Investigating Serum Dairy Fatty Acid Biomarkers and their Association with Type 2 Diabetes and its Underlying Disorders

by

Ingrid Dominique Santaren

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Abstract

Growing evidence suggests that dairy intake is inversely associated with type 2 diabetes risk, however current literature is inconclusive and few studies have evaluated dairy’s effects on the underlying disorders of the disease. This thesis aimed to investigate the relationship between the dairy specific serum fatty acid biomarkers pentadecanoic acid (15:0) and trans-palmitoleic acid (trans 16:1n-7) with incident type 2 diabetes, as well as associations with insulin sensitivity, beta-cell function, and markers of chronic systemic inflammation in the large multi-ethnic Insulin Resistance Atherosclerosis Study (IRAS). This study found that 15:0, a marker of dairy intake in the IRAS cohort, was inversely associated with incident diabetes after 5 years; positively associated with insulin sensitivity and beta-cell function; and negatively associated with plasminogen activator inhibitor-1, independent of demographic, lifestyle, and dietary variables. This study extends current literature pointing to a possible protective role of dairy intake on cardiometabolic risk.
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**Figure 4-1**: Study population flowchart
List of Abbreviations

BMI  Body mass index
CRP  C-reactive protein
CVD  Cardiovascular disease
DI   Disposition index
FFQ  Food frequency questionnaire
FSIGT Frequently sampled intravenous glucose tolerance test
HF   High fat
IGT  Impaired glucose tolerance
IQR  Interquartile range
IRAS Insulin Resistance Atherosclerosis Study
LF   Low fat
NEFA Non-esterified fatty acids
OGTT Oral glucose tolerance test
PAI-1 Plasminogen activator inhibitor-1
SFA  Saturated fatty acid
S_i Insulin sensitivity index
T2DM Type 2 Diabetes Mellitus
TFA  Trans fatty acid
TNF-α Tumor necrosis factor-α
Statement of Contributions

In this thesis, I performed the literature review, identified knowledge gaps, formulated the research questions and study objectives, statistically analyzed all data, interpreted the results, wrote abstracts and presented the current study at local and international conferences (1 oral presentation and 2 posters), wrote a manuscript (1 paper in press) and the current thesis document.
Chapter 1
Introduction

Type 2 diabetes (T2DM), a growing worldwide pandemic, is a major cause of morbidity and mortality and carries considerable direct and indirect economic costs (Chapter 2.1) (1). Although T2DM can be managed using an array of clinical approaches, the disease is progressive and burdensome complications ultimately develop in the majority of individuals with T2DM. In this context, interventions targeting modifiable factors, such as diet, have the potential to be easily translatable and cost-effective primary prevention strategies.

In the past few years, increasing evidence has pointed to the potential protective role of dairy intake on T2DM risk. Meta-analyses of prospective studies have consistently reported a decreased T2DM risk with higher intakes of dairy (Chapter 2.5) (2-4). While most studies have observed an inverse association between dairy intake and T2DM risk, individual observational studies have reported inconsistent results (2). Heterogeneity in published studies may be partly due to the use of self-reported questionnaires to measure dairy intake, which are prone to measurement error. In addition, it is currently unclear though which pathway dairy intake affects T2DM risk, as few studies have evaluated dairy’s effects on the underlying pathophysiology of T2DM.

The particular components of dairy which may contribute to its inverse relationship with T2DM are still unclear. Dairy is a complex food matrix composed of different nutrients, including protein, micronutrients, and lipids (Chapter 2.4.2) (5). Dairy is rich in saturated and trans fatty acids, and with emerging evidence showing a reduced cardiometabolic risk associated with dairy intake, recent studies suggest that different types of these fatty acids may affect cardiometabolic risk differently, with dairy fatty acids potentially having a protective role (6-8).

Dairy specific fatty acids have been validated and used as biomarkers for dairy intake in observational studies (Chapter 2.6) (9-17). Biomarkers provide more objective measures of dairy intake compared to questionnaires, as they overcome some of the limitations associated with self-reported dietary questionnaires. However, existing observational studies that have utilized dairy fatty biomarkers in the evaluation of T2DM risk have shown inconsistent results, with some studies reporting a decrease in T2DM risk (16-18), and others showing no association
(Chapter 2.5.2) (19-21). More importantly, there is insufficient understanding on the potential association of dairy fatty acids in the etiology of T2DM’s underlying disorders of insulin resistance and beta-cell dysfunction (16, 22, 23)

Dairy fatty acids can potentially have effects on chronic low-grade systemic inflammation, a major risk factor for T2DM and CVD. Three cross-sectional studies have reported reductions in circulating inflammatory markers with higher dairy intakes. However, these observational studies were conducted in European and Iranian populations (24-26), therefore further evaluation of dairy intake on inflammatory markers in a multi-ethnic North American population would allow for a broader generalizability of study findings (Chapter 2.5.4). Furthermore, observational studies utilizing dairy fatty acid biomarkers have only examined the associations with two inflammation markers, namely CRP and fibrinogen (Chapters 2.7.2 and 2.8.2) (16, 17, 27). It is clear that further studies on this topic are warranted, specifically aimed at exploring the association between dairy fatty acid biomarkers with a variety inflammatory proteins.

In the light of the current literature and the prevailing gaps in knowledge surrounding the link between dairy intake and T2DM outcomes, the aim of this thesis was to investigate the association between two dairy fatty acid biomarkers, pentadecanoic acid (15:0) and \textit{trans}-palmitoleic acid (\textit{trans} 16:1n-7) with incident diabetes at 5 years, as well as directly measured insulin resistance and beta-cell dysfunction (Chapter 3), and markers of subclinical inflammation (CRP, TNF-\(\alpha\), PAI-1, and fibrinogen) (Chapter 4), in a large multi-ethnic cohort free of T2DM at baseline.
Chapter 2
Literature Review

2.1 Type 2 Diabetes: prevalence and burden of disease

Type 2 diabetes mellitus (T2DM) is a metabolic disease marked by hyperglycemia that arises due to defects in insulin secretion and insulin action (28). Chronic elevations in blood glucose are associated with long-term macrovascular and microvascular complications and premature mortality (28, 29). T2DM is an increasing health burden worldwide (30). In 2013, the global estimate for numbers of people with diabetes was 382 million, and approximately 5.1 million deaths in 2013 were attributable to diabetes (1). T2DM accounts for 90-95% of diabetes cases (28). T2DM is associated with burdensome complications and comorbidities such as cardiovascular disease (CVD), hypertension, dyslipidemia, nephropathy, retinopathy and neuropathy (28, 31).

2.2 Pathophysiology of T2DM

2.2.1 Insulin resistance and beta-cell dysfunction as underlying disorders

While the pathogenesis of T2DM is complex, it is accepted that hyperglycemia in T2DM arises from a combination of longstanding insulin resistance and pancreatic beta-cell dysfunction (28). Resistance to insulin action occurs in various tissues throughout the body, including skeletal muscle, liver, pancreas and adipocytes (32), and manifests as an impairment in insulin-mediated glucose uptake in target tissues (33), as well as impairment in the suppression of hepatic glucose production (34). In addition, increasing beta-cell dysfunction occurs during the evolution of T2DM, as the beta-cells are unable to augment insulin secretion to compensate for the increasing insulin resistance (33). The relationship between declining beta-cell function and increasing insulin resistance may be quantified using the disposition index (DI), which can be depicted graphically through a hyperbolic curve (35). DI is an integrated measure of beta-cell function, reflecting the ability of the beta-cells to upregulate insulin secretion in order to compensate for increasing insulin resistance, and is calculated as the product of insulin sensitivity and insulin secretion. In the early stages of T2DM development, insulin resistance increases and while there
may be concomitant increases in beta-cell activity, the degree of insulin secretion is ultimately inadequate to compensate, and dysglycemia consequently develops.

2.3 Risk factors for T2DM

T2DM is a multifactorial disease arising from a combination of non-modifiable and modifiable factors (36).

2.3.1 Genetic determinants of T2DM

A large body of evidence points to a genetic component in the etiology of T2DM (37). In the Framingham Offspring Study, it was calculated that the lifetime risk of developing T2DM was 3.5-fold and 6-fold higher in individuals with one or two parents with T2DM, respectively (38), compared to those without a family history of the disease. The risk of T2DM also differs by ethnicity, with African Americans and Hispanic Americans having higher prevalence of T2DM; although some of this increased risk is undoubtedly the result of environmental factors, genetic aspects are also likely at play (39). Over the past several years, genome-wide association studies (GWAS) have identified more than 70 single nucleotide polymorphisms (SNPs) found in more than 40 genomic regions associated with T2DM (40). Many of these SNPs and regions have relevance to beta-cell development and function (41).

2.3.2 Chronic low-grade systemic inflammation and T2DM risk

Low-grade systemic inflammation is now recognized as a significant factor in the etiology and progression of T2DM (42-44), and is a common underlying mediator linking obesity, T2DM, and CVD (45). Inflammatory markers may induce insulin resistance in skeletal muscle, adipose tissue, and other insulin sensitive tissues through activation and upregulation of serine kinases such as Jun N-terminal kinase (JNK), which are involved the phosphorylation of insulin receptors and insulin receptor substrate (IRS) proteins, and thus disrupting normal insulin action (46). Inflammatory markers have also been implicated in beta-cell function inhibition and apoptosis (47).

A number of cross-sectional studies have confirmed higher concentrations of circulating inflammatory markers in newly diagnosed or established diabetes states compared to control subjects (43). Cross-sectional studies in populations without diabetes or with impaired glucose
tolerance (IGT)/impaired fasting glucose (IFG) have also demonstrated positive associations of inflammatory markers with insulin resistance, BMI, waist circumference, and other components of the metabolic syndrome (43). Furthermore, increased markers of inflammation have been prospectively associated with T2DM and CVD incidence in various cohorts (44, 48-50). Moreover, recent large trials have tested the efficacy of lowering inflammation on cardiometabolic outcomes. For example, the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) reported reductions in C-reactive protein (CRP) concentrations, as well as CVD events and mortality with rosuvastatin treatment (51).

While a large number of inflammatory markers are available, there is no agreed upon optimal marker or set of markers for measuring chronic subclinical inflammation (52). The following section will briefly review a subset of inflammatory markers which are relevant to the current thesis: CRP, tumor necrosis factor (TNF-α), plasminogen activator inhibitor-1 (PAI-1), and fibrinogen.

CRP is a liver-derived acute phase protein that is recognized as a sensitive biomarker for systemic inflammation (48). Cross-sectional studies have shown elevated levels of CRP in T2DM and metabolic syndrome (44) and a recent meta-analysis of prospective studies also found high CRP concentrations to be associated with an increased T2DM risk (48). TNF-α is a pro-inflammatory cytokine (53) produced and secreted mainly by adipocytes (54, 55) and is also produced by activated macrophages, lymphocytes (55, 56), and endothelial cells (57). High TNF-α concentrations have been associated with T2DM (58, 59), and insulin resistance (58, 60). PAI-1, a marker of fibrinolysis, coagulation (61), and inflammation (62), is expressed in a variety of cells including endothelial cells, hepatocytes and adipocytes (63). Physiologically, PAI-1 acts as an inhibitor of fibrinolysis (64). Elevated PAI-1 levels have been observed in individuals with insulin resistance and impaired glucose tolerance cross-sectionally (65-67), and have been shown to be a significant predictor of T2DM (49, 61). Fibrinogen, another coagulation and fibrinolytic factor, is an acute phase protein produced in the liver (68), through stimulation by cytokines (43). Observational studies have reported that high fibrinogen levels predict T2DM (49, 50).

2.3.3 Lifestyle and dietary risk factors for T2DM

Trials of long-term intensive lifestyle modification programs involving individuals at high risk for T2DM have found significant reductions in progression to T2DM compared to standard
advice: a 34% reduction after 10 years in the U.S. Diabetes Prevention Program Outcomes Study (DPPOS) (69), a 43% reduction after 7 years in the Finnish Diabetes Prevention Study (DPS) (70), and a 43% reduction after 20 years in the Da Qing study (71). Modifiable lifestyle factors that have been linked with T2DM risk include obesity, smoking, physical activity, alcohol consumption, and diet (72). Certain dietary factors, in particular, have been well-established to modify T2DM risk, including whole grains (73), red meat (74), and overall dietary patterns (75). Fat consumption has also been investigated in relation to T2DM. Cross-sectional studies have found higher relative intakes of total fat and saturated fat (SFA) in recently diagnosed T2DM compared to control subjects, and high proportions of SFA associated with insulin resistance (76). Results of prospective studies on total fat consumption, however, are inconsistent (77, 78).

2.3.4 Role of total non-esterified fatty acids on T2DM risk and its underlying disorders

Elevated levels of non-esterified fatty acids (NEFA) in circulation are observed in obesity and T2DM (79). Prospective studies have shown that high NEFA levels are predictive of a higher risk of conversion to T2DM (80, 81), and experimental studies demonstrated a dose-dependent relationship of increasing insulin resistance with increasing plasma NEFA in healthy subjects (82, 83). While acute elevations in NEFA increase glucose stimulated insulin secretion (GSIS), desensitization of GSIS due to sustained high NEFA levels has been observed in humans (84), especially in individuals at risk (85). In vitro and animal studies indicate that lipotoxicity due to chronic elevations in circulating NEFA may contribute to insulin resistance through interruptions of insulin signaling at the level of glucose transport and utilization, such as reduced GLUT-4 translocation (79). Furthermore, long-term NEFA exposure and islet lipid accumulation may induce beta-cell dysfunction through increased nitric oxide production or downregulation of Akt phosphorylation, which induces apoptosis in beta-cells (79, 86).

2.3.4.1 Saturated fatty acid intake

Raised serum SFA are associated with higher fasting insulin and glucose levels cross-sectionally (87), and increased T2DM risk prospectively (76). Previous studies have demonstrated potential mechanisms for direct effects of SFA on insulin resistance and beta-cell dysfunction in vivo and in vitro. Mechanisms include increased expression of inflammatory markers and macrophage infiltration in adipose tissue, and decreased phosphorylation of the insulin receptor and insulin
receptor substrate-1, decreased PPARγ coactivator-1α activation (reduced fatty acid and glucose oxidation), and accumulation of inflammatory markers, diacylglycerol, and ceramide in muscle (88).

2.3.4.2 Trans fatty acid intake

Current literature on trans fat (TFA) intake and risk of T2DM and insulin resistance is limited and inconsistent. Incident T2DM risk in relation to TFA consumption has been evaluated in 3 prospective cohorts. In the Nurses’ Health Study, a 31% increase in risk of T2DM was reported for those in the highest quintile of TFA intake, with stronger associations in women who were obese or had low physical activity (77). The other 2 prospective studies based on the Iowa Women’s Health Study cohort (89) and Health Professionals Follow-up Study (90) did not find an association of TFA intake with T2DM risk in fully adjusted models. Part of the inconsistency in the literature may be due to poor characterization of TFA in both food frequency questionnaires, as well as in the databases that generate intake levels (15). While the exact mechanisms underlying the effects on T2DM are not yet clear, animal studies indicate that TFA intake increases postprandial insulin and glucose, and reduced Akt activation in muscle and adipose tissue (91).

