The Impact of Low-Fat and Full-Fat Dairy Consumption on Metabolic Health: A Randomized Dietary Intervention Trial

Kelsey A. Schmidt

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Reading Committee:

Mario Kratz, Chair

Kristina M. Utzschneider

Sarah E. Holte

Michael E. Rosenfeld

Program Authorized to offer degree:

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ABSTRACT

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Kelsey A. Schmidt

Chair of the Supervisory Committee:
Mario Kratz, PhD
Department of Epidemiology

Background: Dairy is often considered an important part of a healthful diet. However, questions remain as to the type of dairy foods that best promote cardiometabolic health. In the observational literature, there is particularly strong and consistent evidence linking yogurt and biomarkers of full-fat dairy food intake with a reduced risk of type 2 diabetes. Further, emerging evidence challenges the long-standing view that full-fat dairy foods promote cardiovascular disease through the lipid altering affects of its high saturated fat content. Rather, evidence indicates that dairy fat may not negatively impact CVD risk, particularly when consumed in whole foods with a complex matrix. However, trials investigating the effect of dairy foods on cardiometabolic health rarely include full-fat or fermented dairy foods in their interventions and often test only one type of dairy food. Therefore, we aimed to compare the effects of diets rich in a wide variety of either low-fat or full-fat dairy foods, or a diet limited in dairy on glucose tolerance and its determinants as well as cardiovascular disease risk factors.

Methods: This was a parallel-design randomized controlled trial set at an urban research center in the northwestern United States. Seventy-two men and women with metabolic syndrome completed a 4-week wash-in period, limiting dairy intake to ≤ 3 servings of nonfat milk per week. Participants were then randomized to either continue the limited dairy diet, or switch to a diet containing 3.3 servings per day of either low-fat or full-fat milk, yogurt, and cheese for 12-weeks (n=24 per group). Main outcome measures included glucose tolerance as measured by the area-under-the-curve glucose during a standardized 180-minute oral glucose tolerance test

(primary), insulin sensitivity, pancreatic β-cell function, systemic inflammation, liver fat content, body weight and composition, a comprehensive fasting lipid profile, and blood pressure.

Results: In the primary *per-protocol* analysis (n=67), there was no treatment effect on glucose tolerance (p=0.340, overall repeated measures analysis of variance). However, both the low-fat and full-fat dairy diets decreased the Matsuda insulin sensitivity index (p=0.012 overall) and increased the homeostatic model assessment of insulin resistance (p=0.005 overall) as compared to the limited dairy group. Two measures of pancreatic β-cell function, insulinogenic index and glucose sensitivity did not change differentially. Dairy consumption also resulted in weight gain (p=0.006 overall), with an increase in the full-fat dairy diet compared to the limited dairy diet, and the low-fat dairy diet falling in-between. Intervention effects on insulin sensitivity were not, or only mildly, attenuated by adjusting for changes in adiposity. No intervention effects were seen in measures of liver fat content or biomarkers of systemic inflammation. Further, there were no intervention effects on fasting plasma total-, low-density lipoprotein-, and high-density lipoprotein-cholesterol; triglycerides; free fatty acids; or cholesterol content in 38 isolated lipoprotein fractions (p>0.1 for all variables). There was also no intervention effect on diastolic blood pressure. There was a significant intervention effect for systolic blood pressure (p=0.045), with a trend for a decrease in the low-fat dairy diet compared to the limited dairy diet in *post hoc* testing after adjustment for multiple testing. Results from the *intent-to-treat* analysis (n=72) were consistent with those of the *per-protocol* analysis.

Conclusions: Contrary to our hypothesis, consuming 3.3 servings per day of low-fat dairy or an equivalent amount of full-fat dairy, did not improve glucose tolerance in men and women with the metabolic syndrome. Both dairy diets similarly decreased insulin sensitivity through mechanisms largely unrelated to changes in body weight, fat mass, liver fat content, or biomarkers of systemic inflammation. Additionally, dairy fat, when consumed as part of mixed complex whole foods such as milk, yogurt, and cheese, did not adversely impact the serum lipid profile. Overall, our findings indicate that lower dairy intake may be beneficial in individuals with the metabolic syndrome and that full-fat dairy products do not differ from low-fat dairy products in regards to their impact on cardiometabolic health.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

LIST OF ACRONYMS

AUC: area under the curve

AMPK: monophosphate-activated protein kinase

BCAA: branched chain amino acids

BMI: body mass index (kg/m^2)

CRP: C-reactive protein

CVD: cardiovascular disease

DEXA: dual-energy x-ray absorptiometry

FABPs: fatty acid binding proteins

FFAs: free fatty acids

FFQ: food frequency questionnaires

FS-OGTT: frequently sampled oral glucose tolerance test

GIR: glucose infusion rate

GLUT-4: glucose transporter type 4

HbA1c: hemoglobin A1c

HDL: high density lipoprotein

HOMA-IS: Homeostatic Model Assessment-Insulin Sensitivity

HOMA-IR: Homeostatic Model Assessment- Insulin Resistance

HOMA-%B: Homeostatic Model of β-cell function

IFN-γ: interferon gamma

IGT: impaired glucose tolerance

IL: interleukin

IR: insulin resistance

IRS-1: insulin receptor substrate 1

ITT: *intent-to treat*

IVGTT: intravenous glucose tolerance test

LDL: low density lipoprotein

LPL: lipoprotein lipase

MCP: monocyte chemoattractant protein

MR: mineralcorticoid receptor

MetS: metabolic syndrome

NAFLD: non-alcoholic fatty liver disease

NFκB: nuclear factor kappa B

NHANES: National Health and Nutrition Examination Survey

LPL: lipoprotein lipase

OGTT: oral glucose tolerance test

PI3K: phosphoinositide 3-kinase

PKB/Akt: protein kinase B

PPAR: peroxisome proliferator activated receptor

PTH: parathyroid hormone

RCT: randomized controlled trial

ROS: reactive oxygen species

RR: relative risk

SFAs: saturated fatty acids

T2D: type 2 diabetes mellitus

T2D: type 2 diabetes

TLRs: toll-like receptors

TNF-α: tumor necrosis factor alpha

US: United States

VLDL: very low density lipoprotein

WC: waist circumference

WHI: Women's Health Initiative

WHR: waist to hip ratio

WHtR: waist to height ratio

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CHAPTER 1: BACKGROUND AND SIGNFICANCE

1.0 Burden of Obesity and Chronic Disease

1.1 Chronic Disease, Mortality and Economic Impact

Chronic disease places a major health and fiscal burden on individuals and economies around the globe. According to the World Health Organization, chronic disease claims the lives of 41 million people annually world-wide[1], accounting for 73% of total deaths[2]. Chronic disease is also the leading cause of death and disability in the United States (US). According to the Center for Disease Control's National Center for Chronic Disease Prevention and Health Promotion, one in six Americans have at least one chronic disease and four in ten Americans have two or more chronic diseases[3]. Approximately two thirds of US healthcare costs are spent on those with multiple chronic conditions[4], resulting in $3.5 trillion in annual health care costs[3]. Consequently, chronic disease poses both a significant health and economic burden. As a result, an initiative by the Department of Health and Human Services was issued calling for more research on multiple chronic conditions to identify solutions to improve chronic disease prevention and treatment options[5]. Without new treatment and prevention strategies, it is estimated that 171 million Americans will have more than one chronic disease by 2030[6]. This would continue to pose both a physical, psychological, and fiscal burden on individuals and societies.

1.1.1 Burden of Type 2 Diabetes

Type 2 diabetes (T2D) is a chronic disease related to obesity and increases the risk for other chronic diseases independent of body mass index (BMI)[7]. The global burden of diabetes is currently estimated at 451 million people, with the vast majority having T2D[8]. Without intervention, the prevalence is projected to rise to 693 million people by 2045[8]. As of 2016, 8.6% of the US population, or 21 million adults had diagnosed T2D[9]. The prevalence of T2D is concerning because it is associated with a myriad of comorbidities and complications such as non-alcoholic fatty liver disease (NAFLD), periodontal disease, hearing loss, erectile dysfunction, depression, heart disease and stroke, blindness, kidney failure, and lower-limb amputation[10]. Diabetes is a serious health concern, with 277,000 deaths attributable to diabetes in the US in 2017[11], making it one of the top 10 causes of death in the US[12]. T2D also places a

significant financial burden on the US healthcare system. In 2017, $327 billion was spent on diabetes, which includes $237 billion in direct medical costs and $90 billion in decreased productivity, defined as people being absent from work, being less productive while working, not working at all, or lost productivity due to early mortality[11]. Furthermore, individuals with diabetes incur on average $16,750 in medical expenditures annually with $9,600 attributed to their diabetes[11]. T2D is a serious health concern and is one major piece in a complex network of chronic disease plaguing the US.

1.1.2 Burden of Cardiovascular Disease

Cardiovascular disease (CVD), encompassing coronary heart disease, heart failure, stroke, and hypertension; is the leading cause of death globally[2]. In 2015, there were an estimated 423 million CVD cases around the world[13] and in 2017, CVD contributed to an estimated 17.8 million deaths world-wide[2]. In the US, according to National Health and Nutrition Examination Survey (NHANES) data, the 2016 prevalence of CVD was 48.0% or 121.5 million adults[14]. With over 635,000 individuals dying of heart disease and over 140,000 people dying of cerebrovascular diseases in the US in 2016, it is not surprising that CVD accounts for two of the ten leading causes of death in the US, including the primary cause of death[15]. As a result, 14% of the United States' overall national health expenditures are spent on CVD[14]. This equated to $351 billion on CVD spending, including $214 billion in direct costs and $137 billion in lost productively in 2015[14]. It is projected that by 2035, 45.1% of the US population will have some from of CVD and that this will lead to an increase in the financial burden of CVD, costing $749 billion annually by 2035[14]. Without changes in policy and treatment as well as steps to reduce risk factors, these numbers may end up being even larger than predicted.

1.2 The Obesity Epidemic

The obesity epidemic continues to grow, becoming a major threat to the health of individuals and economies around the globe. According to the 2017-2018 NHANES, 42.4% of US adults are obese (BMI $\geq$ 30 kg/m^2), and 9.2% are severely obese (BMI $\geq$ 40 kg/m^2)[16]. Further, this condition is not isolated to adults; using data from the 2015-2016 NHANES, 18.5% of children 2-19 years old were also obese in the US[17]. When comparing these numbers to data from the 1999-2000 NHANES, this indicates a significant and continued increase in the prevalence of

obesity over time[16,17]. Without research identifying key prevention strategies, it is projected that the obesity prevalence will continue to rise. In fact, Wang et al. predicts that by 2030 46.5% of the US population will be obese, with 10% of the US population classified as severely obese[18]. Obesity is a serious health concern because it is associated with an increased risk of a number of health conditions including diabetes, hypertension, hyperlipidemia, CVD, arthritis, gallbladder disease, and certain cancers[19,20]. Consequently, those with a BMI between 35-40 kg/m^2, have a reduced life expectancy of five years as compared to those with a BMI in the upper normal range of 21-25 kg/m^2 [21].

Obesity is not only a health concern, but also a fiscal issue[22-24]. Direct medical costs are up to 42% higher for obese individuals as compared to normal weight individuals[20,24,25]. A 2016 meta-analysis of studies investigating the economic burden of obesity in the US reported that the direct medical costs of obesity was $1,910 per person annually[26]. This translates to nearly $150 billion on the national level[26]. It is estimated that obesity-related costs could account for up to 20% of medical spending in the US[25]. Furthermore, it is predicted that there will be an increase in annual medical costs to treat obesity-related illnesses of $22 billion per year by 2020 and $48 billion per year by 2030[27]. While the cost of health care is often initially covered by private insurance agencies, ultimately these costs are absorbed by employers and employees as higher premiums, copayments, and deductibles increase to cover medical services[24]. Therefore, the cost of obesity impacts each and every American. Additionally, obesity related loss in productivity due to absenteeism and loss of presenteeism costs between $797 to $6,694 per person annually depending on gender and obesity class[24]. Overall, Finkelstein et al. estimates that the total cost of obesity in the US due to medical expenditures, absenteeism and presenteesim is $73.1 billion per year[24]. To put this in perspective, this is equivalent to hiring 1.8 million additional employees at an annual salary of $42,000[24]. If we stay on this current trajectory, it is estimated that by 2030, obesity related loss in productivity could cost $580 billion annually[25]. Moreover, based on the trends of obesity from 2000-2010, it is estimated that obesity will result in the loss of 24.5 million quality-adjusted life-years in the US over the 20 year period between 2010-2030[27]. For all these reasons, obesity has become the most costly preventable disease in the US[25]. The burden and cost of obesity observed in the US is similarly seen across the world as the

prevalence of obesity continues to rise globally[20]. Therefore, obesity is both a serious health concern and a pressing financial issue that needs to be addressed.

1.3 Association Between Obesity and Chronic Disease Risk

1.3.1 Association Between Obesity and Type 2 Diabetes

Obesity is associated with an increased risk of T2D. Abdullah et al. investigated the relationship between adiposity and the risk of diabetes through a meta-analysis including 18 prospective cohort studies, a total sample size of 590,251 participants, and data from various regions of the world including the US, Asia-Pacific, and Europe. They found that obese individuals had a seven times higher risk of diabetes and overweight individuals had an almost three times higher risk of diabetes when compared to their normal weight peers[28]. It should be noted, however, that there were variations in the relative risks (RRs) according to the study population characteristics including gender and study region, as well as study quality characteristics such as sample size, method of diabetes assessment, and method of BMI ascertainment[28]. Notably, obese women had a higher risk for T2D with a RR of approximately eight, whereas obese men had a RR of T2D of approximately six when compared to their normal weight peers[28]. The reasons for this sex difference remain unclear, but Abdullah et al. speculated that this may be a result of gender differences in fat mass and distribution[28]. Additionally, it remains unclear whether there is a linear relationship between obesity and T2D or rather a threshold effect[28].

In order to assess how metabolic health modifies the relationship between adiposity and T2D risk, a meta-analysis by Bell et al. investigated the relationship between obesity and risk of T2D in both metabolically healthy and unhealthy obese and normal weight participants. A total of eight prospective studies were included in this analysis. Obese individuals who were classified as metabolically healthy (defined by BMI, normal cardiometabolic clustering, and insulin profile or risks score) had an increased risk of T2D when compared to metabolically healthy normal weight controls with a RR of 4.03[29]. Though, it should be mentioned that the way metabolic health was defined in this meta-analysis was typically based on the definition of metabolic syndrome (MetS). Therefore, studies could have categorized individuals with impaired glycemic control as metabolically healthy as long as they did not have any of the other MetS criteria. Given that

impaired glycemic control is a clear risk factor for T2D, many would argue that these individuals were not metabolically healthy at baseline. Though, it is unclear how many individuals included in these studies were classified as metabolically healthy while having impaired glycemic control. Further, the one study included in the meta-analysis that excluded individuals with impaired fasting glucose through their definition of metabolic health, found similar increases in risk to the overall risk estimate. Therefore, while it remains unclear how excluding individuals with impaired glycemic control from the studies included in this meta-analysis might have changed the magnitude of the risk estimate, Bell et al. still provides evidence that obesity is an independent risk factor for T2D. These kinds of observations have led some to question the concept of metabolically healthy obese[30].

While the Bell et al. meta-analysis indicates that obesity likely increases the risk of T2D independent of metabolic health, the investigators also provide evidence that it is probable that other metabolic parameters modify the risk of an obese individual's progression to T2D. Combining the results of eight prospective studies, the investigators revealed that metabolically unhealthy obese individuals have a nine fold increased risk of T2D when compared to metabolically healthy normal weight controls[29], which is substantially larger than the four fold increased risk of T2D observed in those classified as metabolically healthy obese. Further, a smaller sub-analysis of the English Longitudinal Study of Ageing with 3,066 individuals found that unhealthy normal-weight individuals showed a higher risk for T2D development when compared to healthy obese subjects[29]. It should be noted that the metabolically healthy obese were on average younger, less likely to be smokers, and had intermediate levels of risk factors when compared to both the metabolically unhealthy obese and non-obese[29]. Additionally, the metabolically healthy obese had a lower BMI when compared to the unhealthy obese counterparts[29]. Altogether, this indicates that there are other contributing factors to the development of T2D besides obesity.

Adiposity can be measured using a variety of tools including BMI, waist circumference (WC), waist to hip ratio (WHR), and waist to height ratio (WHtR)[31]. BMI is an indicator of overall fat mass, whereas WC and WHR are traditionally thought to capture abdominal obesity, where there are increased levels of visceral fat[31]. A meta-analysis by Kodama et al, identified 15 prospective

cohort studies that investigated the relationship between obesity measured as WHtR and at least one additional obesity indicator (BMI, WC, or WHR) and T2D. Per one standard deviation increase in WHtR the RR for T2D was 1.62[31]. Per one standard deviation increase in BMI, WC, and WHR, the relative risks for T2D were 1.55, 1.63, and 1.52 respectively[31]. This meta-analysis corroborates previous findings that obesity is associated with T2D. These results also indicate that WC and WHtR may be slightly stronger indicators for obesity-related T2D when compared to WHR or BMI. This suggests that the relationship between abdominal obesity and T2D is stronger than measures of total adiposity. Overall, the evidence is clear that obesity is associated with an increased risk of T2D.

1.3.2 Association Between Obesity and Cardiovascular Disease

There is a positive association between adiposity and the development of CVD. A prospective cohort of over one million US adults enrolled in the Cancer Prevention Study II showed that BMI is predictive of death from CVD[32]. Men with a BMI above 35 kg/m^2 had a RR of death from CVD of 2.90 as compared to men with a BMI of 23.4-24.9 kg/m^2 [32]. Further, the study indicated that just being overweight confers an increased risk. Women with a BMI above 25 kg/m^2 and men with a BMI above 26.5 kg/m^2 were at a significantly increased risk of death from CVD as compared to those with a BMI of 23.5-24.9 kg/m^2 [32]. Additionally, there appears to be a dose response relationship between adiposity and CVD risk. A meta-analysis of 57 prospective cohort studies that included approximately 900,000 adults concluded that for every 5 kg/m^2 increase in BMI above 25 kg/m^2, cardiovascular mortality increased by 40%[33]. A meta-analysis of 15 prospective studies and randomized clinical trials of CVD risk and abdominal obesity determined that every one centimeter increase in WC resulted in an increase in the RR of CVD by 2% and for every 0.01 unit increase in WHR the RR increased by 5%[34]. This is why the American Heart Association identifies obesity as a major risk factor for heart disease, placing it on par with cigarette smoking, physical inactivity, and high blood cholesterol[35].

