concentration should be investigated. Studies that examine the influence of those other biomarkers on the association between serum CRP and T2D is urgently needed.
9 REFERENCES


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