2.4 Dairy as a nutritional factor in T2DM etiology

2.4.1 Consumption pattern

Dairy products, which are nutrient-dense foods, are considered part of a healthy eating pattern and the current Dietary Guidelines for Americans (2010) recommends 3 cups per day of milk and milk products for adults (92). In the United States, it was reported that almost half of all dairy intake is consumed as cheese, largely as full-fat cheese. Fluid milk intake was mainly consumed as reduced fat (2%) or whole (full-fat) milk, while fat-free (skim) or low-fat (1%) milk were consumed in lower amounts (92). Overall, Americans adults are not meeting the recommended amounts of dairy intake, with intakes averaging at 1.8 servings per day, just over half of the recommended servings (93). Furthermore, based on NHANES 2005-2006 data, dairy foods were a significant contributor to total SFA in the U.S. diet: regular cheese contributed to 8.5%, dairy desserts contributed to 5.6%, reduced-fat milk contributed to 3.9%, whole milk contributed to 3.4%, and butter contributed to 2.9% of total SFA consumption in the U.S. diet.
among those aged 2 and over (92). Total SFA contributed about 11% of total calories in the diet, which is above the recommended goal of 10% (92). As such, the guideline also advises individuals to opt for fat-free or low-fat dairy products to reduce SFA and TFA consumption, and overall caloric load (92).

2.4.2 Nutritional composition of dairy

Dairy foods contain a wide variety of nutrients, contributing about 70% of calcium, 30% of phosphorus, 16% of potassium, 14% of the magnesium, 25% of the riboflavin, 15% of the zinc, and 18% of the vitamin B12 in the U.S. food supply (94). Also, dairy products are the most significant dietary sources of vitamin D (fortified), calcium, potassium, and magnesium nutrients, overall intakes of which are considered inadequate in the U.S. diet (92, 95).

2.4.2.1 Fatty acid profile

Fat is the major energy component in milk (96), constituting about 3.5 to 4.6% of energy from milk, and is present as microscopic globules emulsified in the aqueous phase (6). Milk fat content and fatty acid composition may vary depending on the cow breed, diet, and stage of lactation (97). More than 400 different fatty acids have been identified in milk (6). These fatty acids vary from 4-24 carbons in length (98), and include saturated, trans, monounsaturated, and polyunsaturated fatty acids (96). The majority of the fatty acids are esterified to triacylglycerols (~95%), while the remainder are esterified to phospholipids, cholesterol, diacylglycerols, monoacylglycerols or present as NEFA (96). SFA make up about 60-75% (15, 97), while TFA compose 2-5% of total fatty acids (99).

2.5 Role of dairy consumption in the etiology of T2DM and its underlying disorders

2.5.1 Potential mechanisms for dairy’s role in T2DM etiology

As a complex food with a wide array of nutrients, dairy foods contain a variety of components that may potentially have beneficial effects on cardiometabolic risk, including calcium, vitamin D, protein, magnesium, and fatty acids. In vitro and animal studies have shown that calcium increases insulin secretion in beta-cells, and is essential for insulin-responsive tissues, including the adipose tissue and skeletal muscle (100). Calcium may also suppress adipose tissue oxidative and inflammatory stress (101), and increase weight and fat loss in energy restriction (102, 103).
and fat oxidation (104). Mouse studies have also reported reduced cytokine expression in adipocytes with high calcium intakes (101, 105). Vitamin D may improve insulin resistance by stimulating insulin receptor expression, thus increasing insulin responsiveness for glucose uptake (100). Vitamin D may mediate direct effects on the beta-cell by binding to the vitamin D receptor, or have indirect effects through its role in the regulation of calcium flux in the beta-cell (100). In animal models, whey protein has been shown to decrease weight gain and improve insulin sensitivity (106). Lactoferrin, a milk-derived protein, may also have anti-inflammatory effects by regulating the recruitment and activation of cytokine-releasing immune cells (107, 108). While data are limited, magnesium has been associated with improved insulin sensitivity in observational studies and clinical trials (109). Lastly, fatty acids may also have direct metabolic effects. **Cis-9, trans-11** conjugated linoleic acid (c9,t11-CLA), another naturally occurring fatty acid (110), has been demonstrated to be a ligand and activator of peroxisome proliferator-activated receptors (PPARα), a transcription factor in lipid metabolism, in vitro (111). Also, c9,t11-CLA intake in obese mice has been shown to increase GLUT4 and insulin receptor expression, reduce macrophage infiltration, and downregulate inflammatory markers, including TNF-α, in adipose tissue (112).

### 2.5.2 Dairy consumption and risk of T2DM: observational studies

There is a growing body of evidence suggesting that dairy intake may be associated with a decreased risk of T2DM. Three meta-analyses of prospective cohort studies have shown significant inverse associations of higher intakes of dairy with T2DM risk (RR=0.82-0.93) (2-4). Results across individual epidemiological studies, however, have not been consistent, with studies suggesting either a decrease in T2DM (113-118) or no association (7, 119-122) with higher total dairy intakes. In addition, specific types of dairy products appear to have unique influences on T2DM risk. Two of the meta-analyses found significant decreases in T2DM risk with low-fat (LF) milk intake: RR=0.91 (95% CI, 0.86-0.96) (2), and RR=0.82 (95% CI, 0.74-0.90) (3), while there were no significant associations with high-fat (HF) dairy, milk, cheese, and yogurt intakes (2, 3). Individual studies also report inconsistent results across different types of dairy products, with some reporting inverse associations for LF dairy products (113, 116, 117, 123, 124), LF or skim milk (113, 115, 116), yogurt (7, 113, 116, 117) and cheese (7, 115, 116), while other studies found no association (120, 122).
Although there has been increasing interest between dairy intake and T2DM as a primary outcome, there has been very limited literature on dairy’s association with the underlying pathophysiological disorders of T2DM, as few studies have collected detailed measures of insulin sensitivity and beta-cell function. Results from studies examining insulin resistance have shown either no association or an inverse association and only 1 study has assessed beta-cell function (Table 2-1).

Differences in the study results summarized above may be due to differences in population characteristics, average dairy intakes in the cohorts, differences in measurement methods for assessing usual dairy intake and outcomes, type of dairy food evaluated in the analyses, and covariates adjusted for in statistical analyses.
Table 2-1: Observational studies evaluating associations of dairy intake with insulin resistance and beta-cell function

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Exposure</th>
<th>Result</th>
<th>Co-variates in analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ma et al. (2006) (125)</td>
<td>Cross-sectional</td>
<td>1,036 healthy adults, IRAS</td>
<td>Total dairy</td>
<td>↔ 𝑆₁</td>
<td>Age, sex, ethnicity, clinic, total calorie intake, family history of diabetes, smoking,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>physical activity, protein, fat, fiber, calcium, magnesium, refined and whole grains, fruit,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vegetables, fish, meat, BMI</td>
</tr>
<tr>
<td>Struijk et al. (2012) (122)</td>
<td>Prospective cohort</td>
<td>5,953 healthy adults, Inter99 study</td>
<td>Total dairy, LF dairy, milk and milk products</td>
<td>↔ HOMA-IR, ↔ HOMA-B</td>
<td>Age, gender, intervention group, education, diabetes family history, physical activity,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>smoking, alcohol. Whole grain cereal, meat, fish, coffee, tea, fruit, vegetables, total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>energy intake, change in diet from baseline to 5-year follow-up; waist circumference</td>
</tr>
<tr>
<td>Akter et al. (2013) (126)</td>
<td>Cross-sectional</td>
<td>496 healthy adults</td>
<td>Total dairy; HF dairy</td>
<td>↔ HOMA-IR; ↓ HOMA-IR</td>
<td>Age, sex, workplace, sedentary work, physical activity, alcohol, BMI, parental T2DM, total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>energy intake, fibre, PUFA, SFA</td>
</tr>
<tr>
<td>Wang et al. (2013) (127)</td>
<td>Cross-sectional</td>
<td>6,526 adults; Framingham Heart Study Offspring and Third Generation cohorts</td>
<td>High (&gt;2.07%kcal) vs. low yogurt</td>
<td>↓ HOMA-IR</td>
<td>Age, sex, PAI score, total energy intake, and smoking, DGAI score, BMI</td>
</tr>
</tbody>
</table>

1DGAI, Dietary Guidelines Adherence Index; HF, high fat; HOMA-B, beta-cell function; HOMA-IR, insulin resistance; IRAS, Insulin Resistance Atherosclerosis Study; LF, low fat; PAI, Physical Activity Index; PUFA, polyunsaturated fatty acid; 𝑆₁, insulin sensitivity index.
2.5.3 Dairy consumption and underlying disorders: clinical trials

Results from clinical trials on the effect of dairy intake on insulin resistance have been inconclusive, with studies showing either a decrease in HOMA-IR (105, 128, 129) or no change (130-132). A number of factors have contributed to the inconsistencies in the results of these trials, including small sample sizes, short intervention durations, amount and type of dairy foods tested in each treatment, and energy balance between treatments. No clinical studies have measured dairy’s effect on beta-cell function.

2.5.4 Role of dairy products on markers of subclinical inflammation: observational studies

Only three studies have evaluated dairy’s role on subclinical inflammation cross-sectionally (Table 2-2). No prospective studies, to date, have been conducted.

In a study that evaluated the association of components of the Mediterranean diet with inflammatory markers in 772 adults with high cardiovascular risk, higher dairy intake was inversely associated with CRP ($p$-trend=0.005), and soluble intercellular adhesion molecule-1 (sICAM-1) ($p$-trend=0.04) (26).

Another study of 486 healthy Iranian women (25) found no significant association between total dairy intake, measured using a validated food frequency questionnaire, with inflammatory markers. However, LF dairy intake was significantly and inversely associated CRP, IL-6 and sICAM-1. After further adjustment for dietary variables, only sICAM-1 remained significant. Conversely, HF dairy intake was positively associated with serum amyloid A and sICAM-1.

The ATTICA study analyzed data from 3,042 Greek adults, aged 18-89 years (24). Dietary intake was assessed using a validated food frequency questionnaire (the EPIC-FFQ), and inflammatory markers were measured from a fasting blood sample. In fully adjusted linear regression models, the study found significant reductions ($p<0.05$) of 0.073 mg/l in CRP, 0.068 ng/ml in IL-6, 0.041 mg/dl in TNF-α per 1 serving/week increase in LF dairy consumption. HF dairy consumption (1 serving/week increase) was also significantly associated with a decrease in IL-6 (0.05 ng/ml) and TNF-α (0.047 mg/dl), with borderline significance for CRP (0.021 mg/l) reduction ($p=0.06$). While the study adjusted for a variety of potential confounders, there was no adjustment for total energy intake.
Table 2-2: Observational studies examining associations of dairy intake with markers of subclinical inflammation

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Exposure</th>
<th>Result</th>
<th>Co-variates in analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salas-Salvadó et al. (2008) (26)</td>
<td>Cross-sectional</td>
<td>772 adults, high CVD risk</td>
<td>Total</td>
<td>↓ CRP, sICAM-1</td>
<td>Age, sex, BMI, diabetes, smoking, statin, NSAID, aspirin</td>
</tr>
<tr>
<td>Esmailzadeh &amp; Azadbakht (2009) (25)</td>
<td>Cross-sectional</td>
<td>486 adult women</td>
<td>LF dairy; HF dairy</td>
<td>↓ sICAM-1; ↑ SAA</td>
<td>Age, BMI, waist circumference, smoking, physical activity, total energy intake, estrogen use, menopausal status, family history of T2DM, systolic and diastolic blood pressure, fasting plasma glucose, serum TAG, total cholesterol, HDL and LDL cholesterol, cholesterol intake, meat, fish, fruit, vegetables, whole and refined grains, hydrogenated and non-hydrogenated vegetable oils, percentage of energy from fat and mutual effects of HF- and LF dairy</td>
</tr>
<tr>
<td>Panagiotakos et al. (2010) (24)</td>
<td>Cross-sectional</td>
<td>3,042 adults, ATTICA study</td>
<td>LF dairy; HF dairy</td>
<td>↓ CRP, IL-6, TNF-α; ↓ TNF-α, IL-6</td>
<td>Age, sex, smoking, physical activity, BMI, systolic and diastolic blood pressure, total and HDL cholesterol, triglyceride, medication, Mediterranean diet score</td>
</tr>
</tbody>
</table>

1CRP, C-reactive protein; HDL, high-density lipoprotein; HF, high fat; LDL, low-density lipoprotein; LF, low fat; NSAID, non-steroidal anti-inflammatory drug; SAA, serum amyloid A; sICAM-1, soluble intercellular adhesion molecule-1; TNF-α, tumor necrosis factor-α.
2.5.5 Role of dairy products on markers of subclinical inflammation: clinical trials

Results from randomized clinical trials, again, have not been conclusive with regard to the effect of increasing dairy product consumption on changes in circulating inflammatory markers. About half of the studies show either a potential beneficial effect of high dairy consumption on inflammatory marker concentrations, while the remainder show no effect (101, 105, 128, 130-136). There are several limitations and methodological factors that have likely contributed to the variation in the results of these trials. First, only 4 of the studies considered inflammation as a primary outcome (128, 130-132). As such, these studies may have been underpowered to detect between treatment changes in the inflammatory markers. Also, decreases in inflammatory markers in energy restricted interventions may be confounded by associated decreases in adiposity parameters (128). As previously mentioned, the generalizability and interpretation of the results of these studies are limited due to differences in the amount and type of dairy product used in the interventions, overall diet during the intervention (i.e. isoenergetic or energy restricted), trial duration, and participant selection.

2.6 Serum fatty acids as biomarkers for intake in nutritional epidemiology

Epidemiological studies commonly rely on self-reported food frequency questionnaires (FFQ) to measure usual dietary intake (137). However, a major limitation of the FFQ is the potential for measurement error (137, 138) and inaccuracies in food composition databases (139, 140). Underreporting in energy intake has been observed obese individuals, and in individuals with diabetes (141).