The relationship between obesity and CVD is modified by metabolic health. A meta-analysis of eight prospective cohort studies including 187,918 participants investigated the relationship between obesity, metabolic health, and CVD risk. Fan et al. found that being overweight or obese was associated with a RR of CVD of 2.30 compared to normal weight metabolically

healthy individuals[36]. This risk was altered by sex and metabolic health. Metabolically healthy obese men had a RR for CVD of 1.84 whereas metabolically unhealthy obese men had a RR for CVD of 2.45 when compared to normal weight metabolically healthy individuals[36]. Metabolically healthy obese women had a RR for CVD of 1.95 and metabolically unhealthy obese women had a RR of CVD of 3.21 when compared to normal weight metabolically healthy peers[36]. Similarly when looking at six prospective cohort studies that had CVD mortality as an endpoint, metabolically healthy obese had a RR of 1.37 and metabolically unhealthy obese had a RR of 2.01 for CVD mortality when compared to their normal weight peers[36]. Furthermore, the relationship between metabolically healthy obese and risk of CVD appeared to be stronger when comparing those studies with follow-up periods greater than 15 years to those with less[36]. This is in alignment with a review by Ginsberg and MacCallum, which concluded that individuals with MetS or T2D were at an increased risk for CVD[37]. Within this review, they noted that data from the NHANES III found that in adults 50 years or older, the prevalence of heart disease was greatest in individuals who had MetS and diabetes[37]. This review article further indicates that it is not just obesity, but also metabolic health that plays a key role in determining one's risk for CVD.

2.0 Metabolic Dysfunction as a Risk Factor For Chronic Disease

2.1 Obesity is Associated with Metabolic Dysfunction

As discussed in section 1.3, obesity is associated with chronic diseases including T2D and CVD. These associations support the hypothesis that excess adipose tissue may play an important role in the pathogenesis of chronic disease. Additionally, obesity is associated with a range of cardiometabolic risk factors including systemic inflammation, insulin resistance, impaired glucose tolerance (IGT), NAFLD, hypertension, and dyslipidemia. The association between obesity and these cardiometabolic risk factors will be outlined in the following sections, including some insight into potential mechanisms.

2.1.1 Excess Fat Mass and Chronic Systemic Inflammation

Obesity is associated with increased subclinical chronic systemic inflammation as identified through biomarkers such as C-reactive protein (CRP), pro-inflammatory cytokines, adhesion molecules and increased oxidative stress[7,38,39]. It appears that the level of systemic inflammation may be dependent on the distribution of fat. Specifically, increased visceral adiposity is strongly associated with increased levels of tumor necrosis factor alpha (TNF-α) and Interleukin (IL) six[40]. There are multiple mechanisms by which increased adipose tissue stores contribute to elevated levels of inflammation. Some of these potential mechanisms are outlined below.

Adipose tissue plays an integral role in regulating the amount of fat that is circulating in the blood versus stored. As fat is added to the system through feeding or lipolysis, this fat is either utilized for energy or makes its way into subcutaneous or visceral fat stores depending on the current energy environment. If a person's diet is hyper-caloric, individual adipocytes take up more fat and new adipocytes are generated to accommodate the increased need for fat storage[41,42], leading to weight gain and an increased BMI. If the need for fat storage continues to increase, the storage capacity of the adipose tissue and individual adipocytes can become overwhelmed[41]. When the storage capacity of the adipose tissue is saturated, this leads to an increase in inflammation in adipose tissue and deposition of fat in non-adipose tissues, such as the liver and skeletal muscle[43]. Therefore, excess energy balance exposes cells to an influx of fatty acids, which activate cellular stress pathways including the unfolded protein response pathway, further contributing to stress and inflammation[43].

Obesity-related changes in adipokine concentrations may also contribute to systemic inflammation. Obesity is associated with a decrease in adiponectin and an increase in leptin concentrations, which promotes increased systemic inflammation[38]. Adiponectin has many anti-inflammatory, insulin sensitizing, and anti-atherogenic effects[38]. Therefore, an obesity-related decrease in adiponectin supports the development of inflammation. It is also interesting to note that adiponectin levels are more strongly correlated to visceral fat than total fat[38]. Since visceral fat is more strongly associated with chronic disease than subcutaneous fat, it may be that adiponectin is part of the causal pathway by which adiposity leads to increase inflammation and the development of cardiometabolic disease. Leptin is a hormone that inhibits appetite and food intake and stimulates energy expenditure over time[42]. Leptin is over-expressed in obese individuals likely because of leptin resistance as well as the increased concentration of pro-inflammatory cytokines TNF-α and IL-1[42]. Leptin has pro-inflammatory effects and promotes the production of IL-2 and type II interferon, while inhibiting the production of anti-inflammatory cytokine IL-4 by T-cells[42,44]. Therefore, an obesity-related increase in leptin promotes systemic inflammation. In this way obesity-associated changes in adipokines contribute to the development of systemic inflammation.

Exogenous agents may also trigger obesity-related systemic inflammation. There is preliminary evidence suggesting that dietary agents associated with an obesogenic diet, such as certain saturated fatty acids (SFAs), could interact with adipocyte cell surface markers, inducing a cascade of events that leads to increased inflammation. Adipose tissue plays an active role in innate immunity, displaying toll-like receptors (TLRs) on its cell surface[41]. When TLR2 or TLR4 are activated by bacterial lipoproteins, lipopolysaccharides, SFAs, or potentially other lipids; this leads to the translocation of nuclear factor kappa B (NFκB) resulting in the synthesis of pro-inflammatory factors such as TNF-α, IL-6, and chemokines[41]. In this way, hyperlipidemic states could amplify inflammation. However, it is unclear whether this potential link between an obesogenic diet and systemic inflammation occurs in humans since the majority of the work has been done in murine models.

2.1.2 Excess Fat Mass and Insulin Resistance

Insulin is a hormone that allows cells to take up glucose for energy generation, playing an instrumental role in glucose homeostasis. Insulin resistance (IR) is a metabolic state defined as "an inadequate response by insulin target tissue, such as skeletal muscle, liver, and adipose tissue, to the physiologic effects of circulating insulin"[45]. There is a strong association between obesity and T2D as discussed above. One of the hallmarks of the multiple contributing factors to T2D is IR. It is likely that one of the mediating pathways between obesity and T2D is through the association of obesity with IR. This is a concept that has been documented as early as the 1960s, when Bierman et al. proposed that the obvious common denominator linking obesity and diabetes is the regulation of insulin secretion and sensitivity to its action[46]. Since then, multiple observational studies have been published, indicating that obesity is positively associated with IR.

A cross-sectional study of non-diabetic South American adults found that both BMI and WHtR are associated with Homeostatic Model Assessment-Insulin Sensitivity (HOMA-IS) with a Pearson's correlation coefficient of -0.50 and -0.45 respectively[47]. This association is also found when using more rigorous measurements of insulin sensitivity and adiposity. A study of healthy US men of European and Asian Indian descent assessed insulin sensitivity as the insulin-mediated glucose disposal rate in a 2-hour euglycemic-hyperinsulinemic clamp and adiposity through CT scan and bioelectrical impedance. Total, subcutaneous, and visceral fat were associated with insulin-mediated glucose disposal with correlation coefficients of -0.61, -0.47 and -0.55 respectively[48]. These results indicated that visceral fat is more strongly associated with IR as compared to subcutaneous fat. A third cross-sectional study of obese and normal weight healthy US men by Paradisi et al. similarly found that BMI and WC were significantly associated with the glucose infusion rate (GIR), which is positively associated with insulin sensitivity, with a correlation of -0.394 and -0.461 respectively[49]. Both BMI and WC were also associated with area under the curve (AUC) insulin with correlation coefficients of 0.476 and 0.426 respectively[49]. To further investigate the relationship between adiposity and metabolic parameters, Paradisi et al. looked at whether adiposity assessed by a dual-energy x-ray absorptiometry (DEXA) scan was associated with insulin sensitivity. The results indicated that

the percent of total body fat as measured by DEXA was associated with both GIR and AUC insulin with correlation coefficients of -0.413 and 0.496 respectively[49]. The DEXA scan results also indicated that the fat mass between L1 and L4 were most strongly correlated with GIR and AUC insulin[49], further indicating that visceral adiposity is more detrimental than subcutaneous adipose tissue. Collectively, these studies indicate that regardless of method used to assess adiposity or insulin sensitivity, a relationship between these two factors is consistently detected. Further, those measures of adiposity that are indicative of increased visceral adiposity appear to be more strongly associated with insulin sensitivity than those reflecting subcutaneous or peripheral fat mass.

It is important to note that the relationship between adiposity and insulin sensitivity appears to be modified by ethnic background[48,50]. At a similar age and BMI, Asian Indians had a significantly lower glucose disposal rate during an insulin clamp when compared to Caucasians[48]. There is also evidence that the relationship between adiposity and insulin sensitivity differs for Pima Indians when compared to Caucasians. In Pima Indians, there was a decrease in insulin-mediated glucose disposal rate with increased adiposity up to 28 percent body fat, with no further reduction above this threshold[50]. However, in the Caucasian population, there was a significant linear negative relationship between degree of adiposity and insulin-mediated glucose disposal rate with a correlation coefficient of -0.37[50]. While this is not an extensive list of all ethnic groups with specific differences in their relationship between adiposity and insulin sensitivity, it indicates that there may be key genetic and environmental factors that should be investigated as potential explanatory variables for the relationship between obesity and IR. Further the differences seen by ethnic group may be better understood after there is a more comprehensive understanding of the mechanisms linking increased adiposity with decreased insulin sensitivity.

There are multiple potential pathways linking excess fat mass with IR, including the activation of pro-inflammatory pathways, mechanisms involving elevated circulating free fatty acids (FFAs), accumulation of fat in ectopic depots, and endocrine disruption. Inflamed adipose tissue secretes pro-inflammatory cytokines and chemokines. Monocyte chemoattractant protein (MCP)-1, TNF-α, IL-1, IL-6, and IL-8 are all pro-inflammatory agents secreted by adipose tissue and have been reported to promote IR[42]. For example, TNF-α inhibits the key insulin receptor transducer insulin

receptor substrate 1 (IRS-1) through serine phosphorylation and reduces gene expression of the glucose transporter type 4 (GLUT-4), the key transporter responsible for glucose uptake by adipose and skeletal tissues[51]. Therefore, increased TNF-α can result in insulin receptor impairment or resistance and decreased glucose uptake. Additionally, increased adipose tissue inflammation leads to the activation of T-cells that release other pro-inflammatory cytokines such as interferon gamma (IFN-γ), which impairs the activation of GLUT-4[52]. It should be noted that obese patients' adipose tissue show an over expression of a variety of inflammatory and macrophage genes beyond just TNF-α and IFN-γ that have the potential to influence insulin sensitivity[52].

The alteration in the expression of and sensitivity to adipokines due to obesity may further play a role in the link between adiposity and localized IR. Under normal conditions, leptin improves insulin sensitivity in the liver and skeletal muscle, but impairs insulin signaling in adipocytes, at least in rats[42,53]. It is thought that leptin impacts insulin sensitivity through stimulation of phosphoinositide 3-kinase (PI3K) signaling[54]. Under conditions of obesity, changes in leptin concentrations and sensitivity may contribute to IR in tissues where leptin typically supports insulin sensitivity under normal conditions. One theory is that while leptin is over expressed in obesity[42], leptin can not perform its insulin sensitizing function in skeletal muscle and liver due to obesity-related leptin resistance[54]. Another theory is that the blood brain barrier in obesity restricts the entry of leptin as a response to obesity-induced hyperleptinemia, leading to leptin insufficiency in the brain[54]. In either case, the inhibited ability of leptin to perform its function in obesity contributes to obesity-related IR. Resistin is another adipokine that promotes IR in murine models of obesity[42,54]. However, the association between resistin and IR in humans remains unclear[42]. Excess fat mass decreases the expression of the adipokine adiponectin, which is a powerful insulin sensitizer[38]. In murine models, recombinant adiponectin administration led to improved whole-body insulin sensitivity[54,55], whereas mice deficient in adiponectin were IR[54,56]. In humans, plasma adiponectin levels are negatively associated with IR[54,57]. Adiponectin exerts its effects on metabolism primarily through adiponectin receptor 1 that is expressed ubiquitously and adiponectin receptor 2 that is expressed primarily in the liver to impact various biological activities, including insulin sensitivity[54]. Overall, it is clear that adipokines play an essential role in the link between increased adiposity and IR.

Localized IR in adipocytes can also lead to IR in other insulin target tissues. One mechanism linking localized and systemic IR is increased adipocyte lipolysis, which results in increased levels of circulating FFAs[58]. While plasma FFA levels do not increase in proportion to the amount of body fat, obese individuals have higher circulating FFAs when compared to lean subjects[42]. An increased concentration of FFAs in the plasma results in IR in skeletal muscle and the liver[58]. Elevated FFAs promote IR through inhibiting the insulin-signaling pathway and reducing insulin-stimulated muscle glycogen synthesis[58]. Typically, insulin binds to its receptor, promoting the phosphorylation of the insulin receptor, which activates PI3K[58]. Activated PI3K activates protein kinase B (PKB/Akt), leading to the translocation of GLUT-4 to the cell surface[58]. FFAs activate NFκB, an inhibitor of kappa B–kinase, which inhibits IRS and PKB/Akt[58]. This inhibits insulin signaling, preventing the translocation of GLUT-4 to the membrane for glucose uptake[58]. In this way, increased circulating FFA's contribute to the development of IR. Increased circulating FFAs also results in the deposition of fat into non-adipose tissues such as liver and skeletal muscle. The impact of ectopic fat deposited in the liver will be discussed in section 2.1.4. The increased deposition of lipid metabolites in skeletal muscle stimulates many serine/threonine kinases responsible for phosphorylating proteins in the insulin-signaling pathway[58]. For example, glycogen synthase kinase and PKB/Akt are two key proteins in the insulin-signaling pathway that are inactivated through the serine/threonine kinase protein kinase C[58]. It has been suggested that fatty acids and their metabolites (acyl-coenzyme A, ceramides, diacylglycerol) also impair insulin signaling by up-regulating other protein kinases such as mitogen-activated protein kinase and c-Jun N-terminal Kinase[42]. Therefore, FFAs are one potential mediator by which obesity leads to IR[59].

2.1.3 Excess Fat Mass and Impaired Glucose Tolerance

As discussed in the previous section, obesity is associated with IR. When an individual becomes IR, their blood glucose levels will remain in the homeostatic range as long as their pancreatic β-cells are able to compensate by producing more insulin[59]. However, obesity has not only been suggested to decrease insulin action in skeletal muscle and adipose tissue but also to decrease insulin secretion from pancreatic β-cells[59]. IGT occurs when insulin receptor resistance and β-cell dysfunction simultaneously occur, leading to the impairment of glucose homeostasis[59].

Excess fat mass is thought to lead to IGT predominantly through the effect of gluco-lipotoxicity. Elevated FFAs, associated with increased adiposity, leads to an accumulation of intramyocellular fatty acid metabolites that produce free radical reactive oxygen species (ROS)[58]. ROS can cause tissue damage, including in pancreatic β-cells. Further the inappropriate deposition of fat in the pancreas surrounding and infiltrating the pancreatic islet cells leads to an amplification of the natural age-related decline in insulin output[38]. Additionally, fat in the pancreas leads to decreased insulin output by impairing the exocytosis of insulin filled vesicles as well as triggering β-cell apoptosis[52]. As a result, lipotoxicity leads to β-cell dysfunction through decreased insulin output and β-cell destruction. In this way, increased adiposity can lead to an inability of the pancreatic islet cells to meet the insulin output necessary to fully overcome IR and maintain normal glucose tolerance. Therefore, obesity is associated with IGT due to its relationship to both IR and impaired β-cell function.

2.1.4 Excess Fat Mass, Fat content and Liver Function

Increased weight gain enhances lipogenesis. When the ability of adipose tissue to accommodate the overload of incoming fat becomes overwhelmed, this leads to fat deposition in other organs, including the liver[43,41]. The liver is a vital organ that plays an essential role in metabolism, digestion, and nutrient storage. It is a central player in gluconeogenesis, glycolysis, glycogenolysis, and glycogenesis, thereby serving a key role in the regulation of glucose homeostasis. Fat deposited in the liver is problematic because it impairs liver function. When the liver has a high fat content for reasons other than alcoholism, this is known as NAFLD. NAFLD is the most common chronic liver condition in the US[60]. In a meta-analysis of cohort studies, obesity was associated with a 3.5 fold increased risk of developing NAFLD compared to being normal weight[61]. Further, the investigators observed a dose-dependent relationship between BMI and NAFLD where each 1-unit increase in BMI was associated with a 20% increase in risk of developing NAFLD[61]. It appears that elevated central adiposity is especially problematic. In a meta-analysis of observational studies, when WC was taken as a binary variable with cut-off values in alignment with national or international standards, the pooled effect size was 2.34[62]. Further, when WC was measured on a continuous scale, it was found that each 1-centimeter increase in WC was associated with a 7% increased risk of NAFLD[62]. Elevated WHR, another

measurement of central adiposity, was associated with approximately three times the odds of developing NAFLD compared to individuals without elevated WHR[62]. Overall, increased adiposity is positively associated with an increased risk of NAFLD. In fact, two thirds of patients with NAFLD are obese[42].

It is not surprising that the increased prevalence of obesity is associated with a rise in the prevalence of NAFLD[42], and that the rise in NAFLD plays a role in obesity-associated metabolic complications. In particular, increased central adiposity associated with increased visceral fat, impacts glucose homeostasis partially by altering hepatic function and fat content[38]. According to the "portal theory", concentrated abdominal fat results in an influx of fatty acids, cytokines and hormones into the liver through the portal vein[38]. Omental and mesenteric adipose deposits release high concentrations of FFAs directly into the portal vein[38]. In addition to increased release of FFAs from visceral adipose tissue stores, there is also an increased release of FFAs from subcutaneous tissue in obese versus lean individuals[58]. Increased hepatic FFAs increase lipid synthesis, gluconeogenesis, and contribute to the development of IR in the liver[42]. IR results in hyperinsulinemia, which increases de novo hepatic lipogenesis, decreases FFA oxidation, decreases hepatic very low density lipoprotein (VLDL) secretion and increases efflux of FFAs[42]. This leads to steatosis when the increase fatty acid import or de novo fatty acid synthesis exceeds the ability of the liver to clear lipids through fatty acid oxidation or triglyceride export[42]. In fact, the hepatic triglyceride content is directly related to the amount of FFAs circulating and therefore taken up by the liver[58]. Hepatic fatty acid de novo synthesis is regulated in response to insulin and glucose signaling[58]. Therefore, FFAs in the liver promote increased glucose production, triglyceride synthesis and impair insulin suppression of hepatic glucose output[42].

Obesity-related pathways further contribute to the continued decline of liver health and function over time. As mentioned above, excess adiposity results in increased hepatic exposure to FFAs. Activation of TLR4 by FFAs in the liver promotes inflammation[42]. Inflammation plays a key role in the transition from hepatic steatosis to fibrosis[42]. The adipokine adiponectin usually protects the liver from steatosis and inflammation by increasing the ability of insulin to suppress glucose production, glucose output, and inhibiting hepatic lipogenesis[42]. Leptin on the other hand, stimulates 5' monophosphate-activated protein kinase (AMPK), which activates lipid

oxidation[42]. Further, leptin increases the expression of transforming growth factor-β1, a pro-fibrogenic cytokine, and activates hepatic stellate cells and stimulates production of α-smooth muscle actin, collagen and tissue inhibitor of metalloproteinase[42]. As previously mentioned, obesity results in the increased expression of leptin and the decreased expression of adiponectin. Therefore, obesity-associated alterations in the concentrations of circulating adipokines, such as adiponectin and leptin, are an important mechanism through which obesity affects liver fat content and function.

2.1.5 Excess Fat Mass and Blood Pressure

Excess weight gain is one of the best predictors for high blood pressure and weight loss has proven to be effective at lowering blood pressure[63]. Obesity is associated with an increased risk of hypertension, partially as a result of the increased metabolic demands of the excess adipose tissue depots and decreased fat-free mass[38]. This increased demand on the body's circulatory system can lead to an elevated afterload on the left ventricle and eccentric or concentric left ventricle hypertrophy[38]. This can result in impaired diastolic function[38]. Additionally, increased adiposity can lead to cords of fat cells accumulating between the muscle fibers in the heart, which could result in myocyte degeneration and cardiac conduction defects[38]. This is one explanation for how obesity can lead to increased blood pressure and cardiac inefficiencies.

Another mechanism linking increased adiposity and hypertension is altered concentrations of adipokines. As mentioned previously, excess adiposity leads to the over-expression of leptin and the under-expression of adiponectin[38]. Both of these hormones have been indicated as independent risk factors for heart disease[38]. Leptin increases blood pressure as well as increases sympathetic nerve activity, stimulates generation of ROS, induces platelet aggregation and promotes arterial thrombosis[38]. In contrast, adiponectin is vasoprotective partially through the stimulation of fatty acid oxidation and glucose utilization by activating AMPK in the liver and skeletal muscle[42]. In this way, obesity-related alterations in adipokines can contribute to the development of hypertension.

Other potential mechanisms linking obesity to hypertension include kidney overload, sympathetic nervous system activation, and over-activation of the renin-angiotensin-aldosterone

system. Increased adiposity is positively correlated with increased concentrations of plasma aldosterone[64]. Aldosterone is a hormone that plays a key role in the regulation of blood pressure, acting to stimulate water and sodium uptake through activating the mineralcorticoid receptor (MR) expressed in epithelial tissues, the heart, and adipose tissue[64]. Leptin, adiponectin, and complement-C1q TNF-related protein-1 are all adipocyte-derived factors that influence the production and secretion of aldosterone in adipose tissue[64]. MR expression is positively correlated with BMI in humans and mice[64]. In this way, increased adiposity results in increased aldosterone action and therefore hypertension. Another potential mechanism by which obesity leads to hypertension is through obesity-induced impaired renal excretory function[63]. Obese individuals have impaired sodium excretion due to increased renal sodium reabsorption as a result of increased glomerular filtration rate[63]. Prolonged obesity results in increased arterial pressure; renal vasodilation and glomerular hyper-filtration, neurohumoral activation; and metabolic changes that can cause glomerular injury leading to more severe hypertension and loss of kidney function[63]. Adding to this is an increased activation of the sympathetic nervous system associated with obesity, predominantly due to the obesity-related rise in leptin[63]. Specifically, renal sympathetic activity is increased with obesity[63]. Furthermore, adipose tissue encapsulates a large portion of the kidneys and in obese subjects, can penetrate the medullary sinus, causing compression and increased intra-renal pressure[63]. This may further contribute to obesity-associated hypertension.

2.1.6 Excess Fat Mass and Serum Lipids

Obesity is linked to an increased prevalence of dyslipidemia, characterized by increased levels of FFAs, increased levels of triglycerides, decreased levels of high density lipoprotein (HDL) and abnormal low-density lipoprotein (LDL) composition[42]. Adipocyte size may contribute to the degree to which adipose tissue contributes to dyslipidemia and therefore, chronic disease[42]. As mentioned in section 2.1.1, obesity can lead to adipocyte hypertrophy. Presumably, the more a person is in energy excess and the more fat needs to be stored, the larger the adipocytes typically are. As previously mentioned, increased adiposity and specifically visceral adiposity is associated with increased FFAs[40]. Increased levels of FFAs can inhibit LPL in adipose tissue and skeletal muscle[42]. As a result, serum lipids that are normally taken up by adipose tissue and skeletal muscle instead remain in the blood stream longer, thereby raising concentrations.

Additionally, excess adipose tissue and the associated adipocyte IR leads to an uncontrolled release of FFAs through lipolysis, which leads to increased fatty acid uptake by the liver[42]. FFAs are toxic to the liver, therefore the liver attempts to compensate for the influx of fatty acids by increasing the synthesis and export of VLDL[42]. Increased VLDL concentrations can inhibit lipolysis of chylomicrons in the liver, which causes hypertriglyceridemia[42]. Hypertriglyceridemia triggers cholesteryl ester transfer protein-mediated exchange of triglycerides for cholesterol esters between triglyceride-rich lipoproteins such as VLDL, and lipoproteins that are rich in cholesterol esters like LDL and HDL[42]. This results in reduced levels of circulating HDL-cholesterol and an increased concentration of small dense LDL particles that are associated with cardiovascular diseases[42]. Additionally, obesity is linked to low levels of HDL potentially through the impairment of LPL activity as well as enhanced cholesteryl ester transfer protein mediated lipid exchange associated with obesity[38].

Adipokines are another means by which excess fat mass may contribute to dyslipidemia. Plasma concentration of adiponectin are correlated with plasma HDL-cholesterol both in individuals with and without T2D after adjustment for body mass index, body-fat distribution, and insulin sensitivity (reviewed in[65]). Further, there is a negative correlation between adiponectin and the catabolic rate of apolipoprotein A1, the major protein component of HDL-cholesterol (reviewed in[65]). Therefore, one potential theory is that adiponectin-induced activation of the adiponectin receptor 2 in the liver reduces hepatic apolipoprotein A1 catabolism, thereby increasing apolipoprotein A1 concentrations and therefore HDL-cholesterol concentrations[65]. However, future investigations into this hypothesis are needed to gain a clearer picture on any potential mechanisms by which adiponectin concentrations may impact HDL-cholesterol levels. Therefore it remains unclear whether obesity related alterations in adiponectin concentrations directly impact HDL metabolism in obesity[38]. Adiponectin also induces the activation of LPL, resulting in enhanced VLDL clearance and reduced plasma triglycerides[42]. Since adiponectin concentration is reduced in obesity, this is another mechanism by which obesity negatively impacts serum lipids.

2.2 Metabolic Dysfunction is Associated with Chronic Disease

2.2.1 Chronic Systemic Inflammation is Associated with Type 2 Diabetes

A review of the epidemiological literature by Duncan and Schmidt concluded that there is an association between biomarkers of systemic inflammation and the development of T2D[66]. The inflammatory markers indicated in this analysis include the number of leukocytes in the circulation and blood concentrations of sialic acid, orosomucoid, fibrinogen, IL-6, CRP, plasminogen activator inhibitor-1, adiponectin, and complement C3[66]. Overall, Duncan and Schmidt concluded that inflammatory markers predict diabetes with the most consistent evidence being for leuokocyte numbers and the strongest being for CRP[66].

Evidence linking CRP to T2D is found in populations around the world. In a cross-sectional study with 2,520 urban north Indians of Indo-European descent, it was found that elevated hsCRP, as defined as a concentration greater than 3 mg/L, was positively associated with T2D with an odds ratio of 1.66 as compared to an hsCRP concentration of less than 1 mg/L[67]. This association remained even after adjustment for age, sex, BMI, WC and WHR, indicating that the link between this key biomarker of inflammation and T2D is independent of adiposity. A prospective cohort study of over 12,800 Finnish men and women found that those with elevated levels of CRP had an increased hazard ratio for T2D and this relationship was modified by sex. For individuals with CRP levels at 0.05-0.99, 1.0-2.99, and $\geq$ 3.0 mg/L the hazard ratio for T2D in men was 1.00, 1.46, and 1.85 while the hazard ratio in women was 1.0, 3.83, and 8.37 respectively[68]. This relationship was unaltered after adjusting for age, smoking status, level of obesity, alcoholic drinking habits, and family history of diabetes[68]. A study using data from the Women's Health Initiative (WHI) cohort showed that baseline levels of the inflammatory markers IL-6 and CRP are both predictive of the development of T2D in healthy women[69]. The RRs for T2D in women in the highest versus the lowest quartile of IL-6 was 7.5 and for CRP it was 15.7[69]. In adjusted models that included BMI, family history of diabetes, smoking, physical activity, alcohol consumption and hormone replacement therapy use, CRP remained a statistically significant predictor of development of T2D, while the association between IL-6 and T2D was attenuated to a trend[69]. While the evidence of the association between CRP and T2D is strong compared to other inflammatory markers, it remains unclear whether the observed association is driven by the stability of the inflammatory measure rather than the true strength of

the association[66]. It may be that with more robust measures of inflammation as an exposure variable, we may see similarly strong associations with other inflammatory variables and incident T2D.

In their review, Duncan and Schmidt also pointed out that individuals with multiple elevated inflammatory markers had a larger increase in risk of developing T2D when compared to individuals with only a few or none[66]. Using data from the Atherosclerosis Risk In Communities study, white non-smoking individuals with three or more elevated markers had approximately three times the risk of developing T2D when compared to those with zero to two elevated markers[66]. This was also seen in the WHI study where they found that the relative risk of T2D was highest among women who had both high baseline levels of IL-6 and CRP when compared to those with either just high IL-6 or high CRP, indicating a multiplicative interaction[69]. Overall, it appears that the more evidence of chronic systemic inflammation, the higher the risk of incident T2D.

There are multiple theories on the mechanisms behind the development of T2D, including oxidative stress; endoplasmic reticulum stress; amyloid deposition in the pancreas; ectopic lipid deposition in the muscle, liver and pancreas; lipotoxicity; and glucotoxicity, some of which was discussed in sections 2.1.2 and 2.1.3[70]. It should be noted that each of these mechanisms are either thought to be induced by inflammation or their effects are worsened in the presence of systemic inflammation[70]. That would explain why inflammatory markers including elevated levels of IL-1β, IL-6, and CRP are predictive of T2D[70]. That would also explain why experimental drugs targeting the inflammatory pathway have shown some success in altering determinants of glucose tolerance. Drugs targeting inflammatory pathways including selective blockade of IL-1 R1 activation with either IL-1RA or specific antibodies, and inhibition of the NFkB pathways with salicylate derivatives, lower blood glucose levels and improve β-cell secretory function and insulin sensitivity as well as reduce systemic inflammation[70]. Taken collectively, the evidence indicates that the presence of chronic systemic inflammation is associated with an increased risk for T2D. Systemic inflammation may be in the causal pathway for the development of T2D and therefore, could be an effective target for intervention strategies.

Increased systemic and localized inflammation not only increases one's risk for T2D, but also increases one's risk for CVD. Increased circulating levels of pro-inflammatory cytokines such as TNF-α and IL-6, as well as acute phase proteins such as CRP and fibrinogen, are correlated with an increased risk of developing and dying from CVD[39,71]. In a nested case-control study with over 28,000 healthy postmenopausal women, those in the highest versus the lowest quartile for hsCRP had a RR of 4.4 for a cardiovascular event, though this association was attenuated after adjusting for other risk factors[72]. Additionally, the RR when comparing those in the highest versus the lowest quartile for IL-6 was 2.2, though this observed relationship was no longer significant after adjusting for other risk factors[72]. Further, the authors concluded that models that included markers of systemic inflammation in addition to lipid markers were more accurate in predicting risk for cardiovascular events[72]. A study done using data from the Nurses Health Study and the Health Professionals Follow-up Study similarly found that multiple inflammatory markers were associated with heart disease, but that only CRP was significantly associated with heart disease after adjustment for other risk factors[73]. Specifically, the RR of heart disease was 1.75 for those with a CRP level of at least 3.0 mg/L as compared to those with CRP levels less than 1.0 mg/L[73]. Similar to the evidence linking inflammation to T2D, CRP appears to be the inflammatory marker with the strongest and most consistent association with CVD. A meta-analysis of 11 population based prospective cohort studies concluded that the multi-variable adjusted risk estimate for those with CRP values in the top tertile as compared to the bottom tertile is 2.13[74].

CRP was identified as an inflammatory biomarker linked to CVD as early as 1943[75]. Since that time, more than 30 prospective cohort studies conducted in a variety of populations have confirmed that elevated serum CRP is an independent predictor of future cardiovascular events[75]. This association has been strengthened by recent evidence indicating that targeted intervention for patients with increased hsCRP is effective at reducing CVD risk. For example, in the JUPITER trial, investigators showed that rosuvastatin was able to reduce CRP levels by 37% in men and women with normal low-density lipoprotein and elevated CRP levels[76]. Those randomized to receive rosuvastatin had a hazard ratio of 0.53 for the combined end point of myocardial infarction (MI), stroke, or death from cardiovascular causes, suggesting that rosuvastatin may lower the risk of cardiovascular events partly through its effects on low-grade

chronic systemic inflammation[76]. Overall, it is believed that low-grade chronic systemic inflammation in general and CRP in particular may be key targets for preventing one of the leading causes of death in the US, CVD.

If CRP does increase one's risk for CVD, we would expect that individuals with CRP gene polymorphisms would also be at an increased risk for CVD. However, a review by Hage and Szalai found that there is not a consistent association between CRP single nucleotide polymorphisms and CVD risk[77]. Therefore, while there are several studies indicating that there is an association between serum CRP levels and risk of CVD, the CRP gene polymorphism data calls into questions whether CRP lies on the causal pathway for the development of CVD. It may be that CRP is a marker of other inflammatory pathways that contribute to the development of CVD and does not directly influence CVD risk. Alternatively, the lack of an association in gene polymorphism studies may be due to the studies being under-powered to detect an association[77], or may be due to the fact that using a gene polymorphism to define exposure may not adequately capture true CRP levels, thereby leading to misclassification. Therefore, more research is needed to determine whether the relationship between CRP and CVD is causal[77]. Overall, elevated CRP is an independent and serious risk factor for CVD[78], placed in the same category as high LDL-cholesterol, low HDL-cholesterol, and hypertension[79].

2.2.2 Insulin Resistance is Associated with Chronic Disease

IR is a strong predictor of future development of T2D[58]. A study following 155 subjects with parents who had diabetes found that those who developed T2D had significantly lower insulin sensitivity than those who did not develop T2D as measured by an intravenous glucose tolerance test (IVGTT) ten years prior[80]. Another study including 2,115 individuals followed over six years, found that IR, defined as a Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) above the median, predicted the development of T2D, with a hazard ratio of 2.1[81]. A person can be both IR and glucose tolerant, which explains why an individual could have evidence of IR, but not have T2D[59]. In these individuals, their pancreatic β-cells increase their insulin output to compensate for the decreased insulin sensitivity in order to maintain normal glucose

tolerance[59]. Over time, an increase in IR without adequate compensation by increased insulin release, or a gradual loss of β-cell function, could result in the development of T2D.

IR is also associated with CVD[82]. There is evidence that IR and hyperinsulinemia may lead to endothelial dysfunction[38]. In a cohort of 919 Italian adults followed for 15 years, those who developed CVD had significantly higher baseline HOMA-IR as compared to those who did not develop CVD[83]. Additionally, individuals with IR at baseline, as defined as having a HOMA-IR in the top quartile, were 2.2 times more likely to develop CVD independent of other classic risk factors as compared to those in the bottom quartile[83]. Similar conclusions were made when evaluating data from the San Antonio Heart Study. This cohort study included 2,569 individuals who were free of diabetes at baseline and followed them for eight years. They found that those in the 5th quintile of HOMA-IR at baseline had 2.52 times the odds of a CVD event as compared to those in the first quintile[84]. Further, this relationship was not significantly impacted when adjusted for ethnicity, sex, hypertension, dyslipidemia, glucose tolerance, or obesity[84]. While it is clear that IR is associated with CVD, there remains some debate over whether IR directly contributes to an increased risk for CVD[82], or whether IR is only related to CVD through its association with classic cardiovascular risk factors not typically adjusted for, such as hsCRP[85].