Biomarkers have been proposed as more objective measures of diet compared to FFQ since they overcome the above mentioned limitations of FFQ (15). Optimal biomarkers are those that are not endogenously produced because concentrations reflect intake rather that physiological processes. In the realm of fatty acids, optimal markers include TFA, as well as odd numbered and branched chain fatty acids (142). Fatty acid biomarkers may be measured in different biological media: serum or plasma, erythrocytes, and adipose tissue (143). Serum or plasma measurements reflect short-term intake over the past few days (143) to weeks (144).
2.6.1 Dairy specific fatty acids

Fatty acids predominantly found in dairy foods include pentadecanoic acid (15:0) (10), heptadecanoic acid (17:0) (10), \textit{trans}-palmitoleic acid (\textit{trans} 16:1n-7) (16), vaccenic acid (\textit{trans} 18:1n-11) (99), and conjugated linoleic acid (c9,t11 CLA) (99). These fatty acids have been shown to be correlated with dairy intake and have previously been used as biomarkers for dairy intake. The subsequent sections will focus on 15:0 and \textit{trans} 16:1n-7 as these are the markers available in the current thesis.

2.6.2 Validation of dairy fatty acids as biomarkers for dairy intake

Pentadecanoic acid (15:0), measured in adipose tissue, serum, plasma, or erythrocytes, is considered a biomarker for dairy intake. Several studies report significant positive correlations between 15:0 and self-reported dairy intake ($r=0.3-0.74$) (9-14). Similarly, \textit{trans}-palmitoleic acid (\textit{trans} 16:1n-7), measured in plasma and erythrocytes, has been correlated with dairy intake ($r=0.15-0.30$) (15-17).

2.7 Pentadecanoic acid (15:0) on T2DM risk and underlying disorders in observational studies

2.7.1 Association of 15:0 on T2DM risk

Observational studies have largely shown no association between 15:0 with incident T2DM (19, 20), insulin resistance (23, 145), or beta-cell dysfunction (145), with 2 studies showing a decrease in T2DM risk (8, 18) (Table 2-3). The lack of association in these studies may be due to several methodological factors, including small sample sizes, population characteristics, and the extent of covariate adjustments.
**Table 2-3:** Observational studies evaluating associations of 15:0 with incident T2DM, insulin resistance, and beta-cell dysfunction

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Result</th>
<th>Co-variates in analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krachler et al. (2008) (18)</td>
<td>Nested case-referent (sex- &amp; age-matched)</td>
<td>159, no T2DM at baseline; 291 referents</td>
<td>↓ T2DM risk</td>
<td>Alcohol, BMI, HbA1c</td>
</tr>
<tr>
<td>Patel et al. (2010)(20)</td>
<td>Nested case-cohort</td>
<td>199 incident diabetes cases; 184 noncases</td>
<td>↔ T2DM risk</td>
<td>Age, sex, family history of diabetes, BMI, smoking status, physical activity, alcohol intake</td>
</tr>
<tr>
<td>Kröger et al. (2011) (19)</td>
<td>Nested case-cohort</td>
<td>2,724; 673 incident T2DM cases</td>
<td>↔ T2DM risk</td>
<td>Age, sex, BMI, waist circumference, cycling, sports activity, education, smoking, alcohol, occupation activity, coffee intake, fibre intake.</td>
</tr>
<tr>
<td>Kratz et al. (2014)(22)</td>
<td>Cross-sectional</td>
<td>17, with NAFLD¹; 15 BMI- &amp; age-matched control</td>
<td>↔ insulin resistance, beta-cell function</td>
<td>Age, sex, BMI, liver- spleen ratio (liver fat content)</td>
</tr>
<tr>
<td>Nestel et al. (2014) (23)</td>
<td>Cross-sectional</td>
<td>86 overweight and obese with metabolic syndrome</td>
<td>↔ insulin resistance</td>
<td>Age, sex, systolic blood pressure, and waist:hip ratio or BMI</td>
</tr>
<tr>
<td>Forouhi et al. (2014) (8)</td>
<td>Case-cohort</td>
<td>12,403 incident T2DM; 16,154 non-cases; EPIC-InterAct study</td>
<td>↓ T2DM risk</td>
<td>Age, centre, sex, physical activity, smoking, education, total energy intake, alcohol, and BMI; additional sensitivity analyses: (1) meat, fruit and vegetables, soft drinks, and total dairy products, (2) baseline HbA1c, (3) exclusion of 2348 people with HbA1c ≥6.5% (or ≥48 mmol/mol) at baseline, (4) exclusion of 1048 cases of T2DM diagnosed within the first 2 years after baseline</td>
</tr>
</tbody>
</table>

¹NAFLD, Non-alcoholic fatty liver disease.
2.7.2 Association of 15:0 with markers of subclinical inflammation

15:0’s role on markers of subclinical inflammation has been evaluated as a secondary outcome in one study. A cross-sectional analysis of 2,837 adults from the Multi-Ethnic Study of Atherosclerosis (MESA) found no significant association with CRP levels ($p=0.66$) (27).

2.7.3 Potential mechanisms for 15:0 on T2DM risk

The mechanism in which 15:0 may be associated with T2DM risk or its underlying disorders has not yet been evaluated. 15:0 may be a bioactive fatty acid with direct metabolic effects, or it may be a marker of other potentially beneficial components found in dairy foods: vitamin D, calcium, protein, and magnesium (2, 3), or a healthy dietary pattern.

2.8 Trans-palmitoleic acid ($trans$ 16:1n-7) on T2DM risk and underlying disorders

2.8.1 Association of $trans$ 16:1n-7 on T2DM risk

The majority of published observational studies have found no significant association between $trans$ 16:1n-7 and T2DM risk (Table 2-4). Two studies by Mozaffarian et al. however, reported lower T2DM risk with higher quintiles of plasma $trans$ 16:1n-7 (Q5 versus Q1: HR=0.38, 95% CI: 0.24, 0.62, $p$-trend<0.001 (16); Q5 versus Q1: HR=0.52, 95% CI: 0.32, 0.85, $p$-trend=0.02 (17).
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Result</th>
<th>Co-variates in analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mozaffarian et al. (2010) (16)</td>
<td>Prospective cohort</td>
<td>3,736 adults; Cardiovascular Health Study (CHS)</td>
<td>↓T2DM risk, insulin resistance</td>
<td>Age, sex, race, education, enrollment site, smoking, BMI, waist circumference, CHD, physical activity, alcohol, carbohydrate, protein, red meat, whole-fat dairy, low-fat dairy, total energy; plus diabetes</td>
</tr>
<tr>
<td>Patel et al. (2010) (20)</td>
<td>Nested case-cohort</td>
<td>199 incident diabetes cases; 184 noncases; EPIC-Norfolk study</td>
<td>↔T2DM risk</td>
<td>Age, sex, family history of diabetes, BMI, smoking status, physical activity, alcohol intake</td>
</tr>
<tr>
<td>Kröger et al. (2011) (19)</td>
<td>Nested case-cohort</td>
<td>2,724; 673 incident T2DM cases; EPIC-Postdam study</td>
<td>↔T2DM risk</td>
<td>Age, sex, BMI, waist circumference, cycling, sports activity, education, smoking, alcohol, occupation activity, coffee intake, fibre intake.</td>
</tr>
<tr>
<td>Castro-Webb et al. (2012) (21)</td>
<td>Cross-sectional</td>
<td>232 adults with diabetes and 1512 adults without diabetes</td>
<td>↔T2DM risk</td>
<td>Age, sex, area of residence, physical activity, BMI, waist circumference, income, smoking, 9,11 CLA, 15:0 + 17:0</td>
</tr>
<tr>
<td>Mozaffarian et al. (2013) (17)</td>
<td>Prospective cohort</td>
<td>2617 adults; Multi-Ethnic Study of Atherosclerosis (MESA),</td>
<td>↓T2DM risk</td>
<td>Age, sex, ethnicity, education, field centre, smoking, alcohol, physical activity, BMI, waist circumference, red meat, whole-fat dairy, low-fat dairy, total energy</td>
</tr>
<tr>
<td>Kratz et al. (2014) (22)</td>
<td>Cross-sectional</td>
<td>17, with NAFLD; 15 BMI- &amp; age-matched control</td>
<td>↓insulin resistance¹, ↔beta-cell function</td>
<td>Age, sex, BMI</td>
</tr>
<tr>
<td>Nestel et al. (2014) (23)</td>
<td>Cross-sectional</td>
<td>86 overweight and obese with metabolic syndrome</td>
<td>↔insulin resistance</td>
<td>Age, sex, systolic blood pressure, and waist:hip ratio or BMI</td>
</tr>
</tbody>
</table>

¹Additional adjustment for liver-spleen ratio (liver fat content) attenuated result to non-significance.
2.8.2 Association of trans 16:1n-7 with markers of subclinical inflammation

Two cross-sectional analyses found no significant association between trans 16:1n-7 and CRP levels (17, 27). Analysis of the Cardiovascular Health Study (CHS) found a 13.8% decrease in circulating CRP ($p=0.05$), and a non-linear increase in fibrinogen levels ($p=0.006$) across increasing quintiles of trans 16:1n-7 (16). Inflammatory markers were evaluated as secondary outcomes in these studies.

2.8.3 Potential mechanisms for trans 16:1n-7 on T2DM risk

While the mechanism behind trans 16:1n-7 has yet to be determined, Mozaffarian et al. (16, 17) hypothesized that trans 16:1n-7 may mimic the metabolic effects of an endogenously produced isomer, cis-palmitoleate (17, 146). Animal studies have shown that non-hepatic cis-palmitoleate directly improved hepatic and peripheral insulin resistance and suppressed de novo lipogenesis in the liver (146, 147). Non-hepatic sources of cis-palmitoleate include adipose tissue, specifically gluteofemoral adipose tissue (147). These early studies indicate a possible protective metabolic role of non-hepatic palmitoleate.

2.9 Summary, rationale and objectives

Increasing evidence points to a potential protective role of dairy intake in T2DM etiology. Observational studies largely indicate that higher dairy intake is inversely associated with T2DM risk. However, results from individual studies have been inconsistent, with variation in findings depending on the type of dairy product being evaluated. In addition, dairy intake in observational studies was most often measured using self-reported FFQ, which introduce measurement errors in the exposure variables. Clinical trials, which have mostly been short in duration, had small sample sizes, and varied amounts and types of dairy products in the treatment design, have similarly yielded mixed results. Furthermore, very few studies have evaluated the association of dairy intake with insulin resistance, beta-cell function, and subclinical inflammation, which are key pathophysiological disorders underlying T2DM.

Dietary biomarkers provide nutritional epidemiologists with a more objective measurement of food or food group consumption, in that these measures overcome many of the limitations of self-reported FFQ. Dairy contains fatty acids that have been validated as biomarkers for dairy
intake, yet few studies have utilized these fatty acid biomarkers to measure dairy as an exposure for disease outcomes. Currently the literature on dairy fatty acids and T2DM is inconsistent and existing studies are limited in characterization of the underlying disorders.

The **objective** of the present thesis was to investigate the association between dairy fatty acid biomarkers and T2DM traits in the Insulin Resistance Atherosclerosis Study, a large multi-ethnic cohort with a range of glucose tolerance status.

It was **hypothesized** that pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n-7), would be inversely associated with:

1. directly measured insulin resistance and beta-cell dysfunction, as well as incident diabetes at 5 years, and

2. markers of subclinical inflammation: CRP, TNF-α, PAI-1, and fibrinogen.
Chapter 3
Serum Pentadecanoic Acid (15:0), a Marker of Dairy Food Intake, is Inversely Associated with Incident Type 2 Diabetes and Its Underlying Disorders

3.1 Abstract

**Background** Growing evidence suggests that dairy consumption is associated with lower type 2 diabetes risk. However, observational studies have reported inconsistent results and few have examined dairy’s association with the underlying disorders of insulin resistance and beta-cell dysfunction.

**Objective** We investigated the association of the dairy fatty acid biomarkers pentadecanoic acid (15:0) and *trans*-palmitoleic acid (*trans* 16:1n-7) with type 2 diabetes traits by evaluating (1) prospective associations with incident diabetes after 5 years of follow-up; and (2) cross-sectional associations with directly measured insulin resistance and beta-cell dysfunction.

**Design** The study analyzed 659 adults without diabetes at baseline from the tri-ethnic multi-centre Insulin Resistance Atherosclerosis Study (IRAS). Diabetes status was assessed using oral glucose tolerance tests. Frequently sampled intravenous glucose tolerance tests measured insulin sensitivity (S_I) and beta-cell function (Disposition Index, DI). Serum fatty acids were quantified using gas chromatography. Logistic and linear regression models were adjusted for demographic, lifestyle, and dietary variables.

**Results** Serum 15:0 was a significant biomarker for total dairy intake in the IRAS cohort ($r=0.20$, $p<0.0001$). 15:0 was associated with decreased incident diabetes risk (OR=0.73, $p=0.02$), and was positively associated with log $S_I$ ($\beta=0.84$, $p=0.03$) and log DI ($\beta=2.21$, $p=0.02$), in fully adjusted models. *Trans* 16:1n-7 was a marker of total partially hydrogenated dietary fat intake and was not associated with outcomes in fully adjusted models.

**Conclusion** Serum 15:0 was inversely associated with diabetes risk in this multi-ethnic cohort. This study may contribute to future recommendations regarding the benefits of dairy products on type 2 diabetes risk.
3.2 Introduction

Type 2 diabetes, a growing global epidemic, arises from both non-modifiable and modifiable factors (36). Among the many nutritional exposures that have been investigated in type 2 diabetes, dairy consumption is emerging as a potential protective factor. Three meta-analyses of prospective observational studies have reported that high dairy intake was associated with a lower risk of type 2 diabetes (2-4). However, findings have been inconsistent across individual studies (7, 113, 116-122, 124, 148), and only a limited number of studies have assessed the relationship of dairy intake with the main underlying pathophysiological traits of type 2 diabetes (36), namely: insulin resistance (122, 125-127) and beta-cell dysfunction (122).

The majority of previous observational studies measured usual dairy consumption using self-reported intake from food frequency questionnaires (FFQ), which are susceptible to misclassification error due to under or over-reporting (149). A number of dairy bioactives may potentially underlie the associations described in previous studies, including the fatty acid profile, as dairy is a particularly rich source of saturated (SFA) and naturally occurring trans (TFA) fatty acids (3, 6). Despite limited data, current literature suggests that intake of different types of SFAs and TFAs may affect metabolic and cardiovascular risk differently (138), and there is growing evidence that certain fatty acids, including those from dairy, may play a role in type 2 diabetes prevention (7). Certain fatty acid biomarkers have previously been validated as markers for dairy intake, including pentadecanoic acid (15:0) (9-14) and trans-palmitoleic acid (trans 16:1n-7) (15-17), with fatty acids measured in serum reflecting short-term dietary intake (143). Using dairy derived fatty acid biomarkers has the potential to provide more objective measures of dairy intake and thus they may help to elucidate the role of dairy on the risk of type 2 diabetes and its underlying disorders.