2.2.3 Impaired Glucose Tolerance is Associated with Chronic Disease

An individual with IGT is on the spectrum between someone with normal glucose tolerance and someone with manifest T2D[86]. Having IGT increases the risk for developing T2D[86]. According to the San Antonio Heart Study, participants with the MetS and IGT had a 55% risk of developing diabetes within seven years[7]. In another study including 2,115 individuals followed over six years, 12.5% of individuals with IGT progressed to having T2D, as compared to only 6% of those with normal glucose tolerance at baseline[81]. In a review of the progression of individuals with IGT to T2D that included six prospective cohort studies ranging in size from 177 to 693 participants, the incidence rate of T2D was 57.2/1,000 person-years[87]. The progression of those with IGT to T2D varies by population, but generally ranges from 2-14% per year[86]. Therefore, and not surprisingly, IGT is a strong risk factor for T2D.

IGT also increases one's risk for CVD[86]. Using data from the Whitehall Study, Paris Prospective Study, and the Helsinki Policeman Study, Balkau et al. showed that high but non-diabetic blood glucose levels were associated with an increased risk of heart disease and CVD[88]. Men in the upper 2.5% of the 2-h oral glucose tolerance test (OGTT) distribution were at an 80% increased risk of death from CVD and men in the upper 2.5% of the fasting glucose distribution had a 170% increased risk of death from CVD as compared to those in the bottom 80% of the distribution[88]. Further, elevated hemoglobin A1c (HbA1c) is an independent risk factor for heart disease in individuals with and without diabetes[89]. It should be mentioned that, while there is evidence indicating that IGT is associated with CVD, there is some debate as to whether or not this relationship is causal or rather that they both have common underlying causal mechanisms[90]. However, it is plausible that classic CVD risk factors are worsened in the presence of IGT, conferring an increased risk above these CVD risk factors alone.

2.2.4 Fatty Liver is Associated with Chronic Disease

Increased liver fat is another metabolic risk factor associated with chronic disease. In a meta-analysis by Ballesteri et al. that included 20 studies for a pooled population size of 117,202 participants, NAFLD, as determined by serum liver enzymes or ultrasound, was associated with a RR of 1.97 for incident T2D[91]. This is in accordance with an earlier meta-analysis that similarly concluded that NAFLD, determined by either biochemical, radiological, or histological evidence, was associated with a two-fold higher risk of diabetes[92]. In accordance with these two meta-analyses, a recent prospective cohort study of 41,650 Chinese adults found that those with severe NAFLD according to an ultrasound had a hazard ratio of 2.66 for the development of diabetes as compared to those without NAFLD[93].

Not only is there an established association between NAFLD and T2D, but there is also an association between the presence of fatty liver and CVD[91,94,95]. One study of 129 patients found those with biopsy-proven NAFLD were more than twice as likely to die from CVD as compared to the general population[96]. However, there is still some debate as to whether or not NAFLD is associated with CVD independently of other cardiovascular risk factors[94,95]. Ghouri et al. argues that the evidence is inconsistent for the relationship between NAFLD and CVD and that NAFLD should not be used as an additional indicator of risk for CVD[95]. On the other hand, Targher et al.

indicates that NAFLD is linked to CVD and that this relationship is independent of other cardiometabolic risk factors[94]. Since NAFLD results in elevated serum lipids as discussed in section 2.1.4, it seems likely that elevated liver fat affects CVD risk at least partly through a primary effect on serum lipids. However, more research is needed to determine whether or not NAFLD plays an independent causative role in the development of CVD.

2.2.5 Elevated Blood Pressure is Associated with Chronic Disease

Blood pressure is a risk factor for T2D. In a prospective cohort study of 15,792 US adults who were followed over six years, those with hypertension had a RR of 2.43 for incident diabetes as compared to subjects without hypertension at baseline[97]. Additionally, in a cohort of 4.1 million adults, every 20 mmHg higher systolic blood pressure was associated with a 58% higher risk of diabetes and every 10 mmHg higher diastolic blood pressure was associated with a 52% increased risk of diabetes[98]. Additionally, a systematic review that included 30 studies found that the pooled RR of diabetes for every 20 mmHg higher systolic blood pressure was 1.77[98]. Collectively, these data indicate that there is a strong and consistent association between blood pressure and diabetes. However, it is uncertain whether these observed associations are reflective of a cause-effect relationship.

There is strong evidence indicating that blood pressure is a risk factor for CVD. Observational data shows that for every 20 mmHg increase in systolic blood pressure or every 10 mmHg increase in diastolic blood pressure there is a doubling of mortality from ischemic heart disease and stroke[99]. Additionally, data from the Framingham Heart Study show that blood pressures in the 130/85 – 139/89 mmHg range were associated with a greater than two fold increased risk for CVD in women and a 60% increased risk in men when compared to those with blood pressure values below 120/80 mmHg[100]. Further, Whelton et al. pooled data from nine prospective cohort studies to investigate the relationship between diastolic blood pressure and heart disease and stroke. Those in the highest versus lowest quintile for diastolic blood pressure had almost a five times higher risk of heart disease and more than ten times higher risk for stroke[101]. This indicates that lowering diastolic blood pressure by just 5-6 mmHg could reduce one's risk for heart disease by 20-25%[101]. Another review indicates that for individuals of the same age, being hypertensive

results in a three-fold increased risk for CVD[102]. Overall, this evidence indicates that blood pressure is strongly associated with CVD.

2.2.6 Elevated Serum Lipids are Associated with Chronic Disease

Dyslipidemia often presents in patients with T2D through elevated triglycerides and apolipoprotein B, a shift in the LDL composition to include a higher percentage of small dense LDL particles, and low HDL-cholesterol levels[37,103]. Further, there is consistent evidence prospectively linking elevated triglyceride concentrations with T2D risk. A recent meta-analysis by Ye et al. included 31 observational studies and found that higher triglyceride concentrations, measured as either highest versus lowest quintile or per log 1-mmmol/L increase, both resulted in a 30% increased risk of T2D[104]. Further, there is some evidence that the positive association between triglyceride concentrations and T2D risk is modified by sex. A study by Cheng et al. that included 11,946 adults from the Rural Chinese Cohort Study found that the those in the highest quartile of triglyceride to HDL-cholesterol ratio had a hazard ratio of 2.11 for incident T2D as compared to those in the lowest quartile[105]. Further, they found that this relationship was modified by sex and body mass, with a stronger association seen in women and those with a normal BMI[105]. While there is a clear association between dyslipidemia and T2D, it remains unclear whether alterations in the lipid profile directly affect the development of T2D through effects on either insulin sensitivity or glucose tolerance. One mechanism by which elevated serum lipids may lead to the development of T2D is through hyperlipidemia induced ectopic fat deposition (discussed in sections 2.1.4 and 2.2.4), specifically in the liver. Fat in the liver is associated with an increased risk for T2D (section 2.2.4). In this way, hyperlipidemia could plausibly be causally linked to T2D. However, future research is needed to clarify the relationship between dyslipidemia and T2D risk and whether or not this relationship is causal.

Dyslipidemia is considered a well-established and primary risk factor for CVD. A meta-analysis of 61 prospective cohort studies found that for every 1 mmol/L lower total-cholesterol, the hazard ratio for vascular death was 0.44, 0.66, and 0.83 for those 40-49 years old, 50-59 years old, and 70-89 years old[106]. Further, there is some indication that the relationship between total-cholesterol and coronary health may be modified by sex. A meta-analysis of 97 cohort studies found that for every 1 mmol/L increase in total-cholesterol there was a 20% increased risk of

heart disease in women and a 24% increased risk in men[107]. Further, women were at a 13% reduced risk of heart disease when compared to men with the same total-cholesterol concentration[107]. While total-cholesterol is one lipid marker associated with CVD, elevated LDL-cholesterol is widely viewed as a principle risk factor for the development of CVD and is seen as a more accurate predictor of CVD risk. A meta-analysis of 12 prospective studies found that the relative risk ratio for incident CVD was 1.25 when comparing the highest versus the lowest quantile of LDL cholesterol[108]. Recently, there has been some debate as to whether non-HDL cholesterol and apolipoprotein B concentration may be even better predictors of CVD risk, as compared to LDL-cholesterol, since they are thought to more accurately capture LDL particle number[108]. A meta-analysis of 12 observational studies found that those in the highest quantile as compared to the lowest quantile of apolipoprotein B and non-HDL cholesterol had relative risk ratios of 1.43 and 1.34 respectively[108]. Additionally, it appears that this relationship may be modified by metabolic health. A meta-analysis of 13 observational studies found that participants in the highest reference group for non-HDL cholesterol as compared to those in the lowest reference group had a RR of incident CVD of 1.59 and 1.98 for those in the general population and those with type 2 diabetes respectively[109]. Further, a third meta-analysis indicates that sex may modify the relationship between non-HDL cholesterol and CVD risk. A meta-analysis of nine prospective studies found that those with the highest non-HDL cholesterol at baseline were at a 79% higher risk of heart disease than those in the lowest category and that this risk was modified by sex with men having a 98% increased risk where as women had only a 63% increased risk[110]. Dyslipidemia is also characterized by decreased HDL-cholesterol concentrations. Both epidemiological and clinical studies show that low HDL-cholesterol concentration is a strong independent risk factor for CHD[37]. In fact, it was estimated that for every 1 mg/dL increase in HDL-cholesterol, there was a 2-3% decrease risk of CVD[37]. There are also indications that elevated serum triglycerides are an independent risk factor for CVD[37]. A meta-analysis of six prospective studies found that a 18 mg/dL increase in triglycerides increased the risk for CVD by 14% in men and 37% in women, independent of HDL-cholesterol[111]. Though it should be noted that the Emerging Risk Factors Collaboration argues that using HDL-cholesterol levels or apolipoproteins as part of a lipid assessment for vascular disease is enough and that adding triglycerides to the assessment does not further predict the risk of CVD[112].

Further, it remains under debate whether increased levels of small dense LDL is an independent risk factor for CVD[37], though some argue it is the most clinically relevant biomarker for CVD[113].

2.3 Metabolic Risk Factors are Associated with Chronic Disease Independent of Obesity

Nearly one third of obese adults in the general population are metabolically healthy with normal insulin sensitivity, normal glycaemia, low inflammation, and relatively high cardiorespiratory fitness[29]. This indicates that being obese does not necessitate poor health. Similarly, being normal weight does not indicate good cardiometabolic health. Metabolic risk factors are associated with chronic disease independent of the presence of obesity. For example, a sub-analysis of the English Longitudinal Study of Ageing with 3,066 individuals found that unhealthy normal-weight individuals showed a higher risk for T2D development when compared to the healthy obese group[29]. Though it should be noted that the metabolically healthy obese were on average younger, less likely to be smokers, and had intermediate levels of risk factors when compared to both the metabolically unhealthy obese and non-obese[29]. A meta-analysis of eight prospective cohort studies including 187,918 participants investigated the relationship between obesity and metabolic health and CVD risk. While Fan et al. found that obesity was associated with an increased risk of CVD, normal weight individuals who were deemed metabolically unhealthy (MetS or IR) were also at an increased risk for CVD with a RR of 1.81 when compared to their metabolically healthy and normal weight peers[36]. Around 90% of individuals who develop T2D have a BMI greater than 23.0 kg/m^2 [38]. That means 10% of the approximately 21 million adults diagnosed with T2D[9] are not overweight or obese and are actually considered on the lower end of normal weight. This indicates that there are many contributing factors to the development of T2D aside from obesity alone.

3.0 Dairy Foods, Adiposity and Metabolic Risk Factors for Chronic Disease

The increased prevalence of obesity and MetS globally are partially attributed to the adoption of a modern western lifestyle that features stress, sedentary behavior, energy dense and highly rewarding food, food of low-nutritional value, and disruption of chronobiology, all of which contribute to a chronic positive energy balance[41]. With the rise in prevalence of obesity and chronic diseases such as T2D, it is imperative that areas for targeted intervention and prevention strategies are identified. Many modifiable lifestyle factors have been selected as potential means to help reduce the burden of chronic disease[114]. Because diet is a major factor in the etiology of both obesity and T2D, a major emphasis of research is on identifying modifiable dietary factors that affect energy and glucose homeostasis with the potential to prevent and treat obesity and T2D[114,115]. Dairy foods have been identified as one potential factor that may impact weight and metabolic health[116,117]. Dairy is a complicated food group with various factors that may influence its physiological effects. This may include the fat content of the dairy, fermentation, length of ageing, production processes, and how the dairy cows were fed[118]. As a result, in recent years, the impact of dairy foods on energy and glucose homeostasis has been an active area of research.

The 2015-2020 Dietary Guidelines for Americans recommends the consumption of dairy in the form of milk, yogurt, and cheese as part of a well-balanced diet[119]. This is because dairy products are viewed as a healthy source of protein and numerous micronutrients, including calcium, phosphorus, vitamin A, vitamin D, riboflavin, vitamin B12, potassium, zinc, choline, magnesium, and selenium[119]. Traditionally, dairy fat has been seen as a source of unnecessary calories and SFAs, which could contribute to obesity and, through elevated fasting serum lipids, CVD. Therefore, the Dietary Guidelines for Americans encourages people to consume low-fat or fat-free dairy products. The following sections explore the current evidence (up to April 1st, 2020) on the relationship between dairy foods as a whole (3.1-3.6) and dairy fat in particular (3.1.1, 3.2.2, 3.4.4) with adiposity, T2D, CVD, MetS, and risk factors for these chronic cardiometabolic diseases.

3.1 Dairy Foods and Adiposity

Multiple reviews and meta-analyses shed light on the current state of the literature regarding dairy consumption and adiposity. A review by Dougkas et al. that predominantly evaluated the observed association between dairy foods and adiposity in cross-sectional and prospective studies, indicated that dairy consumption is inversely associated with measures of adiposity[117]. Further a recent meta-analysis of observational studies indicated that this association is dose dependent. Lee et al. found that every 200 g per day increase in total dairy intake was associated with a 10% reduced risk of abdominal obesity[120]. This review also identified that a significantly decreased risk for abdominal obesity was observed for every 200 g per day increase in milk consumption[120]. A review by Sayon-Orea et al. looked specifically at the association between yogurt consumption and adiposity. They included ten prospective cohort studies and concluded that the association between yogurt and the development of overweight or obesity is inconsistent, however there is a consistent inverse association between yogurt consumption and changes in weight and waist circumference[121]. Overall, it appears that total dairy as well as specific dairy foods, such as milk and yogurt, are inversely associated with adiposity in the observational literature.

Numerous randomized controlled trials (RCTs) have tested the effect of dairy consumption on body weight. A recent meta-analysis by Geng et al. included 37 RCTs. Key inclusion criteria included studies that had an intervention greater than or equal to four weeks; had dairy foods as the primary intervention; conducted in adults free of severe illness; and that had outcome data on either body weight, body fat, fat mass, lean mass, or WC. Overall, they found there was no difference in change in body weight between dairy interventions and control groups, but a decrease in body fat and WC, and an increase in lean body mass[122]. However, it is important to note that subgroup analyses revealed that dairy consumption resulted in a significant increase in body weight in *ad libitum* studies, while aiding weight loss modestly in the context of energy restriction[122]. Similarly, the effects of dairy consumption on body fat, WC, and lean body mass were consistent in subgroup analyses of dairy interventions in the context of energy restriction, but these effects were no longer observed in trials conducted in the context of an *ad libitum* diet[122]. The modification of the effect of dairy consumption on adiposity by energy balance has

been reported previously[123]. Therefore, overall, the experimental data suggest that dairy consumption is either inversely or not associated with adiposity in the context of energy restriction, and dairy consumption is either positively or not associated with adiposity in the context of *ad libitum* energy intake.

There are a few limitations to the RCTs that have been conducted on this topic thus far including the fact that they usually included a small sample size, the dairy interventions rarely included a diverse range of dairy foods and typically included low-fat dairy products, it is difficult to blind diet studies, there could be questionable compliance with the dietary intervention, length of the interventions may not have been long enough to significantly impact measures of adiposity, and the dairy interventions may have been done alongside other interventions, making it impossible to discern whether any observed impact was truly due to the dairy intervention itself.

3.1.1 Full-fat Dairy Foods, Dairy Fat, and Adiposity

Expanding on a review conducted by Kratz et al. in 2013[118], an updated literature search identified a total of 33 observational studies that directly measured full-fat dairy food intake or biomarkers of full-fat dairy consumption and measures of adiposity from the years 1999-2020. Of these studies, 19 were prospective, 13 were cross-sectional, and one was retrospective. Twelve studies were conducted in the US and 17 were conducted in Europe. The majority of the studies determined dairy fat or high-fat dairy intake through food frequency questionnaires (FFQ) and eight studies used a biomarker of dairy fat intake.

Overall, 24 studies found an inverse relationship between dairy fat and/or high-fat dairy food consumption and measures of adiposity[124-146] (**Table A1**). Eight of the studies did not find any association between dairy fat and or high-fat dairy food consumption and measures of adiposity[147-154]. Only one study found a positive association between dairy fat consumption and adiposity[155]. The majority of the studies used FFQs to assess dairy fat or full-fat dairy food consumption; however, this method of assessment comes with several limitations. One of the ways to get around the limitations of using an FFQ to assess dairy fat consumption is by using a dairy fat biomarker. When separating out the studies that used a biomarker as their assessment of full-fat dairy intake, we see that seven out of these eight studies found an inverse relationship

between full-fat dairy consumption and measures of adiposity[124,126,127,132,133,145,146]. It should be noted that many of the studies were cross-sectional. Further, two of the studies did not control for any confounding factors. The issue of reverse causality is a problem for all cross-sectional studies listed above. To attempt to get around this issue, we can focus on prospective studies. 19 of the 33 studies were prospective in nature (**Table A1**). Of these studies, 12 found an inverse relationship between full-fat dairy consumption and adiposity. It is also important to note that the location where these studies were conducted may influence the results. It appears that a higher proportion of studies conducted in the US found no association between full-fat dairy consumption and adiposity when compared to studies conducted outside of the US (**Table A1**). Therefore, there are many variables, which may explain some of the inconsistencies observed in the observational literature on this topic.

There are many potential limitations and considerations that must be noted when evaluating this short review of observational studies. These limitations remain concerns for the associations outlined in sections 3.1.1-3.4.1. One issue is that the majority of the studies relied on FFQs. There may be exposure misclassification when using FFQs based on over or under reporting. Specifically, those who are obese are more likely to underreport and under-reporters are more likely to underreport those food items deemed unhealthy[156,157]. Psychosocial factors may further impact the ability of FFQs to adequately capture habitual diet[156,157]. Further, if dairy consumption were only evaluated at baseline, changes in dairy consumption over time would not be captured and may lead to misclassification. This may attenuate any observed associations. Further, a FFQ may not accurately capture all dairy consumed by a participant (i.e. dairy found in mixed dishes). Using a biomarker of full-fat dairy consumption as the exposure variable, while more objective than an FFQ, also has its limitations. There is variability in the fatty acids found in various dairy food sources. There could be differences in the bioavailability, metabolism, or incorporation of dairy biomarkers in specific lipid compartments. Additionally, it is possible that dairy biomarkers are correlated with other dietary or endogenous factors. Another consideration is the population size, which ranged from 60 participants to 97,811 participants. For those with a small sample size, it is questionable as to whether or not there was enough power to detect a meaningful association. For those studies with a very large sample size, the effect size of a statistically significant result may be too small to be clinically relevant. There are also issues

with the generalizability of each study based on participant characteristics and region of the world where the study took place. Another issue is that the majority of studies used dairy consumption as a categorical rather than a continuous variable. As a result, if dairy consumption was relatively high in the population as a whole, this could mask the association because the group with the lowest dairy consumption could still consume a large amount of dairy. On the other hand, if dairy consumption is relatively low in the population, the group with the highest dairy consumption may still not consume the dose of dairy necessary to have a biological impact on outcomes. As is always the case with observational studies, there is the potential for residual confounding. One last limitation is the characterization of the outcome variable could bias the results. Limitations aside, overall, the observational data suggests that full-fat dairy foods are not obesogenic. Instead, the literature suggests that full-fat dairy products may protect against obesity.

The data generated from RCTs is not in alignment with the observational literature. A meta-analysis of 20 RCTs by Benatar et al. found increased dairy consumption as compared to the usual diet resulted in weight gain for both studies that increased low-fat and full-fat dairy foods, though generally those consuming full-fat dairy gained less weight than those eating low-fat dairy[158]. There are some limitations and considerations for the studies included in this meta-analysis that must be highlighted. The majority of studies only included women, they only included studies that tested healthy people, the duration of the dietary interventions were variable, there was a large range in sample sizes, inadequate measure of compliance, and potential for publication bias. There are only two RCTs that specifically compared the effects of full-fat versus low-fat dairy on adiposity. In a 12 week parallel designed RCT, Raziani et al. randomized 164 subjects who had at least two metabolic risk factors to diets containing regular fat cheese (80 g per day), reduced fat cheese (80 g per day), or a carbohydrate control consisting of bread and jam[159]. They found that whole-fat cheese consumption did not result in a significant impact on body weight, WC, BMI, fat mass, percentage of body fat or lean mass when compared to the reduced fat cheese or the carbohydrate control[159]. This indicated that dairy fat, when consumed as cheese, is not obesogenic. In a randomized cross-over study of 18 healthy adults, Engel et al. compared the impact of a diet containing 0.5 L of skimmed milk or whole milk per day for three weeks each. Subjects were instructed to not consume any yogurt, ice-cream or milk

besides the study milk, but were asked to keep their cheese and butter intake the same throughout the study. They found that there was no differential effect of the diets on body weight[160]. While these two studies lend evidence that consuming full-fat dairy foods does not differentially impact adiposity as compared to consuming low-fat dairy foods, these studies are limited by the fact that Raziani et al. only tested cheese and Engel et al. only tested milk in their dairy interventions. In the observational literature, yogurt is consistently associated with reduced change in adiposity measures[121]. Therefore, it remains unclear how the consumption of a wide variety of full-fat dairy foods, particularly yogurt, may impact adiposity. Overall, the experimental data indicates that consumption of full-fat dairy foods likely does not impact adiposity or may even reduce adiposity as compared to the consumption of low-fat dairy foods. However, the limitations of the current literature make it difficult to come to a definitive conclusion.

3.2 Dairy Foods and Type 2 Diabetes Mellitus

There are several reviews and meta-analyses of the observational literature on the topic of dairy foods and T2D[116,161-169], all of which consistently come to the conclusion that total dairy intake is inversely associated with T2D risk. The most recent meta-analysis, by Gijsbers et al., included 22 prospective cohort studies conducted in healthy adults and found that the RR for incident T2D was 0.97 per 200 g of dairy consumed daily[165], indicating that there is a significant inverse relationship between dairy consumption and risk of T2D. Further, Gijsbers et al.'s analysis found that the types of dairy foods might impact T2D risk differentially. For example, there was no association observed for intakes of milk, cheese, or cream[165]. However, the investigators found that there was a consistent, but non-linear, association between yogurt intake and T2D risk with a relative risk of 0.86 for those who consume at least 80 g of yogurt per day as compared to those who ate none[165]. Consistently, the investigators also found a non-linear association between fermented dairy foods and risk of T2D, with a 12% reduced risk of T2D for those consuming 40 g per day of fermented dairy as compared to not consuming any fermented dairy foods. However, it must be noted that this result was primarily driven by one study that included high-fat fermented dairy foods, suggesting that fat content may modify the impact of fermented dairy foods on T2D risk. With this said, when analyzing whether total low-fat dairy intake or full-fat dairy intake impacted risk of T2D, Gijsberg and colleagues found that there was a trend for a lower risk of T2D risk per 200 g per day of low-fat dairy foods, but did not find an

association per 200 g per day of full-fat dairy foods[165]. Overall, this meta-analysis provides evidence that total dairy is significantly and inversely associated with T2D risk. The three most recent reviews on this topic, all published in the previous year[166-168], consistently concluded that total dairy is inversely associated with T2D, indicating that any additional data published after Gibjsberg et al.'s meta-analysis does not greatly impact this outcome. Further, Gibbjsberg and colleagues results indicate that the association between dairy intake and T2D may be dependent on the type of dairy consumed, including fermentation status and fat content. They concluded that there is particularly strong evidence for an inverse relationship between yogurt consumption and a reduced risk of T2D. A review published in 2019 by Guo et al. consistently concluded that there is strong and consistent evidence linking yogurt consumption with a lower risk of T2D[166]. Therefore, overall, the observational literature consistently demonstrates that dairy consumption is associated with a decreased risk for T2D, indicating that including dairy in the diet could serve as a tool to decrease the burden of this condition.

Now turning to the evidence from RCTs, there is no RCT that specifically assessed the effect of dairy consumption on incident diabetes. However, several RCTs have investigated the effect of dairy consumption on glucose homeostasis, insulin sensitivity, or β-cell function. These studies are discussed in section 3.5.1.

3.2.1 Full-fat Dairy Foods, Dairy Fat and Type 2 Diabetes Mellitus

Further expanding on a review conducted by Kratz et al. in 2013[118], an updated literature search identified a total of 33 observational studies that investigated the relationship between full-fat dairy consumption or biomarkers of full-fat dairy intake and incident diabetes[132,145,154,170-199] (**Table A2**). Of these studies, 29 were prospective and four were cross-sectional. Fifteen studies were conducted in the US and 13 were conducted in Europe. Many of the studies determined dairy fat or high-fat dairy intake through FFQs, and 16 of the 33 studies used a biomarker of full-fat dairy intake.

Of the 33 studies identified, 12 found that diabetes incidence was inversely associated with full-fat dairy food intake or biomarkers of dairy fat[132,145,154,172-174,177,180,183,185,196,197], while 16 found no association[170,171,175,176,178,179,181,182,184,186-188,190,191,193,195,198,199]. Three studies found a positive

association between some measure of full-fat dairy food intake and incidence of T2D[175,189,192,194]. This indicates that the majority of the observational evidence suggests that full-fat dairy consumption is either inversely associated or not associated with the development of T2D. This is consistent with the conclusions of a recent meta-analysis of prospective cohort studies[169].

While the majority of the observational evidence assessed dairy consumption through FFQs, studies utilizing biomarkers of full-fat dairy intake came to similar conclusions. Using a dairy biomarker is a more objective measure of full-fat dairy food intake. Therefore, isolating the studies that used a dairy biomarker as their exposure method may further shed light on this topic (**Table A3**). Five of the eight studies that included the dairy biomarker C15:0 found that increased concentrations of C15:0 in the plasma phospholipids were associated with a reduced risk of incident T2D[145,172,173,183,186]. The reduced risk ranged from 20%-60% when comparing the highest reference group (usually quintile or quartile) of C15:0 to the lowest reference group, or hazard ratio or odds ratio in observational studies (**Table A3**). Four out of the six studies that evaluated the association between C17:0 and incident diabetes also found an inverse association with a reduced risk ranging from 26%-57% when comparing those in the highest reference group (usually quintile or quartile) of C17:0 to those in the lowest reference group, or hazard ratio or odds ratio in observational studies (**Table A3**)[145,173,183]. Fourteen studies investigated the association between 16:1n-7 and incident diabetes. Five of the studies found no association[173,186,190,191,195], five studies found a negative association[132,145,177,193,196,200] and three found a positive association with incident diabetes[172,192,194] (**Table A3**). Taking into consideration just those studies that used a dairy biomarker as a measure of full-fat dairy consumption, the observational literature indicates that the consumption of full-fat dairy foods is inversely associated with incident T2D. This conclusion is in alignment with a recent meta-analysis from Imamaura that pooled finding from 16 prospective cohort studies and concluded that higher levels of C15:0, C17:0 and trans-16:1n-7 are all associated with a lower risk of T2D[201].

One of the possible mechanisms through which dairy fat could impact the development of T2D is through changes in adiposity. As indicated in section 3.1.1, the observational literature indicates that full-fat dairy food consumption is mostly inversely associated with weight gain.

The majority of the studies in Table A2 adjust for some measure of adiposity, potentially attenuating the association between dairy fat consumption and T2D. Only five studies did not adjust for adiposity, of which three[145,154,185] found an inverse relationship between dairy fat intake and incidence of T2D and two found no association[154,179]. This suggests that there may be mechanisms by which dairy impacts the risk of T2D at least partly independent of changes in body weight. These mechanisms will be discussed in Chapter 4. Overall, the observational data suggests that full-fat dairy food consumption is either not associated with or is inversely associated with the incidence of T2D.

There are no current RCTs investigating the effect of full-fat dairy consumption on incident T2D. There are a couple of trials that have compared the effect of low-fat versus full-fat dairy food consumption on glucose homeostasis, insulin sensitivity, or β-cell function. These studies are discussed in section 3.5.1.

3.3 Dairy Foods and The Metabolic Syndrome

While there are many studies that have investigated the relationship between dairy consumption and the constituents of the MetS (as found in sections 3.1 and 3.5), fewer studies have investigated the relationship between dairy consumption and incident MetS. A recent meta-analysis by Lee et al. included nine observational studies looking at the relationship between dairy consumption and MetS[120]. The authors concluded that an increase of 200 g per day of total dairy product intake was associated with a 9% decreased risk in MetS[120]. Further, an inverse dose-response relationship was also observed for both milk and yogurt consumption with a 13% reduced risk for every 200 g per day increment of milk intake and a 18% reduced risk for every 100 g per day increment of yogurt intake[120]. Therefore, overall, the current state of the evidence indicates that dairy consumption as a whole and milk and yogurt in particular, may be protective against the development of MetS.

3.3.1 Full-Fat Dairy Foods and the Metabolic Syndrome

Only a couple studies have investigated the association between full-fat dairy consumption and the incidence of MetS. A cross-sectional analysis by Drehmer et al. conducted using the Brazilian Longitudinal Study of Adult Health cohort, found that total dairy and full-fat dairy

were inversely associated with MetS, though similar associations were not observed for low-fat dairy foods[202]. In contrast, similar associations between full-fat dairy consumption and incident MetS are not seen in prospective cohort studies. Babio et al. in a prospective cohort study of men and women from the Prevención con Dieta Mediterránea study, found that full-fat dairy consumption was not associated with incident MetS[142]. Further, there was no association between cheese or whole milk consumption and incident MetS. However, there was a 22% reduced risk of MetS among the highest tertile of whole yogurt consumers as compared to the lowest tertile[142]. Neither Babio et al. nor Drehmer et al. used a dairy fat biomarker to assess full-fat dairy food intake. Instead, they relied on less objective assessment methods of dairy consumption, which are subject to misreporting. Zong et al. in an analysis of 1,176 Chinese men and women 50-70 years old, found that those in the highest quartile compared to the lowest quartile of erythrocyte 16:1n-7 concentrations had a RR of 1.48 after adjusting for multiple lifestyle factors and BMI[195]. While this is only the result of one study, with several limitations, it lends some additional evidence to suggest that full-fat dairy may have a differential impact on risk of MetS as compared to low-fat dairy consumption. However, more research is needed to clarify whether the consumption of low-fat versus full-fat dairy products differ in their impacts on the risk of developing MetS.

3.4 Dairy Foods and Cardiovascular Disease

Numerous observational studies have investigated the association between dairy consumption and CVD, which have been compiled into multiple meta-anlyses[203-207]. Recent meta-analyses have concluded that there is no association between dairy intake and total CVD[206] or CVD mortality[203]. However, it appears that dairy consumption may be associated with certain subtypes of CVD. A meta-analysis by Bechthold et al. that was published late last year included a total of 24 prospective cohort studies in their analysis of the association between dairy intake and the incidence of CVD subtypes in healthy adults. Bechthold et al. found that those in the highest category of dairy intake did not differ from those with the lowest dairy intake with regards to the incidence of various subtypes of CVD including heart disease, stroke, and heart failure[207]. Additionally, every 200 g per day increase in dairy was also not associated with heart disease or stroke. However, every 200 g per day increase in dairy was associated with an 8% increased risk of heart failure[207]. Further, there appeared to be a non-linear dose-response relationship between

dairy consumption and stroke, with benefits found only in those consuming less than or equal to 500g of dairy per day[207]. An association between dairy consumption and a reduced risk for stroke has been indicated in previous meta-analyses[204,205], however Bechthold et al. was the first to suggest that there may be a threshold effect. Overall, the observational literature generally indicates that dairy consumption is not associated with CVD, but indicates that dairy may be associated with certain CVD subtypes including heart disease and stroke. Further investigations are needed to verify whether there is a dose response or a threshold effect between dairy consumption and CVD subtype risk. Further, it remains unclear whether the types of dairy foods consumed, such as variations in fermentation or fat content, may have differential associations with CVD risk.

There is no experimental study that investigates the effect of dairy consumption on incident CVD, heart disease, or stroke. However, several RCT's have reported data on the relationship between dairy consumption and risk factors for CVD such as serum lipids and blood pressure. This evidence is discussed in sections 3.5.3. and 3.5.4. Generally, dairy consumption does not appear to affect classic CVD risk factors such as blood lipids or blood pressure. Therefore, current experimental evidence does not suggest that dairy consumption impacts CVD risk.

3.4.1 Full-fat Dairy Foods, Dairy Fat and Cardiovascular Disease

Traditionally, full-fat dairy has been seen as an unnecessary source of SFAs. Dietary SFAs as compared to mono- or polyunsaturated fats are linked to elevated fasting serum cholesterol as well as an increase in LDL-cholesterol (reviewed in [208]). Both increased LDL and elevated serum cholesterol concentrations are risk factors for CVD (reviewed in [208]). Therefore, it was thought that reducing the consumption of dietary sources of SFAs, such as full-fat dairy, would reduce the risk for CVD. With this said, new evidence indicates that the association between SFAs and CVD is not as robust as previously believed. A review by Siri-Tarino calls into question the presumed link between SFAs and CVD[208]. They indicate that not all SFAs behave equally in the body, as discussed briefly above.

Further expanding on a review conducted by Kratz et al. in 2013[118], an updated literature search identified a total of 42 observational studies that directly measured full-fat dairy food or dairy fat

consumption or biomarkers of full-fat dairy food intake and CVD, heart disease, stroke, and MI from the years 1999-2020 (**Table A4**)[127,133,139,176,209-245]. Eighteen studies were conducted in the US and 11 were conducted in Europe. The majority of the studies determined dairy fat or high-fat dairy intake through FFQs. Only 15 of the 42 studies used a biomarker of full-fat dairy food intake. Of these 42 studies, 20 found no association[176,215,216,218-221,223,228,229,231,232,235-239,242,244,245], 14 found an inverse association[127,133,139,209,211,212,214,217,225-227,230,234,240], and only six of the studies found a positive association between full-fat dairy food intake and CVD outcomes[210,213,222,224,233,243].

As mentioned in previous sections, focusing on studies that evaluated full-fat dairy consumption using a dairy fat biomarker can shed additional light. When concentrating just on those studies utilizing a dairy fat biomarker, eight out of the 15 studies found an inverse relationship between full-fat dairy food intake and incident CVD[127,133,209,211,212,225,226,234], while four studies found no association[229,232,244,245], and three studies found a positive relationship[210,224,243]. Therefore, the majority of the studies indicated that higher intakes of full-fat dairy products were not associated with poor cardiovascular outcomes, which goes against the initial paradigm. This conclusion is confirmed by a meta-analysis by Liang et al. that looked at 13 studies that investigated the association between dairy fat biomarkers and cardiovascular health[246]. When pooling the 12 studies that utilized the dairy biomarker C15:0, they found that there was no difference between those in the top tertile as compared to the lowest tertile of biomarker C15:0 on risk of CVD, though there was a decreased risk of heart failure with a RR of 0.72 in subgroup analyses[246]. Of the seven studies that reported on the association between dairy biomarker C17:0 and CVD, there was an 18% reduced risk of CVD for those in the top tertile as compared to those in the lowest tertile, though there was no significant association between C17:0 and any subtype of CVD[246]. Pooling the four studies that reported on the dairy biomarker trans-16:1n-7, there was not a significant association[246]. Therefore, overall, Liang et al. concluded that the observational literature does not support the hypothesis that full-fat dairy food consumption is positively associated with CVD[246].