The current study aimed to investigate the association between dairy biomarkers and type 2 diabetes traits in a large multi-ethnic cohort by evaluating (1) prospective associations of dairy fatty acid biomarkers with incident diabetes after 5 years of follow-up; (2) cross-sectional associations of dairy fatty acid biomarkers with directly measured insulin resistance and beta-cell dysfunction using frequently sampled intravenous glucose tolerance tests (FSIGT). We hypothesized that pentadecanoic acid (15:0) and trans-palmitoleic acid (trans 16:1n-7),
independent of covariates, would be inversely associated with insulin resistance, beta-cell dysfunction, and incident diabetes at 5 years.

3.3 Subjects and Methods

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicentre epidemiological study assessing the relationship between insulin resistance and subclinical cardiovascular disease. The study consists of a tri-ethnic cohort of 1,625 Hispanic, African American and non-Hispanic white adults aged 40-60 years across a range of glucose tolerance status. Participants were recruited from four clinical centres in San Antonio, Texas; San Luis Valley, Colorado; Los Angeles, California; and Oakland, California. Baseline study visits were conducted between October 1992 and April 1994, and 5-year follow-up examinations were conducted from February 1998 to July 1999, with an 81% response rate (150). Participants who did not attend the follow-up examinations were not systematically different in terms of age, sex, ethnicity or clinic, except for a slightly lower educational attainment compared to those who returned for follow-up (151). Each study centre received ethics approval from their institutional review boards and all participants gave informed consent. A comprehensive presentation of the study objectives, design, and recruitment results has been previously published (152).

The present analysis excluded participants with type 2 diabetes at baseline (n=553) and participants who did not return for follow-up (n=177). With further exclusions for missing insulin sensitivity and beta-cell function measures, 15:0, trans 16:1n-7, and total dairy intake values, the final study sample for the current analysis was 659 (Figure 3-1).
Usual dietary intake over the previous year before baseline was assessed using a 114-item food frequency questionnaire (FFQ) modified from the National Cancer Institute (NCI) Health Habits and History Questionnaire to include ethnic and regional foods relevant to the study population (153). The validity and reproducibility of this FFQ were established in a subsample of 186 women from the IRAS population using eight 24h dietary recalls, followed by a second FFQ (153). Food and beverage intake in the FFQ were quantified through interviews in which participants were asked recall the frequency of consumption of each food, or groups of foods, over the past year. The FFQ contained 9 frequency options, ranging from “never or less than once a month” to “six or more times per day”, and 3 portion sizes: “small, medium, or large compared with other men or women about your age.” Servings per day were standardized to the medium serving size for the food intake analyses by multiplying the intake frequency with the portion size after applying a weighting factor (small=0.5, medium=1.0, large=1.5). One serving, therefore, corresponds to 1 medium-sized portion of the food or food group. Total dairy product intake was calculated by adding 11 dairy food line items from the FFQ: whole milk; 2% milk;
skim milk, 1% or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, non-fat or whole); low-fat flavoured yogurt (2% or non-fat); ice cream; frozen yogurt, ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea. Total milk, total cheese, and total yogurt intakes were also calculated. As trans 16:1n-7 may also be found in foods containing partially hydrogenated fats (17), total partially hydrogenated food intake was calculated by summing the following items from the FFQ: french fries and fried potatoes, salty snacks such as crackers, potato chips, corn chips, tortilla chips, pretzels, margarine on bread or roll, doughnuts, cookies, cakes, pastry, brownies, sopapillas, and pan dulce. A similar approach for summing sources of hydrogenated fats was recently used by Mozaffarian et al. (17). Nutrient and energy intakes were estimated from the FFQ using a nutrient database (HHHQ-DIETSYS analysis software, version 3.0; National Cancer Institute, Bethesda, MD, 1993), expanded for additional nutrients.

Clinical examinations were conducted at baseline and follow-up during two 4 hour visits, which were administered 1 week to 30 days apart. Prior to each clinic visit, participants were asked to fast for 12 hours, refrain from heavy exercise and alcohol consumption for 24 hours, and smoking the morning of the visit. All participants received an oral glucose tolerance test (OGTT) to determine glucose tolerance status (normal, impaired glucose tolerance [IGT] or diabetes) based on the 2010 American Diabetes Association criteria for fasting or 2 hour postload glucose concentrations, and oral hypoglycaemic agent or insulin use (154). Frequently sampled intravenous glucose tolerance tests (FSIGT) were administered following a validated modified protocol (152, 155). Insulin resistance was calculated using minimal-model analysis (MINMOD, version 3.0, 1994) (156) and expressed as the insulin sensitivity index (SI). Insulin secretion was assessed via acute insulin response (AIR), a sensitivity index of beta-cell function measured as the mean plasma insulin concentration from 2 to 4 minute time points after the initial glucose administration (152). The product of SI and AIR yields Disposition Index (DI), an integrated measure of beta-cell function reflecting the ability of beta-cells to compensate for insulin resistance by upregulating insulin secretion (35). DI was used as the measure of beta-cell function for this investigation.

Waist circumference and height were measured to the nearest 0.5 cm and body weight was measured to the nearest 0.1 kg, with the average of the duplicate measurements used in all analyses. A validated physical activity recall was used to determine total estimated energy
expenditure over the past year (152). Total estimated energy expenditure (kcal/kg/week) was calculated by summing energy expenditure activities and energy expenditure from sleep. Smoking status was categorized into 3 groups: never, past, or current. Total usual alcohol intake (g ethanol consumed/day) was evaluated through a separate questionnaire with additional questions about recent use and average lifetime use. Race/ethnicity and age were self-reported. Medical history was assessed using structured interviews.

**Fatty acid analysis**

A complete quantitative profile of fatty acids was extracted from participants’ serum (which were stored at -70°C) using methods previously described (Lipomics Technologies Inc., West Sacramento, CA) (157). Briefly, total lipids were extracted in the presence of internal standards according to the method of Folch (158). Fatty acid methyl esters (FAME) were formed by transesterification of total lipid extracts in sulfuric acid/methanol, and were then extracted into hexane and prepared for gas chromatography. Capillary gas chromatography (Agilent Technologies model 6890) equipped with a 30m HP-88 capillary column (Agilent Technologies) and a flame-ionization detector were used to separate and quantify individual fatty acids. The absolute concentration of each fatty acid in the serum sample was measured by comparing its peak area to the internal standard. A total of 35 serum fatty acids were analyzed and quantified using these methods. For this study, dairy derived fatty acids pentadecanoic acid (15:0) and \textit{trans}-palmitoleic acid (\textit{trans} 16:1n-7) are the exposures of interest and are expressed as the mole percentage (mol\%) of total fatty acids.

**Statistical analysis**

Baseline characteristics across quintiles (Q1-Q5) of increasing total dairy intake (servings/week) are presented. Normally distributed variables are presented as means ± SD, non-normally distributed variables are presented as medians with interquartile ranges (IQR), and categorical variables are presented as number and percent of participants in each quintile, with differences across quintiles tested using ANOVA, Kruskal-Wallis tests, and Chi square tests, respectively.

Dietary determinants of dairy fatty acids in serum were assessed by analyzing the correlations of 15:0 and \textit{trans} 16:1n-7 with total dairy, total milk, total cheese, total yogurt, and total partially hydrogenated food intakes from the FFQ. Furthermore, multivariable-adjusted linear regressions
with 15:0 and *trans* 16:1n-7 as the outcome variables were conducted to assess the contribution of total dairy and total partially hydrogenated food intake to serum levels of these dairy fatty acids. The regressions were iteratively adjusted for Model 1: age, sex, and ethnicity; Model 2: physical activity and total energy intake; Model 3: total dairy or total hydrogenated food intake; and Model 4: BMI.

Logistic regression analysis was used to evaluate the prospective associations between dairy fatty acids and incident diabetes at 5 years, with sequential adjustment in 3 models. Model 1 was adjusted for demographic variables: age, sex, ethnicity, and centre. Model 2 was additionally adjusted for lifestyle variables: physical activity, smoking status, alcohol intake, and education. Model 3 was adjusted for dietary variables: total energy intake, fruit and vegetable, red meat, soft drink, and fibre intakes. Based on significant Spearman correlations of BMI and waist circumference with 15:0 and the outcome variables, we included these measures of adiposity in additional mechanistic models since these variables are likely on the etiological pathway between the fatty acid exposures and outcomes. In the cross-sectional study, univariate analyses between outcomes and exposures were conducted using Spearman’s correlations (*r*). Multiple linear regression analysis was used to assess the cross-sectional association between the dairy fatty acids with insulin resistance (SI) and beta-cell dysfunction (DI), adjusted for the same covariates in the logistic regressions. Both SI and DI were skewed, thus these outcome variables were log transformed to achieve normality. As some participants had an SI of 0, a constant of 1 was added to the values before being log transformed. Subgroup analyses on *a priori* variables of interest, including sex, glucose tolerance status, and ethnicity, were conducted for both logistic and linear regressions. Formal tests of interaction for these variables were carried out using cross-product terms and were considered statistically significant when *p*<0.05.

All analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). A *p*<0.05 was considered significant for all analyses.

### 3.4 Results

Table 3-1 shows the baseline characteristics of participants across quintiles of increasing total dairy intake (servings/week). There were no differences in age, sex, and education levels across these categories. Ethnicity differed significantly across quintiles of total dairy intake with non-Hispanic whites and African Americans having the highest and lowest dairy intakes,
respectively. Glucose tolerance status also differed across quintiles, such that the highest percentage of participants with IGT was in Q1, although SI and DI did not significantly differ across quintiles. Total energy intake, percent energy from SFA, dietary intake of TFA, and serum 15:0 and trans 16:1n-7 significantly increased across quintiles.

Total intakes of dairy ($r=0.20, p<0.0001$), milk ($r=0.13, p=0.0006$), and cheese ($r=0.16, p<0.0001$) were positively correlated with 15:0. Trans 16:1n-7 was not significantly correlated with either 15:0 or reported intakes of dairy foods (data not shown). In contrast, intake of total partially hydrogenated foods, another dietary source of trans 16:1n-7, was positively and significantly correlated with serum trans 16:1n-7 ($r=0.18, p<0.0001$). Linear regression analysis showed that 15:0 in serum was independently and positively associated with total dairy intake, while trans 16:1n-7 was negatively associated with total dairy intake (Table 3-2). Conversely, 15:0 was negatively and trans 16:1n-7 was positively associated with total partially hydrogenated food intake (Table 3-3).

In the prospective analysis, 103 participants out of 659 developed diabetes after 5 years of follow-up. In multivariate logistic regression, 15:0 was associated with a 27% decreased risk for incident diabetes in the fully adjusted model, which included demographic, lifestyle, and dietary variables (Model 3: OR per SD=0.73; 95% CI: 0.56, 0.95; $p=0.02$ (Figure 3-2)). After further adjustment for adiposity variables in a mechanistic model, the associations persisted (with additional adjustment for BMI: OR=0.76; 95% CI: 0.58, 0.99; $p=0.04$; or with additional adjustment for waist circumference: OR=0.77; 95% CI: 0.59, 1.00; $p=0.05$). None of the interaction terms tested in the effect modification analyses were statistically significant ($p>0.05$) (Figure 3-2). However, stratified analysis of a priori variables of interest with 15:0 showed that females, those with normal glucose tolerance, and Hispanic participants had modestly stronger inverse associations with diabetes risk in the fully adjusted model (Figure 3-2). Trans 16:1n-7 was not significantly associated with incident diabetes (Model 3: OR per SD = 0.90; 95% CI: 0.70, 1.15; $p = 0.38$).