Overall, the observational data suggest that full-fat dairy food intake is either not associated with or is inversely associated with poor cardiovascular health. This conclusion is in alignment with

two recent reviews and meta-analyses that concluded that there was no significant difference between low-fat and full-fat dairy products on risk of heart disease and stroke[206,207]. This lack of an association between full-fat dairy intake and CVD risk may be unexpected, given that trials consistently indicate that isolated dairy fat in the form of butter results in dyslipidemia[247].

While there are no RCTs that directly compare low-fat to full-fat dairy consumption with regards to the development of CVD, heart disease or stroke, there are a few trials evaluating the effect of full-fat versus low-fat dairy on CVD risk factors as will be discussed in section 3.5.3.

3.5 Dairy Foods and Risk Factors for Chronic Disease

3.5.1 Dairy and Insulin Resistance, β-cell Dysfunction, and Glucose Intolerance

Both IR and β-cell function are important determinants of glucose tolerance, and therefore risk factors for T2D. Consequently, if dairy consumption supports glucose tolerance, we would anticipate seeing an association between dairy consumption and insulin sensitivity or β-cell function. In a cross-sectional analysis of approximately 4,500 Swedish men and women, non-fermented milk was associated with higher HOMA-IR whereas fermented milk and butter were associated with a lower HOMA-IR[218]. This indicates that while dairy consumption is associated with insulin sensitivity, this association is likely influenced by the type of dairy food consumed, with fermented dairy foods and dairy fat conferring a potential benefit. While we cannot rule out the potential for reverse causality in this cross-sectional analysis, a positive association between dairy intake, particularly fermented dairy intake, and insulin sensitivity is also observed prospectively. A prospective cohort study by Drehmer et al. that included over 10,000 participants from the Brazilian Longitudinal Study of Adult Health found that total dairy intake was inversely associated with fasting glucose, 2-hour glucose, 2 hour insulin, HbA1c, and HOMA-IR independent of obesity[248]. Additionally, this association was strongest for fermented dairy products[248]. There is also evidence from observational studies utilizing full-fat dairy food biomarkers, indicating that full-fat dairy food consumption may be associated with improved insulin sensitivity, but not β-cell function. A cross-sectional study including 32 American men and women, 17 of which had NAFLD, found that plasma trans-16:1n-7 was positively associated with both hepatic and systemic insulin sensitivity as measured by IVGTT, independent of body

fat[249]. It should be noted that this relationship was no longer significant after adjusting for liver fat content[249]. This indicates that liver fat may be on the causal pathway by which full-fat dairy consumption may impact insulin sensitivity. There was no association found between biomarkers of dairy fat intake and β-cell function[249]. However, similar outcomes were not observed in a prospective cohort study of middle-aged Finish men. Linkinen et al. found that neither plasma phospholipid C15:0 nor C17:0 were associated with insulin sensitivity, but 16:1n-7 was negatively associated with insulin sensitivity as measured by the Matsuda insulin sensitivity index from a 2-hour frequently sampled OGTT (FS-OGTT)[190]. Further, investigators also found that there was a positive association between C17:0 and a negative association between 16:1n-7 and the early phase disposition index[190]. It may be that the differential outcomes between these two biomarker studies were due to the fact that one was cross-sectional while the other was prospective. Further, it could be that the types of full-fat dairy foods consumed in the American diet differ from those typically consumed in the Finnish diet, which may have influenced any observed association between full-fat dairy food biomarkers and insulin sensitivity. Alternatively, the fact that Linkinen et al. only used middle-aged men in their study may further have contributed to the differential results. Overall, while there is a paucity of research on the association between dairy foods, types of dairy foods, and measures of insulin sensitivity and β-cell function, the available observational evidence indicates that increased total dairy consumption is associated with better insulin sensitivity, but not β-cell function. Further, the fermentation status and fat content of the dairy foods consumed may influence the association between dairy consumption and insulin sensitivity.

Several RCTs have specifically tested the effect of dairy foods on measures of IR. A meta-analysis by Benatar et al. included RCT's in healthy adults who were randomized to increase their dairy consumption for more than one month and excluded any other interventions. The collective analysis of the four studies investigating the impact of increased dairy consumption on HOMA-IR concluded there was no significant change in this measure[158]. It should be noted that these relationships were unaltered when stratifying the analysis into low-fat versus high-fat dairy interventions[158]. Since the publication of this meta-analysis, emerging experimental evidence suggests dairy foods may increase insulin sensitivity. A meta-analysis by Sochol et al., published in 2019, included 12 RCTs investigating the effect of dairy consumption on insulin resistance as

defined by HOMA-IR[250]. They included RCTs with a dairy intervention either with or without energy restriction. They concluded that dairy consumption decreases HOMA-IR with the pooled standardized difference in means of -1.21 (95% CI -1.74 to -0.67) between the dairy and placebo groups[250]. Further, the authors found that participants with a BMI greater than 25 kg/m^2 had a slightly greater reduction in HOMA-IR as compared to the overall effect[250].

There are a few potential explanations for the differences between the Sochol et al. and Benatar et al. meta-analyses. First, Sochol et al. included studies through 2016, whereas Benatar et al. included studies through 2013. Further, the inclusion criteria for the Benatar et al. analysis were more stringent. In contrast to the Sochol et al. meta-analysis, Benatar et al. restricted their meta-analysis to healthy individuals, had a minimum intervention length, and only included studies where there were no other interventions other than dairy. Therefore, it remains unclear whether the effects of dairy consumption on insulin sensitivity seen in the Sochol et al. meta-analysis are driven by the inclusion of studies that included populations with comorbidities; or by including studies that had a combination of a dairy intervention with another intervention component, such as calorie restriction. It may be that increased dairy consumption only improves insulin sensitivity in populations with compromised metabolic health or only in the context of calorie restriction. Additionally, Sochol et al. did not investigate whether there is a differential impact of dairy consumption on insulin sensitivity between interventions that included full-fat dairy foods or fermented dairy foods as compared to low-fat dairy foods or non-fermented dairy foods. Therefore, further research is needed to clarify the relationship between dairy consumption and insulin sensitivity.

There are a few additional studies investigating the relationship between dairy consumption and insulin sensitivity as assessed by fasting measures that were not included in the Benatar et al. or Sochol et al. meta-analyses that should also be considered. Two of these studies, in alignment with Sochol et al., found that dairy consumption improved insulin sensitivity. A randomized cross-over study by Rideout et al. included 23 healthy individuals who ate a diet high in dairy and a diet low in dairy, each for six months. They found that the increase in fasting insulin was significantly smaller in the high dairy group as compared to the low dairy group[251]. In a 16-week parallel design RCT by St-Onge, overweight and obese boys and girls were randomized to either

high or low milk consumption. The increase in HOMA-IR was smaller for the high dairy group as compared to the low-dairy group[251].

Studies by O'Connor et al.[252], Raziani et al.[159], Dugan et al.[253], Van Loan et al.[254], and Maki et al.[255] all found that there was no effect of dairy consumption on insulin sensitivity. O'Connor et al. in a randomized cross-over study had 17 hyperinsulinemic adults assigned to a high dairy diet consisting of 4-5 servings of dairy daily or an adequate dairy diet consisting of two or less servings of daily dairy for six weeks each. They found that the high dairy diet did not differ from the low dairy diet with respect to insulin sensitivity as measured by fasting insulin or HOMA-IR[252]. Another RCT by Dugan et al. included 37 adults with MetS who were randomized to either a low-fat dairy intervention that included low-fat yogurt, milk and cheese or a carbohydrate control group that included juice and granola bars. These diets were followed for six weeks. There was no significant difference in fasting plasma insulin or HOMA-IR between the groups[253]. A parallel design RCT by Raziani et al. randomized 164 subjects to one of three groups; a regular fat cheese group (80 g per day), reduced fat cheese group (80 g per day), or a non-cheese carbohydrate control group consisting of jam and bread. They followed their respective diets for 12 weeks. There was no difference in the change in fasting insulin or HOMA-IR among the groups[159]. In a 15-week RCT in 71 low-dairy consuming overweight or obese adults, Van Loan et al. found that there was no effect of eating more than four servings of dairy per day as compared to less than or equal to one serving of dairy per day in the context of energy restriction on fasting insulin[254]. In a randomized cross-over trial, Maki et al. had 43 men and women at risk of developing T2D follow diets rich in dairy products (2% milk and low-fat yogurt) and another diet rich in non-diet soda and non-dairy pudding for six weeks each. While the dairy rich group reduced fasting insulin, it did not differentially change the HOMA 2- insulin sensitivity[255]. While these studies varied in duration, interventions, and participant populations, they all came to the same general conclusion that increased dairy consumption does not result in increased IR as measured by fasting values. A notable gap in the existing literature is that these interventions rarely included full-fat or fermented dairy products, and that the outcome measurements were not commonly assessed using dynamic testing.

There were three additional RCTs that found that dairy consumption leads to decreased insulin sensitivity as measured by fasting values. Hoppe et al. studied the effects of non-fat milk intake (53 g of protein in the form of skim milk daily) versus an equally weighted serving of protein as meat over seven days on fasting insulin and insulin sensitivity in 24 eight year old boys. They found that fasting insulin and HOMA-IR increased in the milk-consuming group[256]. The meat consuming group didn't have a significant difference in any of the aforementioned parameters and only had a significant decrease in fasting glucose[256]. It must be noted that the total energy intake was substantially higher in the milk group compared to the meat group. As a result, it is unclear whether the results are an effect of the increased milk consumption or the excess energy intake. In a separate randomized cross-over study by Turner et al., 47 overweight and obese men ate three weight-stable diets for four weeks each: a diet high in lean red meat with minimal dairy, a diet high in low-fat dairy and no red meat, and a diet with no red meat nor dairy. Fasting insulin was significantly higher in the dairy diet as compared to the red meat diet and control diet[257]. HOMA-IR was significantly higher when consuming the dairy diet as opposed to the red meat diet, and - in women - higher in the dairy diet as opposed to the control diet[257]. Eelderink et al. conducted a randomized cross-over study with 45 overweight men and women. The dietary interventions were a high dairy diet consisting of 5-6 dairy portions and a low-dairy diet consisting of less than one dairy portion of semi-skimmed yogurt, reduced fat cheese, semi-skimmed milk and buttermilk per day for six weeks. HOMA-IR increased on the high dairy diet as compared to the low-dairy diet phase[258].

It should be noted that in almost all of the studies dairy consumption only included low-fat dairy foods, while, as evidenced in section 3.2.1, observational data more strongly suggest a potential role for full-fat dairy in reducing the risk for T2D. Further, the interventions were relatively short with the diets lasting seven days to six weeks. Taken all together, the studies using fasting measures of insulin sensitivity indicate that increased dairy consumption either does not affect or increases insulin sensitivity.

Few studies have investigated the effect of dairy diets on β-cell function through fasting measures. The study designs for Eelderink et al., Maki et al., and Hoppe et al. were described above. Both Eelderink et al. and Maki et al. found that diets rich in dairy as compared to low-

dairy diets did not differentially affect HOMA-β[258,255]. Hoppe et al. found that both the consumption of non-fat milk or an equally weighted serving of protein increased HOMA-β[256], though there was no assessment to determine whether the increase observed was differential between the two intervention groups. Overall, based on fasting values, this indicates that dairy consumption likely does not affect β-cell function in adults at risk for metabolic disease. However, it remains unclear whether dairy consumption impacts β-cell function in younger and healthier populations or in studies utilizing full-fat dairy interventions.

Several RCTs have also investigated the effect of dairy consumption on glucose tolerance as assessed by fasting glucose. The Benatar et al. meta-analysis discussed earlier identified eight studies that evaluated the impact of increased dairy consumption on fasting glucose in healthy individuals. When evaluating these studies collectively, it was found that increasing dairy consumption modestly increased fasting glucose[158], though it appears that this result was primarily driven by one study. Since the publication of this meta-analysis, trials investigating the effect of dairy consumption on fasting glucose have all concluded that dairy intake does not have an impact on fasting glucose[159,160,251-254,256-261].

Based on the evidence presented above, it would appear that increased dairy consumption either does not impact insulin sensitivity or improves insulin, and does not impact β-cell function or glucose tolerance as assessed by fasting measures. However, using fasting measures as a method for assessing glucose tolerance, insulin sensitivity and β-cell function can be problematic. One issue is fasting levels of insulin are primarily a measure of hepatic insulin sensitivity and not systemic insulin sensitivity[262]. Further, fasting insulin values can be affected by insulin clearance[263]. Though, it should be noted that while fasting insulin is an imperfect assessment of insulin sensitivity, it is highly correlated with clamp and IVGTT techniques[264,265]. Using fasting plasma glucose as a measure of glucose tolerance is also problematic. Fasting plasma glucose is less sensitive than an OGTT in detecting impaired glucose tolerance[266]. Further, one main issue with using fasting values as a measure of insulin sensitivity and glucose tolerance is it only provides insight into these measures in the fasting state. Therefore, the fasting values do not take into consideration the ability of the body to respond to rising or falling glucose concentrations that occur regularly during daily living[267]. Another limitation in using fasting blood to measure

insulin sensitivity and β-cell function is that the homeostatic models commonly employed are not ideal. Both HOMA-IR and homeostatic model of β-cell function (HOMA-%B) use basal concentrations of glucose and insulin to provide measures of insulin sensitivity and β-cell function through an equation derived from a mathematical assessment of the interaction between β-cell function and IR[268]. It should be noted that HOMA-%B is particularly suboptimal because the results for β-cell function only make sense in the context of also reporting the result for HOMA-IR. Someone may appear to have a low β-cell function value, but this may be explained by high insulin sensitivity[268]. Generally, HOMA-IR is a good technique for large scale epidemiological studies where other techniques of insulin sensitivity assessment are close to impossible to gain access to because it is simple and relatively inexpensive[269]. One draw back to the use of HOMA-IR or HOMA-%B is that they are derived from an equation based on data from the 1970's and tend to underestimate IR and overestimate β-cell function[268]. Therefore, while HOMA measures can be a good measure of relative change, it may be inaccurate as an absolute measure of IR and β-cell function. Another limitation of the HOMA-IR model is the assumption that hepatic and peripheral insulin resistance are equivalent[262].

It is because of the limitations of measuring insulin sensitivity and β-cell function through methods that rely on fasting values, that it is important to look at trials that use dynamic measures of metabolic health such as a FS-OGTT, an IVGTT, a hyperinsulinemic euglycemic clamp, or a mixed meal tolerance test. There are nine studies that utilized one of these techniques to investigate how dairy consumption impacts insulin sensitivity and β-cell function[252,255,257,258,260,270-273]. In the three trials that investigated the impact of increased dairy consumption in the context of a hypocaloric diet, they all found that there was no impact of increased dairy consumption on IR and β-cell function as measured through dynamic testing[271-273]. However, the five trials that investigated this question using dynamic testing in the context of an *ad libitum* diet had mixed results. The study designs for St-Onge et al., Turner et al., Eelderink et al., O'Connor et al. and Maki et al. have been described previously. The design of the fifth relevant study by Gardner et al. will be summarized below. The results from St-Onge et al. indicate that those assigned to high milk consumption had a lower AUC insulin when compared to the low milk consumers, but there was no effect seen on AUC glucose[260]. It should be noted that those assigned to the low milk consumption group were also asked to consume

sugar-sweetened beverages. Therefore, we cannot decipher whether a similar result would have occurred if a different control had been selected. In a randomized cross-over study by Turner et al., the Matsuda insulin sensitivity index showed a 14.7% reduced sensitivity in women after the dairy diet as compared to the red meat diet or the control diet lacking meat or dairy, but there was no difference in men[257]. There was also no difference in AUC glucose, insulin, or c-peptide between diet groups[257]. A randomized cross-over study by Gardner et al. included 28 healthy adults who followed a diet including milk, a diet including soy protein isolate, and a diet including whole bean derived soy milk for four weeks each. There was no difference in AUC insulin or 1 hour or 2 hour glucose observed between the milk consumption diet phase and the soy including diets[270]. Another randomized cross-over study by Eelderink et al., found that there was no difference in AUC glucose or insulin during a 2 hour FS-OGTT during their high dairy diet as compared to their low dairy diet phase[258]. Further, there was no effect on endogenous glucose production or glucose clearance rate[258]. However, there was a trend for a decrease in the Matsuda insulin sensitivity index in the high dairy group as compared to the low dairy group[258]. The randomized cross-over trial by Maki et al. found that the dairy rich group did not differentially change the Matsuda insulin sensitivity index or 2-hour insulin during a liquid meal tolerance test[255]. O'Connor et al., in a randomized cross-over study, found that the high dairy diet did not differ from the low dairy diet with respect to insulin sensitivity as measured by the Matsuda insulin sensitivity index or AUC insulin during a 2-hour OGTT[252]. Therefore, the study by St-Onge et al. shows a possible beneficial impact; while Maki et al. O'Connor et al., and Gardner et al. showed no effect; and Turner et al. and Eelderink show a potential negative effect of increased dairy intake in the context of an *ad libitum* diet on IR and β-cell function as measured through dynamic testing. Therefore, collectively the effect of dairy consumption on IR and β-cell function as assessed by dynamic testing in the context of an *ad libitum* diet remains inconclusive.

Overall, the impact of total dairy consumption on IR and β-cell function remains uncertain due to the limitations and inconsistencies in the current literature. The existing body of evidence is limited and is subject to several important limitations, including the following: most of the studies utilized non-fat or low-fat dairy as well as unfermented dairy products in their dairy interventions, even though the observational evidence indicates that it may be full-fat dairy

and/or potentially fermented dairy such as yogurt that may be beneficial for metabolic health. Further, the majority of the studies were not specifically designed to test the impact of dairy foods on IR and β-cell function. The majority of the studies relied on fasting measures instead of using dynamic testing. Additionally, most studies relied on a *per-protocol* analysis instead of reporting an ITT analysis. An ITT analysis is preferred because it is more conservative and less influenced by bias[274]. Since an ITT analysis includes all individuals that were randomized, this approach minimizes confounding between the groups[275]. Further, for the studies that were conducted *ad libitum*, they often failed to control for changes in weight or fat mass either experimentally or statistically. Some additional limitations include small sample sizes, the fact that it is difficult to blind diet studies, there could be questionable compliance with the dietary intervention, and the fact that the length of the interventions may not be long enough to impact the risk factor(s) being investigated. Therefore, we need more rigorously designed RCTs that address these limitations before we can come to a conclusion as to whether or not total dairy consumption has an effect on insulin sensitivity and β-cell function.

Given that there is particularly strong and compelling evidence linking biomarkers of full-fat dairy intake with a reduced risk of T2D (3.2.1), it is important to assess whether trials indicate that full-fat dairy foods beneficially impact glucose tolerance, insulin sensitivity, or β-cell function. There are only two experimental studies that have directly compared the effects of low-fat and full-fat dairy consumption on measures of glucose tolerance and insulin sensitivity. The details of the RCT by Raziani et al. and Engel et al. have been described above. The investigators found that a diet containing full-fat cheese did not differ from a diet rich in low-fat cheese or a carbohydrate control on changes in fasting glucose, fasting insulin or HOMA-IR[159]. In a randomized cross-over study by Engel et al., there was no difference in fasting insulin, fasting glucose, or HOMA-IR when comparing the skimmed milk to whole milk dietary phases[160]. Therefore, the current experimental evidence does not indicate that full-fat dairy has any effect on insulin sensitivity, β-cell function, or glucose tolerance. However, Raziani et al. only used cheese and Engel et al. only used milk in their dairy interventions and both studies relied on fasting measures of glucose tolerance, insulin sensitivity, and β-cell function. Therefore, there is a need for rigorous RCTs to further evaluate the impact of a wide variety of low-fat versus high-

fat dairy foods on glucose tolerance using dynamic testing in order to clarify whether the observed association between full-fat dairy food biomarkers and T2D may be causal.

3.5.2 Dairy and Elevated Liver Fat Content

While liver fat content has been identified as a risk factor for chronic disease, few studies have reported on the relationship between dairy consumption and liver fat. A cross sectional study by Kratz et al. investigated the relationship between full-fat dairy consumption as measured by dairy fat biomarkers and liver fat in 17 men and women with NAFLD and 15 BMI and age matched controls without NAFLD. Liver fat was measured through computed tomography-derived liver-spleen ratio. The results indicated that dairy fat consumption as measured by plasma phospholipid trans-16:1n-7, plasma phospholipid C17:0, plasma free fatty acid C15:0, and plasma free fatty acid C17:0 was inversely associated with liver fat content after adjusting for age, sex, and BMI[249]. However, it should be noted that since this was a cross-sectional study, the results must be interpreted cautiously because we cannot rule out the potential for reverse causation or unmeasured confounding. Further, we cannot determine whether the observed correlation is causal. A RCT by Chen et al. randomized 100 obese Chinese women with NAFLD and MetS to follow a diet with 220 g of whole-fat yogurt or 220 g of whole milk per day for 24 weeks. Twenty participants underwent an MRI scan and were included in the analysis of liver fat. They found that the yogurt intervention significantly decreased the hepatic fat fraction and the intrahepatic lipid content in comparison to the milk group[276]. This indicates that fermented dairy products may differentially impact liver fat content as compared to non-fermented dairy foods. However, given the paucity of research, further evidence is needed to determine whether or not dairy consumption is associated with liver fat content and further, whether any observed association is causal. Additionally, future investigations should help clarify whether different types of dairy differentially affect hepatic fat content.

3.5.3 Dairy and Dyslipidemia

One primary risk factor for CVD is dyslipidemia in the form of low HDL-cholesterol, high LDL-cholesterol, or high triglycerides in fasting serum. A recent meta-analysis of observational studies by Lee et al. found that total dairy consumption was not associated with hypertriglyceridemia or low HDL-cholesterol[120]. However, increased milk consumption was

associated with a reduced risk of low HDL-cholesterol[120]. Further, increased full-fat dairy consumption as measured by dairy biomarkers, is generally not associated with an unfavorable lipid profile. Warenjo et al. found that dairy consumption, as measured by the sum of C15:0 and C17:0 in serum phospholipids was inversely associated with the fasting serum triglyceride and total cholesterol concentrations[127]. These results were confirmed in a more recent study by the same research group using a larger sample size where they found an inverse association between the dairy blood biomarkers C15:0 and total cholesterol as well as C17:0 and triglycerides[133]. Additionally, Pranger et al. found that plasma triglyceride C17:0 is inversely associated with total cholesterol, LDL-cholesterol, total triglycerides, and the triglyceride: HDL-cholesterol ratio; and positively associated with HDL-cholesterol concentrations[146]. However, these associations were not seen for either C15:0 or trans C16:1n-7[146]. Therefore, overall, the observational evidence is mixed, but does not suggest that dairy consumption in general or full-fat dairy in particular is associated with dyslipidemia.

The experimental evidence similarly indicates that total dairy consumption does not promote dyslipidemia. Using nine RCTs that evaluated the impact of increased dairy consumption on HDL- and LDL-cholesterol, a meta-analysis by Benatar et al. concluded that there were no dairy associated changes in these lipid markers[158]. Further, the results were unchanged when stratified by low-fat versus high-fat dairy consumption[158]. Since then several RCTs have published data on this topic, all of which came to the conclusion that dairy consumption does not have an effect on serum lipids. The study designs for Dugan et al.[253], Raziani et al.[159], Eelderink et al.[258], and Rideout et al.[251] were described previously. All studies found that dairy consumption did not affect serum lipid endpoints such as LDL-cholesterol, total cholesterol, or triglycerides. All the studies found no impact on HDL-cholesterol, except Eelderink et al. found that the high dairy phase resulted in lower fasting serum HDL-cholesterol concentrations as compared to the low dairy diet phase[258]. Three additional RCTs similarly found that dairy consumption generally had no effect on serum lipids. A study by Benatar et al., in a parallel designed RCT, compared 180 healthy volunteers who were randomized to a reduced dairy intake group, a group that kept their baseline dairy consumption, or an increased dairy group for one month. They found that there was no effect on HDL-cholesterol, LDL-cholesterol or triglycerides[259]. In a randomized crossover trial by Thorning et al., 14 overweight women consumed a diet rich in cheese, a high

meat control diet, and a high carbohydrate control diet for two weeks each[277]. There was no difference between the groups in their effects on fasting serum total cholesterol, LDL-cholesterol, or triglyceride concentrations[277]. During the cheese and meat diets, participants had a smaller reduction in HDL-cholesterol when compared to when they were on the carbohydrate diet[277]. The authors concluded that a diet rich in cheese resulted in a less atherogenic lipid profile than a low-fat high carbohydrate diet. Bendtsen et al.[278] conducted a 24-week RCT where 96 participants were randomized to follow either a high dairy diet with energy restriction or a low dairy diet with energy restriction. The high dairy diet asked participants to consume at least 1,200 mg of calcium per day through dairy foods and the low dairy diet limited calcium consumption to no more than 600 mg per day. There was no difference between the diets in fasting serum total-, LDL-, HDL-cholesterol, or triglyceride concentrations[278]. All studies varied in participant population, dairy intervention and length of the intervention and yet they all were in agreement with the conclusion from the Benatar et al. meta-analysis that dairy consumption generally does not significantly impact serum lipids. With this said, these studies are subject to some limitations such as the fact that the majority of the studies included predominantly low-fat dairy as their dairy intervention even though observational studies suggest that full-fat dairy may lower CVD risk, as was discussed in section 3.4.1. Further discussions of some of the limitations can be found in section 3.5.1.

One driver for recommending low-fat dairy over full-fat dairy foods is the hypothesis that consuming full-fat dairy foods promotes dyslipidemia due to its high SFA content. However, given that biomarkers of full-fat dairy intake are consistently not associated with an unfavorable lipid profile, it is important to assess whether consumption of full-fat dairy foods results in dyslipidemia in clinical trials. Trials consistently indicate that isolated dairy fat in the form of butter results in dyslipidemia[247]. Specifically, a recent meta-analysis concluded that safflower, sunflower, rapeseed, flaxseed, corn, olive, soybean, palm, and coconut oil; or beef-fat, all reduced LDL- and total-cholesterol as compared to an equal amount of butter[247]. Further, sunflower, soybean, and palm oil were more effective at reducing triglycerides than butter[247]. While there is consistent evidence that dairy fat consumed as butter promotes dyslipidemia, recent trials suggest that a harmful impact of dairy fat on serum lipids is not observed when the same amount of dairy fat is consumed in the form of cheese[279,280]. These results suggest that

dairy fat may differentially impact serum lipids when consumed as butter as compared to dairy foods with a complex matrix. Further, trials comparing low-fat to full-fat dairy consumption generally indicate that consumption of full-fat dairy foods does not result in dyslipidemia. In a cross-over study, Nestle et al. asked overweight and moderately obese men and women to follow diets rich in either low-fat milk and yogurt, non-fermented full-fat dairy products, or fermented dairy products for three weeks each[281]. Generally, they found that there was no difference in serum lipids, markers of chronic inflammation, or lipid peroxidation among the three diet groups. Though it should be noted that they found that HDL-cholesterol significantly increased during the non-fermented full-fat dairy phase as compared to the low-fat dairy diet and LDL-cholesterol increased in both the non-fermented and fermented full-fat dairy diets as compared to the low-fat dairy diet[281]. However, this study had many limitations. The study order was not randomized; the non-fermented full-fat dairy group only included calorie dense full-fat dairy foods such as butter, cream, and ice cream; and the length of study was likely too short to detect certain effects of the dairy foods. Raziani et al. randomized men and women with the MetS to a regular-fat cheese, reduce fat cheese, or a carbohydrate control diet for 12 weeks. They also found that the diets had no effect on LDL-, HDL-, or total-cholesterol, triacylglycerols, free fatty acids, LDL:HDL cholesterol ratio, or the TC:HDL cholesterol ratio[159]. In a randomized cross-over study including 18 healthy adults, Engel et al. had participants follow a diet consisting of 0.5L of whole milk or skim milk per day for three weeks. They were asked to not consume any other dairy products, except they were allowed to keep their butter and cheese consumption as it was at baseline. They found that there was no effect of whole milk on LDL-cholesterol, a trend for an increase in total cholesterol, and an increase in HDL-cholesterol as compared to skimmed milk[160]. In a 6-week cross-over study, Steinmetz et al. had participants consume skim or whole milk while following a diet based on the American Heart Associations. They found that there was no difference in the change in total cholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL cholesterol:HDL-cholesterol ratio, or total triglyerides[282]. Therefore, currently, there is only one trial that even slightly suggests that full-fat dairy promotes dyslipidemia in the form of elevated LDL-cholesterol[160]. However, there are also two trials that indicate that full-fat dairy increases HDL-cholesterol levels[160,281]. The majority of the evidence indicates that full-fat dairy consumption does not cause dyslipidemia. Indeed, a review of RCTs that investigated the effect of dairy fat consumption in the form of whole dairy foods on the fasting serum lipid profile indicates that

consumption of full-fat dairy products does not affect HDL- or LDL-cholesterol[158]. However, there are important limitations of the current evidence that need to be addressed in future studies. The majority of these studies were short in duration and did not include a range of dairy foods in their interventions. Therefore, more experimental research is needed to clarify the effect of dairy fat consumption on the blood lipid profile.

3.5.4 Dairy and Blood Pressure

Blood pressure is another established risk factor for CVD. Thus, one of the ways dairy foods may impact CVD risk is through altering blood pressure. The meta-analysis by Lee at al. discussed in the previous section also concluded that dairy is not associated with blood pressure in the observational literature[120]. However, it may be that different types of dairy impact risk of hypertension differentially. For example, studies that measured full-fat dairy food consumption through full-fat dairy food biomarkers begin to suggest a potential association between full-fat dairy consumption and hypertension. One study found that dairy consumption, as measured by the sum of C15:0 and C17:0 in serum phospholipids was not associated with blood pressure[127]. A more recent study by the same research group with a larger sample size found that there was a negative correlation between C17:0 and systolic and diastolic blood pressure[133]. Further, a cross-sectional study in the Netherlands found that C15:0 and C17:0 are both negatively correlated with both systolic and diastolic blood pressure, whereas trans-16:1n-7 is not[146]. In a prospective cohort study of men and women, with 2,900 participants, they found that the dairy biomarkers C15:0 and trans-16:1n-7 as measured in the plasma phospholipids were negatively associated with systolic and diastolic blood pressure[226]. Therefore, studies using dairy biomarkers begin to indicate a potential protective role of full-fat dairy consumption against the development of hypertension.

The association between dairy foods and blood pressure may be dependent on the type of dairy foods consumed. In a cross-sectional analysis of approximately 4,500 Swedish men and women, investigators assessed the correlation between non-fermented milk, fermented milk, cheese, butter and cream with both diastolic and systolic blood pressure. Only butter was positively correlated with diastolic blood pressure and only non-fermented milk was positively correlated with systolic blood pressure[218]. This indicates that dairy fat consumed as part of the food matrix

as opposed to consumed in isolation (i.e. butter) may impact blood pressure differently. Further, it also indicates that fermentation may further be a factor in whether or not dairy impacts risk of hypertension. Overall, the observational evidence remains mixed as to the association between dairy consumption and blood pressure, but generally does not indicate that dairy is associated with elevated blood pressure.

The experimental evidence further supports the conclusions drawn from the observational evidence, indicating that dairy consumption does not affect blood pressure. Using seven studies that evaluated blood pressure in the Benatar et al. meta-analysis, there was no effect of dairy consumption on blood pressure[158]. Further, this outcome was not impacted by stratification by dairy fat content[158]. Since then several RCTs have published data on this topic, all of which came to the same conclusion that dairy consumption does not have an impact on blood pressure[159,251,253,278]. The study designs for Dugan et al., Raziani et al., Rideout et al., and Bendtsen et al. have previously been described. These studies ranged in intervention length of six weeks to 12 months, some studied healthy individuals while others studied those with MetS, and the studies predominantly used low-fat unfermented dairy products. Even under this range of conditions, all of the studies found that there was not a significant effect of dairy consumption on blood pressure. In contrast, a recent study by Rietsema et al. found that a high dairy diet resulted in reduced systolic and diastolic blood pressure as opposed to a low dairy diet[283]. In this study, 52 overweight men and women participated in a randomized cross-over study where they followed a low dairy diet with less than or equal to one dairy portion per day or a high dairy diet with 5-6 reduced-fat dairy portions per day for six weeks. Overall, the majority of the evidence indicates that dairy consumption does not have an impact on blood pressure. However, this may be a result of the limitations of the studies published. These limitations have already been discussed in section 3.5.1.

Given that observational studies using biomarkers of full-fat dairy intake begin to indicate that full-fat diary foods may impact blood pressure, it is important to assess whether full-fat dairy food consumption differentially affects blood pressure as compared to low-fat dairy food consumption in clinical trials. There are two RCTs that directly compared the effects of low-fat to full-fat dairy consumption on blood pressure. Raziani et al, discussed above, found that there

was no differential effect of consuming reduced fat cheese, whole fat cheese, or a carbohydrate control on either diastolic or systolic blood pressure[159]. Similarly, Alonso et al. found that full-fat dairy consumption in the form of milk and yogurt did not significantly differ in the change in blood pressure from the low-fat dairy diet[284], though it is unclear whether differences between the diets would have been observed with a longer intervention period. Alonso et al. in comparison to Raziani et al. begins to suggest that dairy consumption may affect blood pressure. The difference in outcomes between these trials may be due to the fact that Alonso et al. used yogurt and milk for their dairy interventions, whereas Raziani et al. relied on cheese. This indicates that different types of dairy may work differentially to impact blood pressure. Further, Raziani et al. conducted their intervention in individuals with MetS, whereas Alonso et al. recruited healthy participants. Overall, given the limitations in the amount of available literature, more research is needed to clarify whether full-fat dairy has differential impacts on blood pressure as compared to their reduced-fat or skim counterparts.

3.5.5 Dairy and Low-Grade Systemic Chronic Inflammation

Few observational studies have reported on the association between full-fat dairy food consumption as assessed by dairy fat biomarkers, and markers of systemic inflammation. In a prospective cohort study of men and women, with 2,900 participants, dairy biomarkers C14:0, C15:0 and trans-16:1n-7 as measured in the plasma phospholipids were not associated with CRP[226]. Another study looking at data from 327 healthy women from the Nurses Health Study, found that higher erythrocyte trans-palmitoleate levels were associated with lower levels of IL-6 and CRP[132]. A third study found that plasma phospholipid concentrations of both trans-16:1n-7 and C17:0 were inversely associated with hsCRP, but C15:0 was not[146]. More studies are needed to investigate whether or not dairy biomarkers are associated with markers of systemic inflammation. At this point, the observational evidence is too limited to come to definitive conclusions, but does not suggest that dairy in general or full-fat dairy in particular are pro-inflammatory.

Only a few RCTs have investigated the impact of dairy foods on low-grade chronic inflammation. The meta-analysis by Benatar et al., described previously, found that there was no significant effect in the six studies evaluating dairy consumption and CRP[158]. This result

remained the same after stratifying by low-fat versus full-fat dairy consumption[158]. Since then a few additional RCTs have been published looking at the effect of increased dairy consumption on CRP. The study designs for Raziani et al.,[159] Eelderink et al.[258], Benatar et al.[259], and Bendtsen et al.[278], and Dugan et al.[285] have previously been described. All of these trials found that there was no effect of dairy consumption on CRP. Therefore, the current literature appears to indicate that dairy consumption does not have an effect on CRP. Additionally, a few RCTs have used IL-6 as a marker of low-grade chronic inflammation. Stancliffe et al. and Wennesberg et al. concluded that increased dairy consumption did not have an effect on systemic inflammation, as measured by IL-6[261]. Once again, these studies are subject to limitations described previously, which is why there needs to be further studies evaluating the relationship between dairy consumption and low-grade chronic inflammation.