In univariate analyses, 15:0 was positively correlated with S1 ($r=0.14, p=0.0003$) and DI ($r=0.11, p=0.006$), while trans 16:1n-7 was negatively correlated with S1 ($r=-0.12, p=0.003$), and not significantly correlated with DI. Multiple regression analyses showed that 15:0 was positively associated to both log S1 ($\beta=0.84; \text{SEM} = 0.38; p = 0.03$) and log DI ($\beta=2.21; \text{SEM}=0.93$);
$p=0.02$) after adjustment for demographic, lifestyle, and dietary variables (Table 3-4). Further adjustment for BMI (Table 3-4) and waist circumference (data not shown) in mechanistic models attenuated results to non-significance, although the direction and magnitude of these associations were similar. $Trans\ 16:1n-7$ was negatively associated with log $S_i$ after adjustment for age, sex, ethnicity and study centre ($\beta=-0.56;\ SEM=0.21;\ p=0.0096$). Further adjustment with lifestyle and dietary variables attenuated results to non-significance. $Trans\ 16:1n-7$ was not associated with log DI. None of the interaction terms tested in the effect modification analyses were statistically significant ($p>0.05$). Significant results from subgroup analyses may be found in the Supplementary Tables S3-1 to S3-3. Briefly, in the fully adjusted models, $15:0$ was positively associated with log $S_i$ and log DI in females, and positively associated with log $S_i$ in Hispanics. On the other hand, $trans\ 16:1n-7$ was negatively associated with log $S_i$ in African Americans.
Table 3-1: Baseline characteristics of IRAS participants, overall and across quintiles of total dairy intake (servings/week) from the FFQ\(^1\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Quintile 1 (0-2.07)</th>
<th>Quintile 2 (2.10-4.10)</th>
<th>Quintile 3 (4.13-6.65)</th>
<th>Quintile 4 (6.69-9.73)</th>
<th>Quintile 5 (9.80-31.08)</th>
<th>(p^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>659</td>
<td>132 (20.03)</td>
<td>131 (19.88)</td>
<td>132 (20.18)</td>
<td>132 (20.03)</td>
<td>131 (19.88)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.68 ± 8.56</td>
<td>54.74 ± 8.84</td>
<td>55.52 ± 8.27</td>
<td>53.69 ± 7.96</td>
<td>55.99 ± 9.24</td>
<td>53.50 ± 8.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Males</td>
<td>297 (45.07)</td>
<td>61 (46.21)</td>
<td>59 (45.04)</td>
<td>60 (45.11)</td>
<td>69 (52.27)</td>
<td>48 (36.64)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>362 (54.93)</td>
<td>71 (53.79)</td>
<td>72 (54.96)</td>
<td>73 (54.89)</td>
<td>63 (47.73)</td>
<td>83 (93.36)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>277 (42.03)</td>
<td>36 (27.27)</td>
<td>50 (38.17)</td>
<td>56 (42.11)</td>
<td>66 (50.00)</td>
<td>69 (52.67)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>152 (23.07)</td>
<td>55 (41.67)</td>
<td>32 (24.43)</td>
<td>28 (21.05)</td>
<td>16 (12.12)</td>
<td>21 (16.03)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>230 (34.90)</td>
<td>41 (31.06)</td>
<td>49 (37.40)</td>
<td>49 (36.84)</td>
<td>50 (37.88)</td>
<td>41 (31.30)</td>
<td></td>
</tr>
<tr>
<td>Glucose Tolerance Status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Normal Glucose Tolerance</td>
<td>447 (67.83)</td>
<td>81 (61.36)</td>
<td>93 (70.99)</td>
<td>103 (77.44)</td>
<td>85 (64.39)</td>
<td>85 (64.89)</td>
<td></td>
</tr>
<tr>
<td>Impaired Glucose Tolerance</td>
<td>212 (32.17)</td>
<td>51 (38.64)</td>
<td>38 (29.01)</td>
<td>30 (22.56)</td>
<td>47 (35.61)</td>
<td>46 (35.11)</td>
<td></td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0009</td>
</tr>
<tr>
<td>Never</td>
<td>306 (46.43)</td>
<td>48 (36.36)</td>
<td>59 (45.04)</td>
<td>65 (48.87)</td>
<td>53 (40.15)</td>
<td>81 (61.83)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>259 (39.30)</td>
<td>55 (41.67)</td>
<td>53 (40.46)</td>
<td>56 (42.11)</td>
<td>58 (43.94)</td>
<td>37 (28.24)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>94 (14.26)</td>
<td>29 (21.97)</td>
<td>19 (14.50)</td>
<td>12 (9.02)</td>
<td>21 (15.91)</td>
<td>13 (9.92)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
<td>------------</td>
<td>------------</td>
<td>-----------</td>
<td>------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake category, g ethanol/week</td>
<td>3.52 (0-39.54)</td>
<td>2.24 (0-34.35)</td>
<td>7.26 (0-57.82)</td>
<td>0 (0-15.47)</td>
<td>8.86 (0-63.92)</td>
<td>3.52 (0-25.92)</td>
<td></td>
</tr>
<tr>
<td>Highest education level completed, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>87 (13.20)</td>
<td>21 (15.91)</td>
<td>16 (12.21)</td>
<td>12 (9.02)</td>
<td>20 (15.15)</td>
<td>18 (13.74)</td>
<td></td>
</tr>
<tr>
<td>≥ 12 years</td>
<td>572 (86.80)</td>
<td>111 (84.09)</td>
<td>115 (87.79)</td>
<td>121 (90.98)</td>
<td>112 (84.85)</td>
<td>113 (86.26)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.24 (24.83-27.24)</td>
<td>27.24 (24.81-29.99)</td>
<td>26.58 (24.10-29.33)</td>
<td>28.09 (25.56-30.80)</td>
<td>27.20 (24.77-29.55)</td>
<td>27.71 (24.75-33.07)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.28 ± 12.52</td>
<td>90.53 ± 12.74</td>
<td>87.87 ± 11.79</td>
<td>89.92 ± 11.06</td>
<td>91.53 ± 11.57</td>
<td>91.59 ± 14.92</td>
<td></td>
</tr>
<tr>
<td>Total estimated energy expenditure (kcal/kg/week)</td>
<td>272.28 (250.88-305.33)</td>
<td>272.03 (247.84-304.72)</td>
<td>270.05 (251.20-303.87)</td>
<td>267.22 (249.16-295.37)</td>
<td>284.22 (255.35-320.87)</td>
<td>272.96 (251.64-309.56)</td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity (x10⁴ min⁻¹[µU/ml]⁻¹)</td>
<td>1.71 (0.95-3.01)</td>
<td>1.73 (0.98-2.80)</td>
<td>1.92 (1.00-3.25)</td>
<td>1.73 (1.00-3.11)</td>
<td>1.53 (0.92-2.50)</td>
<td>1.65 (0.85-3.16)</td>
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</tr>
<tr>
<td>Acute insulin response (µU/ml)</td>
<td>51.5 (29.5-87.0)</td>
<td>50.50 (30.00-92.25)</td>
<td>51.0 (24.5-93.0)</td>
<td>60.0 (36.0-84.0)</td>
<td>49.5 (30.5-82.0)</td>
<td>49.5 (25.0-81.5)</td>
<td></td>
</tr>
<tr>
<td>Disposition Index</td>
<td>85.68 (43.44-154.59)</td>
<td>103.86 (43.10-151.16)</td>
<td>89.70 (48.02-149.49)</td>
<td>98.05 (57.38-179.41)</td>
<td>66.28 (41.22-140.27)</td>
<td>76.44 (36.25-153.00)</td>
<td></td>
</tr>
<tr>
<td>Dietary variables</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kcal/week)</td>
<td>12,416.84 (8,999-16,181.69)</td>
<td>9,559.99 (7,181.22-13,685.13)</td>
<td>11,457.43 (7,858.01-13,914.89)</td>
<td>12,257.03 (9,288.20-15,780.76)</td>
<td>13,505.43 (10,679.84-17,341.15)</td>
<td>15,005.27 (11,425.64-19,989.34)</td>
<td></td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>35.44 ± 8.44</td>
<td>34.68 ± 8.66</td>
<td>35.62 ± 9.51</td>
<td>35.64 ± 8.84</td>
<td>34.94 ± 7.91</td>
<td>36.29 ± 7.14</td>
<td></td>
</tr>
<tr>
<td>% Energy from SFA</td>
<td>12.05 ± 3.39</td>
<td>11.18 ± 3.42</td>
<td>11.74 ± 3.66</td>
<td>12.17 ± 3.31</td>
<td>12.25 ± 3.25</td>
<td>12.88 ± 3.08</td>
<td></td>
</tr>
<tr>
<td>Dietary intake TFA (g/week)</td>
<td>15.05 (7.56-25.90)</td>
<td>11.69 (6.09-21.00)</td>
<td>12.67 (6.23-22.19)</td>
<td>14.81 (9.57-22.54)</td>
<td>16.59 (8.62-29.89)</td>
<td>19.75 (11.83-33.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Total Dairy consumption (servings/week)</td>
<td>5.53 (2.59-8.75)</td>
<td>0.96 (0.35-1.54)</td>
<td>3.05 (2.59-3.54)</td>
<td>5.53 (4.80-6.13)</td>
<td>8.05 (7.28-8.75)</td>
<td>12.46 (11.06-15.23)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total Cheese consumption (servings/week)</td>
<td>1.51 (0.49-3.26)</td>
<td>0.28 (0-0.58)</td>
<td>0.98 (0.32-1.96)</td>
<td>1.58 (0.77-3.01)</td>
<td>2.24 (0.98-3.89)</td>
<td>3.5 (1.96-7.00)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total Milk consumption (servings/week)</td>
<td>0.98 (0-3.50)</td>
<td>0 (0-0.21)</td>
<td>0.56 (0-0.98)</td>
<td>0.98 (0.21-3.50)</td>
<td>2.54 (0.56-4.66)</td>
<td>3.71 (1.75-7.00)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total yogurt consumption (servings/week)</td>
<td>0.210 (0-1.12)</td>
<td>0 (0-0.21)</td>
<td>0.11 (0-1.05)</td>
<td>0.21 (0-1.12)</td>
<td>0.56 (0-2.52)</td>
<td>0.88 (0-3.82)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum Dairy Fatty Acid (mol% of total fatty acids)</td>
<td>0.25 ± 0.06</td>
<td>0.23 ± 0.05</td>
<td>0.24 ± 0.05</td>
<td>0.26 ± 0.05</td>
<td>0.25 ± 0.06</td>
<td>0.26 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pentadecanoic acid (15:0)</td>
<td>0.30 ± 0.10</td>
<td>0.30 ± 0.11</td>
<td>0.32 ± 0.10</td>
<td>0.32 ± 0.09</td>
<td>0.30 ± 0.10</td>
<td>0.29 ± 0.09</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Total dairy intake was defined as sum of whole milk; 2% milk; skim milk, 1% or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, non-fat or whole); low-fat flavoured yogurt (2% or non-fat); ice cream; frozen yogurt, ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea. Continuous variables are presented as mean ± SD if normal or median (IQR) if distribution is non-normal. Categorical variables are presented as n (%).

2 Range of total dairy intake per quintile (servings/week).

3 For continuous variables, p-value from ANOVA or Kruskal-Wallis comparison across quintiles for normal and skewed variables, respectively. For categorical variables, p-value from chi square value for comparison across quintiles.
Table 3-2: Regression analysis assessing the contribution of total dairy intake to serum 15:0 and trans 16:1n-7<sup>1</sup>

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n</th>
<th>β</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>659</td>
<td>0.002</td>
<td>0.0005</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>646</td>
<td>0.002</td>
<td>0.0005</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 3</td>
<td>646</td>
<td>0.002</td>
<td>0.0005</td>
<td>0.0002</td>
</tr>
<tr>
<td>Model 4</td>
<td>645</td>
<td>0.002</td>
<td>0.0005</td>
<td>0.0001</td>
</tr>
<tr>
<td>trans 16:1n-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>659</td>
<td>-0.002</td>
<td>0.0008</td>
<td>0.021</td>
</tr>
<tr>
<td>Model 2</td>
<td>646</td>
<td>-0.002</td>
<td>0.0009</td>
<td>0.07</td>
</tr>
<tr>
<td>Model 3</td>
<td>646</td>
<td>-0.003</td>
<td>0.0009</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 4</td>
<td>645</td>
<td>-0.003</td>
<td>0.0009</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<sup>1</sup>Total dairy intake was defined as sum of whole milk; 2% milk; skim milk, 1% or buttermilk; cottage and ricotta cheese; cheese; flavored yogurt (2%, non-fat or whole); low-fat flavoured yogurt (2% or non-fat); ice cream; frozen yogurt, ice milk; milk in coffee or tea; and cream or half-and-half in coffee or tea.

Model 1: Total dairy intake adjusted for age, sex, and ethnicity.
Model 2: Additionally adjusted for physical activity and total energy intake.
Model 3: Additionally adjusted for total hydrogenated food intake.
Model 4: Additionally adjusted for BMI.
Table 3-3  Regression analysis assessing the contribution of total partially hydrogenated food intake to serum 15:0 and trans 16:1n-7.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n</th>
<th>β</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>659</td>
<td>-0.0006</td>
<td>0.0004</td>
<td>0.11</td>
</tr>
<tr>
<td>Model 2</td>
<td>646</td>
<td>-0.0007</td>
<td>0.0004</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 3</td>
<td>646</td>
<td>-0.001</td>
<td>0.0004</td>
<td>0.008</td>
</tr>
<tr>
<td>Model 4</td>
<td>645</td>
<td>-0.001</td>
<td>0.0004</td>
<td>0.010</td>
</tr>
<tr>
<td>trans 16:1n-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1(^1)</td>
<td>659</td>
<td>0.003</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 2(^2)</td>
<td>646</td>
<td>0.003</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 3(^3)</td>
<td>646</td>
<td>0.003</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 4(^4)</td>
<td>645</td>
<td>0.003</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\) Total partially hydrogenated food was defined as sum of french fries and fried potatoes, salty snacks such as crackers, potato chips, corn chips, tortilla chips, pretzels, margarine on bread or roll, doughnuts, cookies, cakes, pastry, brownies, sopapillas, and pan dulce.

Model 1: Total partially hydrogenated food intake adjusted for age, sex, and ethnicity.
Model 2: Additionally adjusted for physical activity and total energy intake.
Model 3: Additionally adjusted for total hydrogenated food intake.
Model 4: Additionally adjusted for BMI.
Table 3-4: Cross-sectional multiple regression analyses evaluating associations of serum 15:0 and trans 16:1n-7 with log S_I and log DI

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Log S_I</th>
<th>Log DI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>β</td>
</tr>
<tr>
<td>15:0</td>
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<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>659</td>
<td>0.689</td>
</tr>
<tr>
<td>Model 2</td>
<td>645</td>
<td>0.909</td>
</tr>
<tr>
<td>Model 3</td>
<td>645</td>
<td>0.844</td>
</tr>
<tr>
<td>Model 4</td>
<td>644</td>
<td>0.504</td>
</tr>
<tr>
<td>trans 16:1n-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>659</td>
<td>-0.556</td>
</tr>
<tr>
<td>Model 2</td>
<td>645</td>
<td>-0.439</td>
</tr>
<tr>
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<td>-0.375</td>
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</table>

DI, Disposition Index; S_I, Insulin sensitivity index.
Model 1 was adjusted for age, sex, ethnicity, and centre.
Model 2: Additionally adjusted for physical activity, smoking status, alcohol intake, and education.
Model 3: Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes.
Model 4: Additionally adjusted for BMI.
Figure 3-2: Logistic regression analyses, overall and stratified by subgroups, for serum 15:0 with incident diabetes risk after 5 years

ORs per SD and 95% CI of each regression are shown, as well as interaction p values from the effect modification analyses. ORs were adjusted for age, sex, ethnicity, centre, physical activity, smoking status, alcohol intake, education, total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes. Overall: OR per SD = 0.73; 95% CI: 0.56, 0.95; p = 0.02. GT, glucose tolerance; IGT, impaired glucose tolerance.
3.5 Discussion

In the present study, we found that serum 15:0 was a significant biomarker for total dairy intake in this multi-ethnic cohort. In addition, 15:0 was positively associated with S₁ and DI, and was associated with a decreased incident diabetes risk after 5 years of follow-up, independent of demographic, lifestyle, and dietary variables. Further adjustment for BMI attenuated the cross-sectional results, suggesting that the associations may be partially mediated by adiposity. In contrast, trans 16:1n-7 was negatively associated with S₁, although further adjustment with lifestyle and dietary variables attenuated this result to non-significance. Although there was some evidence of stronger associations of 15:0 with diabetes and its underlying traits in specific subgroups, these results should be interpreted cautiously as the interaction terms were non-significant.

Few previous studies have examined the associations between 15:0 (18) and trans 16:1n-7 (16, 17) biomarkers with type 2 diabetes risk, as well as the underlying disorders of insulin resistance and beta-cell function. These studies largely demonstrated that higher levels of these fatty acids were associated with a lower risk in diabetes. In agreement with the present study, a sex- and age-matched nested case-referent prospective study of 159 Swedish participants free of diabetes at baseline showed that a higher proportion of 15:0 in erythrocyte membranes was associated with a 29% decrease in incident diabetes (OR = 0.71; 95% CI: 0.52, 0.97; p = 0.033) after 5 years of follow-up with limited adjustment for confounding variables (alcohol intake, BMI, HbA1c, smoking, and physical activity) (18). On the other hand, the EPIC cohort did not find significant decreases in diabetes risk with levels of 15:0 (19, 20). Furthermore, one previous cross-sectional study found no significant association of 15:0 with insulin resistance or beta-cell function (22).

Unlike previous studies, the present study did not find serum trans 16:1n-7 to be correlated with total dairy intake or serum 15:0, or to be inversely associated with diabetes risk. In the Cardiovascular Health Study (CHS), Mozaffarian et al. (16) found that trans 16:1n-7 was highly correlated with other biomarkers of dairy fat intake, including 15:0 (r = 0.64), and that higher levels of circulating trans 16:1n-7 were associated with lower risk of incident diabetes (Q5 versus Q1: HR = 0.38, 95% CI: 0.24, 0.62, p trend < 0.001). Furthermore, this study found that trans 16:1n-7 was associated with a 16.7% lower insulin resistance as measured by HOMA-IR (p
trend < 0.001). A more recent study by Mozaffarian et al. (17) also found trans 16:1n-7 to be significantly associated with lower incident diabetes in a multi-ethnic cohort (MESA), with similar findings across different ethnicity subgroup analyses. In contrast, high levels of trans 16:1n-7 in a European cohort were not significantly associated with a lower diabetes risk (19, 20), and adipose tissue trans 16:1n-7 was not associated with diabetes prevalence in a Costa Rican cohort (21). Inconsistencies in the results of these studies are perhaps due to differences in population characteristics, dairy intake behaviours, biological media used to measure fatty acids, and covariates used in the analyses.

Presently, the mechanism underlying the inverse relationship of 15:0 with diabetes risk is not known. It is possible that 15:0, given its significant correlation with total dairy intake, may be a marker for other beneficial components of dairy, such as calcium, vitamin D, magnesium, protein, probiotics or prebiotics (2, 3, 159). Alternatively, as previous studies have shown effects of other types of SFA on beta-cells and insulin sensitive tissues (88), 15:0 may have an as yet to be described direct effect on one or more of the traits underlying diabetes.

Optimal dietary biomarkers for epidemiological research are those that cannot be endogenously produced in the body. In terms of fatty acids, these include odd numbered, branched chained, and trans fatty acids (149). A number of fatty acids have been validated as biomarkers of dairy intake, including 15:0 and trans 16:1n-7. Studies have shown that 15:0, measured in adipose tissue (9, 11, 13, 14), serum (10-12, 14), plasma (15), and erythrocyte (15), is a valid biomarker for dairy intake with strong correlations between the fatty acid and dairy intake measured through dietary records. Our findings are consistent with these aforementioned validation studies on 15:0. In agreement with a previous study (17), partially hydrogenated foods were a source of trans 16:1n-7 in this cohort, as demonstrated by the significant positive association with total partially hydrogenated food intake in the fully adjusted linear regression. Trans 16:1n-7 in plasma (15-17, 160) and erythrocytes (15) has also been shown to be highly correlated to self-reported dairy intake. In contrast, trans 16:1n-7 was not significantly correlated with total dairy intake or 15:0 in our study. The samples in which the fatty acid measures were conducted in the IRAS cohort were collected in the early 1990s, prior to large-scale reformulation of foods to reduce TFA content (17, 161). This may explain the high correlation found in the current study between trans 16:1n-7 and total partially hydrogenated food intake, and not with total dairy intake or 15:0.
There are several strengths to this study. To the best of our knowledge, this is the first study to simultaneously examine the association of 15:0 and trans 16:1n-7 with incident diabetes, as well as its main underlying pathophysiological traits of insulin resistance and beta-cell dysfunction. The design of the IRAS cohort also allowed for the evaluation of these associations across multiple ethnicities and glucose tolerance status. In addition, insulin resistance and beta-cell dysfunction were assessed precisely using FSIGT. Other observational studies on dairy fatty acids and diabetes outcomes did not have measures with this degree of precision (16-19). As previously mentioned, using biomarkers in our analyses gave us a more objective measure of dairy intake compared to estimated obtained from FFQ data. Moreover, we adjusted for a broad range of potential demographic, lifestyle and dietary confounders in the analyses. On the other hand, given the observational design, this study is limited in that it cannot infer causal relationships between the exposure and outcomes. Also, although adjustments for several potential confounders were considered in the analyses, other confounding factors may still be unaccounted for.

3.6 Conclusion

In conclusion, serum 15:0 was a significant and independent short-term fatty acid biomarker for total dairy intake in the IRAS cohort. This fatty acid was positively associated with insulin sensitivity and beta-cell function, as well as a 27% decreased risk of incident diabetes after 5 years. Unlike previous studies, trans 16:1n-7 was not correlated with total dairy intake in this cohort, but rather with intake of partially hydrogenated fats. Further studies are required to evaluate the association between dairy fatty acid biomarkers and diabetes outcomes and its mechanisms, in order to inform future public health recommendations regarding dairy intake.
Supplemental Tables

S3-1: Multiple regression analyses of associations of 15:0 with log S$_I$ and log DI, stratified by sex$^1$

<table>
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<th>Log DI</th>
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<td>Female</td>
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<tr>
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<td>$p$</td>
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<td>$\beta$</td>
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<td>0.52</td>
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$^1$Interaction tests were run on Model 1, testing the product of 15:0 and sex: log S$_I$ interaction $p=0.05$ and log DI interaction $p=0.29$. DI, Disposition Index; S$_I$, Insulin sensitivity index.
Model 1: 15:0 adjusted for age, ethnicity, and centre
Model 2: Additionally adjusted for physical activity, smoking status, alcohol intake, and education
Model 3: Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes
Model 4: Additionally adjusted for BMI
Multiple regression analyses of associations of \( \text{trans} \ 16:1n-7 \) with log \( S_I \) and log \( DI \), stratified by glucose tolerance status

<table>
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<td>Model 4</td>
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</table>

Interaction tests were run on Model 1, testing the product of \( \text{trans} \ 16:1n-7 \) and glucose tolerance status: log \( S_I \) interaction \( p=0.68 \) and log \( DI \) interaction \( p=0.80 \). DI, Disposition Index; IGT, impaired glucose tolerance; \( S_I \), insulin sensitivity index.

Model 1: \( \text{Trans} \ 16:1n-7 \) adjusted for age, sex, ethnicity, and centre
Model 2: Additionally adjusted for physical activity, smoking status, alcohol intake, and education
Model 3: Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes
Model 4: Additionally adjusted for BMI
**S3-3:** Multiple regression analyses of associations of 15:0 and *trans* 16:1n-7 with log $S_I$, stratified by ethnicity

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1Interaction tests were run on Model 1, testing the product of 15:0 or *trans* 16:1n-7 and ethnicity. For 15:0: interaction $p=0.25$ and for trans 16:1n-7, interaction $p=0.72$. $S_I$, Insulin sensitivity index.

Model 1: Dairy fatty acids adjusted for age, sex, and centre
Model 2: Additionally adjusted for physical activity, smoking status, alcohol intake, and education
Model 3: Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes
Model 4: Additionally adjusted for BMI
Chapter 4  
Association Between Serum Pentadecanoic Acid (15:0) with Markers of Subclinical Inflammation

4.1 Abstract

Background: Low-grade systemic inflammation is an important risk factor for type 2 diabetes (T2DM). Increasing evidence indicates an inverse association between dairy consumption and T2DM risk, nevertheless there are limited studies exploring dairy’s potential modulation of circulating inflammatory marker concentrations.

Objective: We aimed to investigate the cross-sectional association of dairy fatty acid biomarker, pentadecanoic acid (15:0), with markers of subclinical inflammation in a large multi-ethnic cohort of non-diabetic adults.

Design: This study analyzed baseline data of 637 adults from multi-centre Insulin Resistance Atherosclerosis Study (IRAS). Serum 15:0 was quantified using gas chromatography. Inflammatory markers C-reactive protein (CRP), tumor necrosis factor (TNF-α), plasminogen activator inhibitor-1 (PAI-1), and fibrinogen were measured from fasting blood samples. Multiple linear regression models were adjusted for demographic, lifestyle, dietary variables, with additional adjustment for metabolic intermediates as pathway models. Subgroup analyses on a priori variables of interest, sex, glucose tolerance status, and ethnicity, were conducted for significant interaction terms.

Results: Serum 15:0 was negatively associated with log PAI-1 in fully adjusted models (β=-2.42, p<0.0001). This association was maintained with further adjustment for metabolic intermediates. 15:0 was also negatively associated with log CRP, in the minimally adjusted model (β=-1.56, p=0.03). Further adjustment for lifestyle, dietary and metabolic variables attenuated results to non-significance. Subgroup analyses by ethnicity showed a negative association between 15:0 and TNF-α in Hispanics in the fully adjusted model, with further adjustment for insulin sensitivity (β=-1.08, p=0.04).
Conclusion: Serum 15:0 was inversely associated with PAI-1 independent of demographic, lifestyle, dietary and metabolic variables. Findings from this study suggest that dairy may modulate chronic inflammation.

4.2 Introduction

Diabetes currently affects more than 382 million people worldwide (1), and with a projected increase to 439 million by 2030 (30), it is clearly a rising international health burden. Type 2 diabetes (T2DM) is characterized by hyperglycemia arising from chronic insulin resistance and pancreatic beta-cell dysfunction, the two main pathophysiological disorders underlying the disease (28). Although the etiologies of insulin resistance and beta-cell dysfunction are not entirely clear, there has been increasing interest in chronic subclinical inflammation as a key risk factor for these phenotypes (42, 43). In light of the increasing global burden of T2DM, there is an urgent need to identify easily accessible and cost-effective interventions which target the pathophysiological disorders underlying this condition.

There are a number of components in dairy that may directly impact cardiometabolic outcomes via the modulation of inflammation, including calcium (101, 162), lactoferrin (107, 108), peptides (163), vitamin D (2), and fatty acids (112). A number of observational studies suggest that dairy consumption may be inversely related to T2DM (2-4) and CVD risk (164), however few studies have assessed dairy’s role on markers of systemic inflammation. Only three cross-sectional studies have evaluated the association between dairy intake and inflammatory markers, with results showing reduced levels of CRP, IL-6, TNF-α, and soluble intercellular adhesion molecule-1 (sICAM-1), with higher intakes of low-fat dairy products in fully adjusted models (24, 25). Intakes of high fat dairy products showed conflicting results, with one study showing an inverse association with IL-6 and TNF-α (24), while the other study found high fat dairy to be positively associated with serum amyloid A (SAA) and sICAM-1 (25). There was no significant association between total dairy intake and inflammatory markers in the second study (25). The third observational study evaluated high dairy intake within the Mediterranean diet and found an inverse association with CRP and sICAM-1 (26).
Results from intervention trials have also been inconsistent, partly due to small sample sizes, short treatment durations, participant characteristics, and type and amount of dairy products consumed (165).

Pentadecanoic acid (15:0) has previously been used as an objective biomarker for dairy intake (9-14) as it provides an alternative to measuring dairy intakes using self-reported questionnaires, which are prone to measurement error (149). Biomarker use, therefore, is a way to better capture dairy exposure as it relates to outcomes. While a few observational and clinical studies have examined the relationship between dairy intake and markers of subclinical inflammation, very few studies have evaluated the association of dairy specific fatty acids with inflammation (17, 27, 112). Previous studies using dairy fatty acids have shown an inverse association with incident T2DM (18) and CVD risk (27), although the relationship of these fatty acids with underlying pathways involving inflammation have received limited attention.

The current study aimed to investigate the association of serum pentadecanoic acid (15:0) with markers of subclinical inflammation (CRP, TNF-α, PAI-1, and fibrinogen) in a large multi-ethnic cohort with a range of glucose tolerance status. Specifically, we evaluated associations with C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), and fibrinogen, which have been previously shown to be associated with insulin resistance (53, 66, 166), and predictive of incident T2DM in IRAS (49, 61), and in other cohorts (48, 50, 58, 167). We hypothesized that serum 15:0 would be inversely associated with markers of subclinical inflammation.

4.3 Subjects and Methods

The current paper analyzed data from the Insulin Resistance Atherosclerosis Study (IRAS), a multicenter epidemiologic study exploring associations between insulin resistance and CVD risk factors. A detailed methodology of the study has been published previously (152). The goal was to recruit equal representation of participants across ethnic groups (African American, Hispanic, non-Hispanic Whites), glucose tolerance status (normal, impaired glucose tolerance [IGT], and diabetes), sex, and age (40–49, 50–59, and 60–69 yrs) from four clinical centres: Los Angeles, CA, Oakland, CA, San Luis Valley, CO, and San Antonio, TX. Baseline clinic visits were
conducted from October 1992 to April 1994. All study participants gave written informed consent and institutional review boards of the centres approved the study.

The study recruited 1,625 adults. For the present analysis, participants without T2DM at baseline were included (n=1,072). With further exclusion of participants with missing measures of 15:0 and inflammatory markers (n=390), and participants with CRP > 10 mg/l (n=45), the final study sample for this analysis was 637 (Figure 4-1). Participants included in the final study sample did not systematically differ from those who were not included in terms of age, sex, and glucose tolerance status, although there were slight differences in the proportions of the 3 ethnicities in this study sample.

**Figure 4-1: Study population flowchart**

Baseline examinations were conducted during 2 clinic visits, occurring 1 week apart (152). Prior to each visit, participants were asked to fast for 12 h, avoid alcohol and heavy exercise for 24 h, and smoking on the morning of the clinic visit. Plasma samples for were centrifuged and stored at -70°C within 90 minutes of collection (168). Samples were analyzed for inflammatory markers at the Laboratory for Clinical Biochemistry Research at the University of Vermont (61). CRP
was measured using an in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, CA) with an interassay coefficient of variation (CV) of 8.9% (169). TNF-α was measured in duplicate in titrated citrated plasma using a Quantikine HS Human TNF-α immunoassay (R&D Systems, Minneapolis, MN), following manufacturer’s instructions, with a CV of 8.4-11.8% (166). PAI-1 in citrated plasma was measured using a two-site immunoassay (170), which was sensitive to free PAI-1 but not to PAI-1 complexed with tissue-type plasminogen activator (t-PA) (171); the CV was 14%. Fibrinogen was also measured in citrated plasma, using a modified clot-rate assay using the Diagnostica STAGO ST4 instrument (Diagnostica, Inc, Parsippany, NJ) (172), with a CV of 3.0% (173).

A 2-hr 75-g oral glucose tolerance test (Orange-dex, Custom Laboratories, Baltimore, MD, USA) was administered to determine glucose tolerance status based on the 1999 World Health Organization criteria (174). Incident diabetes cases were identified using OGTT, or current oral hypoglycaemic or insulin use. A frequently sampled intravenous glucose tolerance (FSIGT) test was administered to measure insulin sensitivity (insulin sensitivity index, Si), using minimal model analysis (MINMOD, version 3.0, 1994) (156). The FSIGT followed a validated modified protocol with an insulin injection at 20 minutes, and a reduced sampling protocol of 12 blood samples (152, 155).

Waist circumference and height were measured to the nearest 0.5 cm and weight was measured to the nearest 0.1 kg. Average values of the duplicate measures of these variables were used in the analysis. Total estimated energy expenditure (kcal/kg/week) was by assessed by summing energy expenditure activities and energy expenditure from sleep during the past year using a validated physical activity recall (152). Alcohol intake (g ethanol consumed/day) over the past month was assessed using a questionnaire, which also included questions on recent use and average lifetime use. Smoking status was categorized into 3 groups: never, past, or current. Ethnicity and age were self-reported, and medical history was assessed using structured interviews.

A semi-quantitative 114-item food frequency questionnaire (FFQ) assessed usual dietary intake over the previous year prior to baseline. The FFQ was modified from the National Cancer Institute (NCI) Health Habits and History Questionnaire to include ethnic and regional foods,
and was validated in a subsample of 186 women of the study population (153). An expanded nutrient database (HHHQ-DIETSYS analysis software, version 3.0; National Cancer Institute, Bethesda, MD, 1993) was used to estimate nutrient and energy intakes. Participants were asked to recall the usual frequency and portion sizes of foods and beverages over the past year. Servings per day were standardized to the medium serving size by multiplying the intake frequency with the portion size after applying a weighting factor (small = 0.5, medium = 1.0, large = 1.5). Total dairy intake was calculated by summing 11 line items pertaining to dairy foods.

**Fatty acid analysis**

Serum samples (stored at -70°C) were analyzed for a quantitative fatty acid profile as described previously (Lipomics Technologies Inc., West Sacramento, CA) (157). Briefly, total lipids were extracted in the presence of internal standards using the method of Folch (158). Fatty acid methyl esters (FAME) were formed by trans-esterification of total lipid extracts in sulfuric acid/methanol, extracted into hexane, and prepared for gas chromatography. Individual fatty acids were separated and quantified using capillary gas chromatography (Agilent Technologies model 6890) equipped with a 30m HP-88 capillary column (Agilent Technologies) and a flame-ionization. The absolute concentration of each fatty acid in the serum sample was measured by comparing its peak area to the internal standard. A total of 35 serum fatty acids were analyzed and quantified using these methods. In the present study the dairy derived fatty acid, pentadecanoic acid (15:0), was the primary exposure of interest and is expressed as the mole percentage (mol%) of total fatty acids.

**Statistical analysis**

Baseline characteristics across tertiles (T1-T3) of increasing 15:0 (mol% of total fatty acids) are shown. Normally distributed variables are presented as means ± SD, non-normally distributed variables are presented as medians with interquartile ranges (IQR), and categorical variables are presented as number and percent of participants in each tertile, with differences across tertiles tested using ANOVA, Kruskal-Wallis tests, and Chi square tests, respectively.
Unadjusted Spearman’s correlation coefficients (\(r\)) between the inflammatory markers and 15:0 were examined. Univariate distributions of all exposure variables were assessed, and CRP, TNF-\(\alpha\), and PAI-1 were log-transformed to achieve normality.

Multiple linear regression analyses were conducted to assess the cross-sectional relationships between 15:0 and inflammatory markers: log CRP, log TNF-\(\alpha\), log PAI-1, and fibrinogen. The regressions were sequentially adjusted for potential confounders in 5 models. Model 1 was adjusted for demographic and lifestyle variables: age, sex, ethnicity, centre, physical activity, smoking status, alcohol intake, and education. Model 2 was further adjusted for dietary variables: total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes. Models 3-5 were additionally adjusted for waist circumference, \(S_i\), and 2-hr postload glucose, respectively. These variables were considered as metabolic intermediates that may mediate the relationship between 15:0 and the plasma inflammatory marker concentrations. Effect modification analyses were conducted to assess potential interactions between 15:0 and variables of \textit{a priori} interest: sex, ethnicity, and glucose tolerance status. Stratified regression analyses were conducted for significant interaction terms (\(p<0.05\)).

All analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at \(p<0.05\).

### 4.4 Results

\textbf{Table 4-1} displays baseline characteristics of participants across tertiles of 15:0 (mol\% of total fatty acids). Ethnicity significantly differed across tertiles, with non-Hispanic whites having the highest levels of 15:0. Glucose tolerance status, smoking, and alcohol intake also differed significantly. Waist circumference decreased and \(S_i\) increased significantly across tertiles. The dietary variables, \%E from fat, \%E from SFA, and total dairy intakes increased across tertiles. The inflammatory markers did not significantly differ across groups, except for PAI-1, with Q3 having the lowest concentrations.

In univariate spearman correlation analysis, 15:0 was negatively correlated with CRP (\(r=-0.08, p=0.04\)) and PAI-1 (\(r=-0.10, p=0.009\)). 15:0 was not significantly correlated with fibrinogen or TNF-\(\alpha\) concentrations.
In multiple linear regression analysis, 15:0 was negatively associated with log PAI-1 after adjustment for age, sex, ethnicity, centre, physical activity, smoking status, alcohol intake, education (Model 1: $\beta=-2.49$, SEM=0.56, $p<0.0001$), and with further adjustment for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intakes (Model 2: $\beta=-2.42$, SEM=0.56, $p<0.0001$) (Table 4-2). Significance of the association was maintained with additional adjustment with metabolic intermediates: waist circumference (Model 3: $\beta=-1.89$, SEM=0.52, $p=0.0003$), SI (Model 4: $\beta=-2.09$, SEM=0.54, $p=0.0001$), or 2-hr glucose (Model 5: $\beta=-2.06$, SEM=0.55, $p=0.0002$). 15:0 was also negatively associated with log CRP, but only in the minimally adjusted model (Model 1: $\beta=-1.56$, SEM=0.70, $p=0.03$). 15:0 was not significantly associated with log TNF-α or fibrinogen levels.

Interaction $p$-values for variables of a priori interest were not significant, except for an interaction of ethnicity on the association of 15:0 with log TNF-α ($p=0.04$). In multiple linear regression, 15:0 was negatively associated with log TNF-α after adjustment for demographic, lifestyle, and dietary variables (Model 2 $\beta=-1.08$, SEM=0.52, $p=0.04$) in Hispanics (Table 4-3). Results remained significant after additional adjustment for SI (Model 4: $\beta=-1.08$, SEM=0.52, $p=0.04$), while the association was attenuated with adjustment for waist circumference (Model 3: $\beta=-0.86$, SEM=0.51, $p=0.09$) and 2-hr glucose (Model 5: $\beta=-0.97$, SEM=0.52, $p=0.06$). 15:0 was not significantly associated with log TNF-α in African American or non-Hispanic whites.
Table 4-1: Baseline characteristics of IRAS participants, overall and across tertiles of serum 15:0 (mol% of total fatty acids)¹

<table>
<thead>
<tr>
<th>Variable</th>
<th></th>
<th>Tertiles of 15:0 (mol% of total fatty acids)²</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (0.002-0.223)</td>
<td>T1 (0.223-0.270)</td>
<td>T2 (0.270-0.622)</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td>T1 (0.223-0.270)</td>
<td>T2 (0.270-0.622)</td>
</tr>
<tr>
<td>n (%)</td>
<td>637</td>
<td>212 (33.28)</td>
<td>213 (33.44)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.57±8.60</td>
<td>55.33±8.53</td>
<td>54.23±8.69</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>291 (45.68)</td>
<td>111 (52.36)</td>
<td>90 (42.250)</td>
</tr>
<tr>
<td>Females</td>
<td>346 (54.32)</td>
<td>101 (47.64)</td>
<td>123 (57.75)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>276 (43.33)</td>
<td>69 (32.55)</td>
<td>88 (41.31)</td>
</tr>
<tr>
<td>African American</td>
<td>148 (23.23)</td>
<td>78 (36.790</td>
<td>44 (20.66)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>213 (33.44)</td>
<td>65 (30.660</td>
<td>81 (38.03)</td>
</tr>
<tr>
<td>Glucose Tolerance Status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Glucose Tolerance</td>
<td>439 (68.92)</td>
<td>130 (61.320</td>
<td>151 (70.89)</td>
</tr>
<tr>
<td>Impaired Glucose Tolerance</td>
<td>198 (31.08)</td>
<td>82 (38.68)</td>
<td>62 (29.11)</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>291 (45.68)</td>
<td>79 (37.26)</td>
<td>101 (47.42)</td>
</tr>
<tr>
<td>Past</td>
<td>248 (38.93)</td>
<td>86 (40.57)</td>
<td>75 (35.21)</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>47 (22.17)</td>
<td>37 (17.37)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>Alcohol intake category, g ethanol/week</td>
<td>3.52 (0-44.59)</td>
<td>8.75 (0-104.16)</td>
<td>2.61 (0-25.77)</td>
</tr>
<tr>
<td>Highest education level completed, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12 years</td>
<td>79 (12.40)</td>
<td>31 (14.62)</td>
<td>31 (14.55)</td>
</tr>
<tr>
<td>≥ 12 years</td>
<td>558 (87.60)</td>
<td>181 (85.38)</td>
<td>182 (85.45)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>27.14 (24.74-30.11)</td>
<td>27.52 (24.81-30.97)</td>
<td>27.50 (25.06-30.19)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.73±12.45</td>
<td>92.11±14.02</td>
<td>89.16±11.53</td>
</tr>
<tr>
<td>Total estimated energy expenditure (kcal/kg/wk)</td>
<td>272.82 (251.31-306.54)</td>
<td>272.07 (248.44-315.31)</td>
<td>279.23 (253.47-308.83)</td>
</tr>
<tr>
<td>Insulin sensitivity (x10^4 min⁻¹[µU/ml]⁻¹)</td>
<td>1.79 (1.01-3.08)</td>
<td>1.64 (0.93-2.78)</td>
<td>1.71 (1.00-2.70)</td>
</tr>
<tr>
<td>Disposition Index</td>
<td>86.78 (45.36-164.07)</td>
<td>84.93 (39.6-140.42)</td>
<td>78.53 (42.52-140.44)</td>
</tr>
<tr>
<td>Dietary variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total E intake (kcal/week)</td>
<td>12,302.82 (9,006.60-16,162.25)</td>
<td>11799.32 (8501.92-15743.32)</td>
<td>12511.20 (9104.32-16518.28)</td>
</tr>
<tr>
<td>% E from fat</td>
<td>35.31±8.60</td>
<td>34.12±9.05</td>
<td>35.71±8.43</td>
</tr>
<tr>
<td>% E from SFA</td>
<td>12.00±3.44</td>
<td>11.40±3.74</td>
<td>12.01±3.27</td>
</tr>
<tr>
<td>Dietary intake TFA (g/week)</td>
<td>14.95 (7.28-25.90)</td>
<td>15.23 (6.65-27.65)</td>
<td>15.51 (7.35-26.43)</td>
</tr>
<tr>
<td>Total Dairy consumption (servings/week)</td>
<td>5.46 (2.59-8.54)</td>
<td>3.64 (1.42-8.02)</td>
<td>5.64 (2.80-8.96)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Total Milk consumption (servings/week)</td>
<td>0.98 (0-3.50)</td>
<td>0.49 (0-2.77)</td>
<td>0.98 (0-3.50)</td>
</tr>
<tr>
<td>Total Cheese consumption (servings/week)</td>
<td>1.47 (0.49-3.01)</td>
<td>0.98 (0.21-2.14)</td>
<td>1.54 (0.49-3.01)</td>
</tr>
<tr>
<td>Total yogurt consumption (servings/week)</td>
<td>0.21 (0-1.47)</td>
<td>0 (0-1.02)</td>
<td>0.21 (0-1.54)</td>
</tr>
<tr>
<td>Serum Dairy Fatty Acid (mol% of total fatty acids)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentadecanoic acid (15:0)</td>
<td>0.25±0.06</td>
<td>0.19±0.03</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td>Markers of subclinical inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>3.35 (2.61-4.32)</td>
<td>3.37 (2.59-4.42)</td>
<td>3.28 (2.62-4.25)</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>17 (10-28)</td>
<td>18 (11-32)</td>
<td>19 (10-30)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>270.14±48.35</td>
<td>270.86±48.20</td>
<td>274.09±47.36</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.59 (0.72-2.79)</td>
<td>1.77 (0.73-3.53)</td>
<td>1.59 (0.79-2.56)</td>
</tr>
</tbody>
</table>

1Continuous variables are presented as mean ± SD if normal or median (IQR) if distribution is non-normal. Categorical variables are presented as n (%).
2 Range of 15:0 per tertile (mol% of total fatty acids).
3 For continuous variables, *p*-value from ANOVA or Kruskal-Wallis comparison across quintiles for normal and skewed variables, respectively. For categorical variables, *p*-value from chi square value for comparison across quintiles.
Table 4-2: Cross-sectional multiple regression analyses evaluating associations of serum 15:0 with markers of subclinical inflammation: log TNF-α, log PAI-1, fibrinogen, and log CRP

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>n</th>
<th>β</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log TNF-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>623</td>
<td>-0.11</td>
<td>0.26</td>
<td>0.66</td>
</tr>
<tr>
<td>Model 2</td>
<td>623</td>
<td>-0.07</td>
<td>0.26</td>
<td>0.79</td>
</tr>
<tr>
<td>Model 3</td>
<td>621</td>
<td>0.07</td>
<td>0.25</td>
<td>0.78</td>
</tr>
<tr>
<td>Model 4</td>
<td>596</td>
<td>-0.02</td>
<td>0.26</td>
<td>0.93</td>
</tr>
<tr>
<td>Model 5</td>
<td>623</td>
<td>0.01</td>
<td>0.26</td>
<td>0.97</td>
</tr>
<tr>
<td>Log PAI-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>623</td>
<td>-2.49</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 2</td>
<td>623</td>
<td>-2.42</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 3</td>
<td>621</td>
<td>-1.89</td>
<td>0.52</td>
<td>0.0003</td>
</tr>
<tr>
<td>Model 4</td>
<td>596</td>
<td>-2.09</td>
<td>0.54</td>
<td>0.0001</td>
</tr>
<tr>
<td>Model 5</td>
<td>623</td>
<td>-2.06</td>
<td>0.55</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>623</td>
<td>-13.84</td>
<td>35.27</td>
<td>0.69</td>
</tr>
<tr>
<td>Model 2</td>
<td>623</td>
<td>-5.73</td>
<td>35.06</td>
<td>0.87</td>
</tr>
<tr>
<td>Model 3</td>
<td>621</td>
<td>12.46</td>
<td>34.38</td>
<td>0.72</td>
</tr>
<tr>
<td>Model 4</td>
<td>596</td>
<td>1.98</td>
<td>35.20</td>
<td>0.96</td>
</tr>
<tr>
<td>Model 5</td>
<td>623</td>
<td>0.49</td>
<td>35.20</td>
<td>0.99</td>
</tr>
<tr>
<td>Log CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>623</td>
<td>-1.56</td>
<td>0.70</td>
<td>0.027</td>
</tr>
<tr>
<td>Model 2</td>
<td>623</td>
<td>-1.34</td>
<td>0.69</td>
<td>0.05</td>
</tr>
<tr>
<td>Model 3</td>
<td>621</td>
<td>-0.73</td>
<td>0.64</td>
<td>0.26</td>
</tr>
<tr>
<td>Model 4</td>
<td>596</td>
<td>-1.13</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>Model 5</td>
<td>623</td>
<td>-0.97</td>
<td>0.68</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Model 1 (demographic and lifestyle): 15:0 adjusted for age, sex, ethnicity, centre, physical activity, smoking status, alcohol intake, and education.
Model 2 (dietary): Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intake.
Model 3 (metabolic intermediates): Model 2 additionally adjusted for waist circumference.
Model 4 (metabolic intermediates): Model 2 additionally adjusted for insulin sensitivity.
Model 5 (metabolic intermediates): Model 2 additionally adjusted for 2-hr glucose.
Table 4-3: Multiple regression analyses of associations of serum 15:0 with log TNF-α, stratified by ethnicity

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>African American</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>β</td>
<td>SEM</td>
</tr>
<tr>
<td>Model 1</td>
<td>272</td>
<td>0.27</td>
<td>0.38</td>
</tr>
<tr>
<td>Model 2</td>
<td>272</td>
<td>0.19</td>
<td>0.37</td>
</tr>
<tr>
<td>Model 3</td>
<td>271</td>
<td>0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>Model 4</td>
<td>257</td>
<td>0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>Model 5</td>
<td>272</td>
<td>0.27</td>
<td>0.37</td>
</tr>
</tbody>
</table>

1Interaction testing the product of 15:0 and ethnicity ($p=0.03$), adjusted for age sex, and ethnicity.

Model 1 (demographic and lifestyle): 15:0 adjusted for age, sex, centre, physical activity, smoking status, alcohol intake, and education.

Model 2 (dietary): Additionally adjusted for total energy intake, fruit and vegetables, red meat, soft drinks, and fibre intake.

Model 3 (metabolic intermediates): Model 2 additionally adjusted for waist circumference.

Model 4 (metabolic intermediates): Model 2 additionally adjusted for insulin sensitivity.

Model 5 (metabolic intermediates): Model 2 additionally adjusted for 2-hr glucose.
4.5 Discussion

In this cross-sectional analysis of IRAS participants without diabetes at baseline, we found that serum 15:0 was inversely associated with log PAI-1 in fully adjusted models, while a minimally adjusted inverse association with log CRP was attenuated with further adjustment of dietary and metabolic intermediate variables. TNF-α and fibrinogen concentrations were not associated with 15:0 levels. In subgroup analysis, 15:0 was negatively associated with log TNF-α in Hispanics after adjustment of demographic, lifestyle and dietary variables, and insulin sensitivity. The significant finding in Hispanics may be due to TNF-α having, on average, a higher concentration compared to the other ethnicities, and as a result, a higher TNF-α range to detect changes in concentration.

Notably, previous IRAS analyses established that higher CRP and TNF-α concentrations were cross-sectionally associated with insulin resistance (53, 166), and that PAI-1 was a strong and independent predictor of incident T2DM, compared to CRP and fibrinogen (49). It is notable that in the current analysis, circulating concentrations of PAI-1 were most strongly influenced by 15:0, even after multivariate adjustment.

To the best of our knowledge, the association of 15:0 with markers of subclinical inflammation has only been evaluated in one observational study. This association was assessed in a cross-sectional analysis, as a secondary outcome, in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort of US adults. The study reported a non-significant association between increasing quintiles of 15:0 and circulating CRP ($p=0.66$) (27). While these associations were adjusted for a number of potential confounders, there was no adjustment for total energy intake or other dietary variables. Our study, on the other hand, found 15:0 to be inversely associated with CRP in the minimally adjusted model, as well as PAI-1 in the fully adjusted model. Also, while the current study found 15:0 to be inversely associated with CRP in Hispanics, the MESA study did not report results of potential effect modification by ethnicity. Differences in these results may be due to differences in population characteristics, including their usual dairy intake, and potential confounders adjusted for the in multivariate analyses. Furthermore, as the MESA
study only evaluated CRP concentrations, further well-designed studies with measures of other inflammatory markers are warranted.

Circulating concentrations of 15:0 have been previously shown to be correlated with self-reported intakes of dairy (9-14). Since odd numbered fatty acids are not endogenously produced by humans, 15:0 is an optimal biomarker for dietary intake (149). We have previously shown that in IRAS, 15:0 is correlated with total dairy intake \( (r=0.20, p<0.0001) \), milk \( (r=0.13, p=0.0006) \), and cheese \( (r=0.16, p<0.0001) \) and that 15:0 was positively associated with total dairy intake in multivariate adjusted linear regression (Chapter 3).

To the best of our knowledge, the mechanism through which 15:0 affects levels of circulating markers of subclinical inflammation has not yet been studied. 15:0 composes a very small portion of total fatty acids measured in serum (<1%), and it is unclear whether this fatty acid is a biologically active compound or a marker of other potential protective bioactives found in dairy such as vitamin D, calcium, proteins, or other dairy specific fatty acids, such as c9, t11-CLA (2, 3, 175). Experimental and clinical studies may help elucidate whether supplementation or enrichment of dairy products with this fatty acid may be a potential therapeutic agent for cardiometabolic regulation (16).

This study has several strengths. First, we used a fatty acid biomarker in our analysis, providing a more objective measure of dairy intake. Second, IRAS is a well-characterized cohort with precise and detailed measurements of outcomes, exposures, and covariates. A limitation of the study is that the associations between the dairy fatty acid and inflammatory markers were analyzed cross-sectionally, which limited the evaluation of temporality. Furthermore, this study only had data for one dairy fatty acid, thus further studies on other known dairy fatty acid biomarkers are warranted. Another limitation is the potential for residual confounding in our analyses, beyond the demographic, lifestyle, dietary, and metabolic variables included in our models.

4.6 Conclusion

In conclusion, our study suggests that serum 15:0, a biomarker of dairy intake, may be inversely and independently associated with the inflammatory marker PAI-1. This study is among the first
of evaluate the role of dairy fatty acids on a variety of subclinical inflammatory markers. Our findings warrant further clinical and experimental studies to determine if there is a causal link between dairy consumption and its components, such as dairy specific fatty acids, with subclinical inflammation.
Chapter 5
Final Discussion

5.1 Summary of results

There is increasing evidence that dairy food intake is inversely associated with T2DM risk (2-4). However, the literature to date is unclear about dairy’s role on the underlying pathophysiology of T2DM. The aim of this thesis was to investigate the prospective association of dairy intake with incident T2DM after 5 years, and the cross-sectional associations with T2DM’s underlying disorders of insulin resistance, beta-cell dysfunction, as well as low-grade systemic inflammation in a large multi-ethnic cohort. Fatty acids measured in serum, specifically 15:0 and trans 16:1n-7, were used as biomarkers for dairy intake.

In this IRAS cohort, among participants with no diabetes at baseline, serum 15:0 was a significant biomarker for dairy intake. 15:0 was associated with lower T2DM risk after 5 years of follow-up, independent of demographic, lifestyle, and dietary variables. The significance of the association was maintained after additional adjustment for adiposity in mechanistic models. In the cross-sectional analyses, 15:0 was positively associated with insulin sensitivity, beta-cell function, in fully adjusted models. Further adjustment for BMI or waist circumference attenuated the association, suggesting that adiposity may partially mediate the relationship observed. 15:0 was also negatively associated with PAI-1 in the fully adjusted model, and after further adjustment for metabolic intermediates (waist circumference, Si, or 2-hr glucose). On the other hand, serum trans 16:1n-7 was a marker of partially hydrogenated fat intake, but not dairy intake, in this cohort and was not significantly associated with incident T2DM or its underlying disorders.

These results suggest that dairy’s protective role on T2DM risk may occur through changes in insulin sensitivity, beta-cell function, and systemic inflammation. Although the exact mechanisms have not yet been elucidated, 15:0 may possibly exert direct effects along the pathways of these underlying disorders or it may reflect beneficial effects of other components.
found in dairy foods, or a healthy dietary pattern (2, 3). Further studies evaluating the role of 15:0 on T2DM’s underlying pathophysiology are warranted.

5.2 Limitations

There are some limitations to consider in this study. First, as the association of 15:0 and trans 16:1n-7 with insulin sensitivity, beta-cell function, and inflammatory markers were examined cross-sectionally, the causality between serum fatty acids levels on these outcomes cannot be inferred and the temporality of the associations cannot be determined. Furthermore, although we found significant associations with certain subgroups in stratified analyses with a priori variables of interest, the interaction terms were largely not significant. This may be due to limited statistical power in each subgroup, and as such, further studies that are adequately powered for subgroup analyses are warranted to confirm the results of this study. Also, while potential confounding factors were considered in the analyses, there may be residual confounding by other factors.

While a number of other dairy specific fatty acids have been identified and used as biomarkers for dairy intake in previous studies, including 17:0, vaccenic acid, and c9,t11-CLA (9-11, 21, 99), the IRAS dataset only had serum levels of 15:0 and trans 16:1n-7 available. This limited the number of exposures used in this study, and it would have been of interest to compare the associations of T2DM outcomes among different dairy fatty acids.

Subclinical inflammation was measured using four inflammatory markers in the second study, and the only independent inverse association was found for 15:0 and PAI-1. Currently, there is no consensus on the optimal number or specific cluster of inflammatory markers that may define chronic systemic inflammation. Further studies on other markers of inflammation are warranted, as 15:0 may possibly have significant associations with other inflammatory markers, as suggested by observational (24-26) and clinical trials evaluating dairy intake (165).

5.3 Strengths

The large IRAS cohort included participants from three ethnic groups with a range of glucose tolerance status, allowing for a degree of ethnic generalizability of the study results. In addition, all metabolic, anthropometric, lifestyle, and dietary data in the cohort were assessed using
detailed and validated measures. In particular, FSIGT provided direct measures of insulin sensitivity and beta-cell function. Previous studies on dairy or dairy fatty acids and T2DM outcomes did not have these specific measurements. Also, diabetes status was assessed using the gold standard OGTT. In addition, demographic, lifestyle, dietary, and metabolic variables were considered in the regression models, to account for potential confounders in the analyses.

A major strength of this study is the use of dairy specific fatty acids as biomarkers for dairy intake. Biomarkers are powerful tools in nutritional epidemiology as they overcome the many limitations of self-reported dietary intake, and thus provide more objective measures of dietary exposure (15). Previous studies on 15:0 and trans 16:1n-7 have largely focused on diabetes risk as the primary outcome, and these studies had limited or no characterization of the underlying disorders of diabetes. Moreover, studies on 15:0 and trans 16:1n-7 with subclinical inflammatory were limited in that they have only evaluated 2 inflammatory markers (16, 17, 27), unlike the current study where measurements for 4 markers were available. This study is novel in that it was able to examine how 15:0 and trans 16:1n-7 were associated with long-term T2DM risk, and more importantly, how these dairy fatty acid biomarkers impacted the underlying pathophysiology of the disease.

5.4 Implications and future directions

The present thesis has provided evidence that 15:0 may be associated with lower T2DM risk, and further, that 15:0 may potentially lower long-term diabetes risk through pathways related to insulin sensitivity, beta-cell function, and systemic inflammation. This study corroborates the potential protective role of dairy foods on T2DM etiology and supports current U.S. dietary recommendations for 3 servings of dairy food intake per day (92).

In the design of the study, it was initially hypothesized that trans 16:1n-7 was a biomarker for dairy intake, as previous studies had reported (16, 17). In contrast, trans 16:1n-7 was in fact correlated with partially hydrogenated fat intake (and not dairy intake) in IRAS, another source of this fatty acid. Notably, baseline serum collection in this cohort was conducted prior to the widespread reduction of TFA in US food supply in the early to mid-2000s (176), and it has been proposed that this lowering of TFA has contributed to the decreased plasma TFA concentrations that have been documented in the U.S. population (176). Given this secular change in TFA
intake, it is likely that serum trans 16:1n-7 in the IRAS cohort reflected partially hydrogenated fat intake more so than dairy food intake, in contrast to more recently assembled U.S. cohorts (17).

Given the richness of the IRAS dataset, the next steps for this project may involve the use of statistical methods that allow for the evaluation of patterns of multiple exposures and outcomes, helping elucidate the role of dairy on T2DM and other cardiometabolic risk factors. A recent paper has proposed novel statistical methods that combine dietary and nutritional biomarker data in order to strengthen the power of analyses to detect relationships between diet and disease (137). One such approach is Principal Component Analysis (PCA), which is a variable reduction technique that creates new exposure variables, called principal components, which explain most of the variation of the original set of variables (177). PCA may also be used to identify novel fatty acid patterns (177) to evaluate the complex physiological and statistical interrelationships between the variables (178).

Confirmation of findings in this study in other populations is needed to establish the role of 15:0 with T2DM outcomes, as the current literature is scarce, especially on the relationship with T2DM’s underlying disorders. In addition, as 15:0 was measured in serum in this study, which reflects short-term intake (143), it would be interesting to determine prospective associations between T2DM outcomes with 15:0 measured in adipose tissue, which reflects long-term cumulative intake (143). Furthermore, well-designed clinical trials on 15:0 would also help determine whether intakes of this fatty acid have direct effects on T2DM outcomes, or if it is simply a biomarker of dairy intake. Moreover, although a number of animal and in vitro studies have examined the metabolic mechanisms of SFA on insulin sensitivity and beta-cell function (88), 15:0 in particular has not yet received any attention, and as such it is currently unclear whether 15:0 has direct metabolic effects or if it is simply a marker of other beneficial components in dairy, or a healthy dietary pattern.

5.5 Conclusion

In conclusion, this thesis investigated the association between dairy fatty acid biomarkers and incident T2DM after five years, as well as its underlying disorders, in order to shed light on potential pathways through which dairy intake may be inversely related to T2DM. This study
provides evidence that serum 15:0 is associated with lower T2DM risk in IRAS, and that this fatty acid may impact T2DM etiology through favourable effects on insulin resistance, beta-cell function, and PAI-1 concentration. On the other hand, trans 16:1n-7 was found to be a marker of partially hydrogenated fat intake in this cohort, and was not significantly associated with any of the T2DM outcomes. Overall, the results of the study support the potential beneficial effects of dairy on cardiometabolic outcomes.

This novel study will extend current knowledge on dairy consumption and T2DM, and potentially help elucidate dairy’s effects on the underlying pathophysiological disorders that impact T2DM. The findings of this study warrant further investigation on the role and mechanisms of dairy fatty acids in T2DM etiology, and may contribute to the development novel therapies for T2DM prevention or treatment.
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