4.0 Mechanism Through Which Dairy Foods May Impact Energy and Glucose Homeostasis

4.1 Effect of Dairy Protein and Calcium on Energy Homeostasis

4.1.1 Dairy Protein

Typically dairy foods are composed of 20-25% protein, which is considered the most satiating macronutrient per kilocalorie[286]. Therefore, it is possible that incorporating more dairy foods in the diet would increase total protein consumption, thereby making the diet more satiating and reducing *ad libitum* energy intake. Dairy is predominantly made up of two major proteins. In milk, approximately 20% of the dairy protein is made up of whey protein and approximately 80% is casein protein[287]. A proposed mechanism by which dairy proteins contribute to energy homeostasis is through acting on anorexigenic hormones to increase satiety, though evidence in this area is still preliminary[288]. It has been suggested that the type of dairy protein may have differing impacts on satiety. In a 12-week randomized parallel designed trial, overweight or obese adults were randomized to a carbohydrate control, casein protein supplementation or whey protein supplementation group. At six-weeks and 12-weeks of intervention, those on the whey protein supplementation showed significantly higher satiety and fullness as measured by a visual analogue scale when compared to both the casein and control groups[288]. One of the proposed explanations for why whey proteins may be more satiating than casein is that whey proteins have bioactive components, such as lactalbumin and branched chain amino acids (BCAAs), that have a faster rate of digestion and absorption, leading to a faster peak in plasma amino acids[288]. Approximately 21-26% of whey protein is made up of BCAAs[289,290]. Beyond improving satiety, there are other proposed mechanisms by which BCAAs could contribute to improved energy homeostasis. For example, it has been suggested that the availability of the BCAA leucine plays a role in diverting energy from adipose tissue to skeletal muscle for protein synthesis[289]. Additionally, BCAAs may stimulate insulin secretion through promoting the release of glucagon-like peptide-1[290]. BCAAs may also activate the mechanistic target of rapamycin, impacting various tissues and organs to impact satiety, body fat, and glycemia to improve glucose management[290]. Further, a recent review of the impact of dairy amino acids on glucose homeostasis concluded that trials of leucine and isoleucine supplementation have demonstrated

beneficial impacts on glucose response[290]. In these ways, dairy proteins may act to improve energy balance and metabolic health.

4.1.2 Calcium

Dairy foods are a rich source of many micronutrients, including calcium. In 2000, Zemel et al. showed that there is an inverse relationship between dietary calcium intake and obesity in the US population using NHANES III data[291]. However, this observed association is not fully substantiated by experimental studies. A meta-analysis by Booth et al. found that of the 19 RCTs that evaluated the effect of calcium supplementation on adiposity, with an average of 900 mg/d of calcium, there was no significant effect on body weight or fat mass as compared to controls[292]. However, it may be that calcium impacts adiposity differentially in subjects with varying demographics. A meta-analysis by Li et al. that included 33 RCTs similarly found that calcium supplementation of at least 1,000 mg per day did not result in a significant decrease in body weight overall[293]. However, they found that calcium supplementation led to a significant decrease in weight in certain subgroups including subjects with a normal BMI, in children and adolescents, adult men and premenopausal women[293].

There are many potential mechanisms by which calcium could mediate the impact of dairy consumption on energy homeostasis. It has been hypothesized that calcium impacts energy homeostasis by binding fats in the gastrointestinal tract, leading to reduced fat absorption[294]. Rats fed a high calcium diet gained less weight than the controls, though there was no statistically significant difference in the amount of food consumed or energy expenditure between the two groups[295]. However, rats on the high calcium diet had a reduced amount of digestible energy as a result of increased fecal fat excretion when compared to the control animals[295]. This phenomenon was confirmed in humans in a 10 person cross-over study by Jacobsen et al., with approximately 350 kj/day lost due to fecal fat excretion on the high calcium diet[296]. Though it should be noted that the changes in adiposity observed in clinical trials investigating the role of dietary calcium are too large to be explained only by the effect of increased fecal fat excretion, suggesting that other mechanisms are at play[289].

Other hypotheses for how calcium impacts energy homeostasis are as follows: changes in calcitrophic hormones, which act on adipocytes to change the intracellular calcium concentration, can result in a shift in lipolysis and lipogenesis[291]. It may be that increased dietary calcium reduces adiposity through the suppression of calcitriol [1,25-(OH)(2)-vitamin D] or parathyroid hormone (PTH)[289,291,297]. In mice fed a high calcium diet, weight gain and fat mass were significantly reduced as compared to the control mice[291]. Additionally the high calcium diets resulted in a significant inhibition of adipocyte fatty acid synthase expression and activity, while significantly stimulating lipolysis[291]. Another hypothesis is that dietary calcium related reductions in PTH further result in decreased body weight as a result of increased stimulation of the sympathetic nervous system, leading to increased diet-induced thermogenesis and fat oxidation rate, resulting in increased energy expenditure and therefore reduced body fat[298]. Another proposed mechanism is that reduced calcitriol levels as a result of a high calcium diet result in a decrease in adipose tissue cortisol production, contributing to reduced visceral adipose tissue mass[289]. While there is some evidence for calcium serving as a mediator between dairy intake and its beneficial health effects, a recent review by Mozzafarian concludes that there is no convincing evidence that calcium is the driver of the cardiometabolic health benefits of dairy consumption[299]. Overall, further research is needed to confirm whether or not calcium mediates the relationship between dairy consumption and energy homeostasis, and by which mechanism(s).

4.2 Dairy Fat Effects on Glucose Tolerance

Dairy fat is the most complex fat consumed by humans and includes a diverse range of SFAs from C2:0 to C28:0 in addition to various mono- and polyunsaturated fatty acids[300,301]. SFAs have historically been viewed as an unhealthful fat. However, it is important to emphasize that, while dairy is rich in SFAs, the C12–C16 long-chain SFAs that are indicated to drive the unhealthful effects of SFAs only make up around 40-50% of the total fatty acids in dairy[118]. The other short-, medium-, and branched-chain SFA in dairy have substantially different physiological and metabolic effects[302]. In particular, the beneficial potential of butyric acid (C4:0) and phytanic acid found in dairy will be discussed below (sections 4.2.1 and 4.2.2). Dairy fat also includes a diverse range of mono- and polyunsaturated fatty acids. Palmitoleic acid, is one monounsaturated fatty acid identified in dairy that has the potential to impact glucose

tolerance, as discussed below (section 4.2.3)[303-305]. Therefore, it may be that the beneficial health effects of the short-chain SFAs, medium-chain SFAs, branched-chain SFAs, monounsaturated fatty acids, or polyunsaturated fatty acids attenuate the deleterious effects of the long-chain SFAs found in full-fat dairy foods. In this way, dairy fats have the potential to beneficially impact cardiometabolic health.

4.2.1 Butyric Acid

Dairy fat is a unique source of short-chain fatty acids such as butyrate. Butyrate has gained interest due to the fact that it has been hypothesized that dietary fiber may exert some of its beneficial metabolic effects through the generation of butyrate and other short-chain fatty acids as an endproduct of its metabolic breakdown by the gut microbiota[306-308]. In mice, administration of butyrate prevents the development of IR when on a high-fat diet[309], reduces obesity, and improves glucose homeostasis (reviewed in[307,308]). It has been proposed that butyrate induces these effects through activation of peroxisome proliferator activated receptor (PPAR)-γ, promoting lipid oxidation; stimulating gut hormones PYY and GLP-1; and through neuro-signaling between the gut-brain axis[307].

Research using a rat model indicates that butyrate may also beneficially impact liver health. Rats with diet induced NAFLD treated with MIYAIRI 588, a butyrate producing probiotic, had reduced diet induced hepatic lipid deposition, improved triglyceride content, IR, and hepatic inflammation[303]. Additionally, treatment resulted in activation of hepatic AMPK and PKB/AKT and the expression of lipid metabolism related proteins[303]. Hepatic AMPK decreases hepatic lipogenesis and can inhibit reactive oxidative stress and inflammation (reviewed in[303]). Given that butyrate can be transferred from the gut to the liver via the portal vein[310], it is therefore possible that dietary butyrate from dairy consumption could impact hepatic fat content through the mechanism described above. Liver fat is a risk factor for chronic diseases including T2D. In this way, increased butyric acid through the increased consumption of dairy may decrease liver fat, resulting in improved glucose tolerance.

A recent review of the literature on the potential cardiometabolic health benefits of butyrate indicates that the majority of the evidence supports the role of butyrate in reducing obesity and

improving metabolic health[307]. However, most of the work done on this topic has been conducted in cell lines and in animal models. Therefore, additional studies are needed to determine whether butyrate similarly beneficially impacts energy and glucose homeostasis in humans[308].

4.2.2 Phytanic Acid

Dairy products, ruminant meat, and some marine fats are the only major dietary sources of the branched chain fatty acid phytanic acid and its metabolic precursor phytol (reviewed in[311]). Phytanic acid has been suggested to have health promoting properties including being protective against MetS and T2D through impacting fatty acid and glucose metabolism[312]. This is thought to primarily occur through phytanic acids stimulation of PPAR-α, PPAR-γ, and the retinoid X receptor[312]. Phytanic acid is another potential active component of dairy that may result in alterations in hepatic lipid metabolism. Phytanic acid is a known potent activator of the master switch of fat oxidation in the liver, PPAR-α[311]. In other words, activation of PPAR-α skews the hepatic metabolism towards fatty acid catabolism rather than *de novo* lipogenesis. Therefore, increasing the levels of phytanic acid in the liver should result in increased expression of genes involved in fatty acid oxidation, resulting in reduced liver fat accumulation[311]. Though it should be noted that data on the effects of phytanic acid on liver fat content and glucose metabolism, in both human and animal models, are scarce partially because of the prohibitively high cost of phytanic acid[311]. Further, there is some research indicating that phytanic acid consumption may have harmful neurological affects[312], so studies looking at the risk versus benefits are warranted. Therefore, while phytanic acid may be a fatty acid found in dairy foods that acts to improved glucose tolerance; further research is needed to test this hypothesis.

4.2.3 Palmitoleic Acid

Dairy fat is one of the few sources of both dietary cis- and trans-palmitoleic acid[249], which could be an active agent by which dairy consumption leads to reduced liver fat content and improved glucose tolerance. This hypothesis was generated based on research in mouse models. Fatty acid binding proteins (FABPs) are lipid chaperones that assist in transferring fatty acids from extra to intra-cellular membranes and are indicated to play a critical role in metabolism[304]. Mice deficient in the FABPs aP2 and mal1 (aP2-mal1$^{-/-}$) in adipose tissue are protected from diet-induced obesity and appear to be protected against IR, T2D, and NAFLD[313]. Changes in the liver lipid

profile in aP2-mal1$^{-/-}$ mice indicated that there is an intracellular hepatic mechanism that could account for the lack of lipid accumulation in liver cells of aP2-mal1$^{-/-}$ mice. Specifically, the results of this study indicate that the beneficial metabolic effects observed in aP2-mal1$^{-/-}$ mice is likely a result of altered hepatic fatty acid metabolism. Since FABP deficient mice are resistant to certain metabolic complications and have a unique lipid profile, this suggests that there is a link between lipid metabolism, composition, and the improved metabolic response[304]. Cao et al. looked at FABP deficient mice and found that enhanced de novo lipogenesis in adipose tissue led to increased plasma cis-C16:1n7-palmitoleate concentrations under both normal chow and high fat feeding[304]. This suggests that cis-palmitoleate might be a key lipokine underlying changes in metabolic health observed in FABP deficient mice. Therefore, this data from mouse models indicated that the flux of the lipokine C16:1n7-palmitoleate may lead to improved metabolic responses resulting in reduced liver fat content[304,313]. These findings also suggested that there may be a hormone effect of certain fatty acids found in foods, such as cis- and trans-palmitoleate in dairy products, on liver fat oxidation and de novo lipogenesis. Other potential mechanisms by which palmitoleate may impact liver fat content and glucose tolerance include blocking stearoyl-CoA desaturase 1 activity and inhibiting the expression of fatty acid synthase and elongation of very long chain fatty acid protein 6, resulting in reduced insulin resistance and NAFLD through its impacts on lipogenesis[314].

In support of the hypothesis that increased palmitoleic acid concentrations improve liver fat content, Kratz et al. reported an inverse association between plasma biomarkers of dairy fat intake, including plasma trans-16:1n-7, and liver fat content[315]. Though it should be noted that this study was cross-sectional and is subject to the limitations of this study design, including the inability to draw causality. All together, these findings suggest that dairy fat intake may reduce de novo lipogenesis and/or stimulate fat oxidation in the liver, thereby reducing hepatic triglyceride content[316]. In fact, NAFLD is associated with impaired fatty acid oxidation and elevated de novo-lipogenesis with approximately 26% of free fatty acids that accumulate in the liver of NAFLD patients derived from hepatic de novo lipogenesis (reviewed in[317]). Additionally, the results identify palmitoleate as one dairy-related fatty acid that has the potential to act as a biologically active agent that impacts hepatic liver fat content, and therefore glucose homeostasis.

A recent review by de Souza et al. analyzed the current state of the literature on the role of cis-palmitoleic acid in the development of chronic metabolic and inflammatory disorders. They concluded that preliminary evidence in cell culture and animal models indicate beneficial effects of cis-palmitoleic acid including improved glucose metabolism and glucose tolerance, induction of oxidative metabolism, and a reduction in inflammation[314]. Beyond beneficial impacts on liver fat content as described above, other proposed mechanisms include decreasing the lipid content, increasing glucose uptake, increasing glucose oxidation, and increasing glycogen synthesis in skeletal muscle; increasing insulin secretion and β-cell proliferation in the pancreas; decreasing lipid content, increasing lipolysis, increasing glucose uptake and increasing glucose oxidation in adipose tissue; decreasing inflammation and decreasing gluconeogenesis in the liver; and decreasing inflammation through reductions in MCP-1, IL-6, TNF-α, and IL-1β, decreased mRNA expression of NF-κB, TLR-4 and HIF-1α, and decreased translocation of NF-κB in macrophages[314]. While there is consistent evidence from in vitro and in vivo animal studies, indicating beneficial metabolic impacts of palmitoleic acid, there is insufficient data from RCTs to determine whether palmitoleic acid improves metabolic health in humans[314]. Therefore, future research is needed to clarify the beneficial potential of both cis- and trans-palmitoleic acid.

4.3 Mechanisms by Which Dairy Consumption May Impact Glucose Homeostasis

Dairy consumption may affect glucose homeostasis through an acute impact on insulin secretion by pancreatic β-cells. Consumption of milk and yogurt results in a higher insulin response than would be expected given their glycemic index[318]. In a study by Ostman et al. ten healthy men and women consumed approximately 25g of carbohydrates in the form of white-wheat bread, a lactose solution, regular milk, or fermented milk products. While the milk products had a statistically significant lower glycemic index as compared to the white-wheat bread reference group, their insulinemic index was not statistically significantly different[318]. This indicates that dairy consumption exposes individuals to higher diurnal concentration of insulin than would be experienced by individuals who consumed other foods with a similar glycemic load. It may be that a greater acute increase in post-prandial insulin from pancreatic β-cells helps maintain glucose concentrations within the normal range, resulting in improved glycemic control. One proposed mechanism by which dairy consumption leads to the observed insulinotropic response

is by a quicker protein absorption rate, leading to a higher peak in plasma concentrations of nutrients, triggering the insulin response[288]. Further, specific amino acids are known to directly act on the pancreatic β cells, to stimulate insulin release, especially BCAAs[319,320]. Further, BCAA also promote the release of the incretin glucagon-like peptide-1 in intestinal cells in vitro, which may be a further explanation for dairy's insulinotropic effects[321]. Frid et al. tested this theory in 14 adults with diet-controlled T2D. They served a high glycemic index breakfast with whey protein on one day and exchanged whey supplement for lean ham and lactose on a separate day. They found that the insulin response was higher when whey protein was included in the meals and that this reduced 120 min AUC glucose by 21%[322]. In a study by Nilsson et al., 12 participants given a test drink consisting of 25g of glucose plus whey proteins had a 56% smaller AUC glucose and a 60% larger AUC insulin when compared to a reference drink consisting of only 25g of glucose[323]. There is also some evidence that the BCAAs proline, glutamic acid, glutamine, isoleucine, lysine, and phenylalanine positively impact glucose homeostasis[290], though it is unclear if the dose of BCAA provided from the regular consumption of dairy products would be sufficient to improve glucose control. So, while there is preliminary evidence that dairy proteins aid in glucose homeostasis, there are no long-term or medium-term studies that investigate whether higher diurnal insulin concentration on a dairy rich diet result in improved glycemic control.

While it is possible that dairy consumption aids in increased insulin sensitivity, it is also possible that dairy consumption has the opposite effect. Since dairy consumption results in an elevated insulin response as compared to its glycemic content[318], increased dairy consumption would expose an individual to higher doses of insulin than would be experienced if individuals consumed other foods with a similar glycemic content. There is some evidence that sustained hyperinsulinaemia results in decreased glucose tolerance. A study by Del Prato et al., found that after four days of sustained hyperinsulinaemia, there was a decrease in whole body glucose disposal of 20-40%[324]. However, while dairy consumption does result in an elevated insulin response, normal consumption of dairy products would not lead to the sustained hyperinsulinaemic conditions used in the Del Prato study. It is unclear to what extent an individual would need to be exposed to hyperinsulinaemic conditions in order to observe a deleterious impact on insulin sensitivity and glucose tolerance.

5.0 Summary

Chronic disease is the leading cause of death and disability in the US. Both T2D and CVD are chronic diseases that place a significant health and financial burden on individuals and societies around the globe. Obesity is one risk factor for both T2D and CVD. Further, obesity is associated with metabolic dysfunction including chronic systemic inflammation, IR, IGT, liver fat, high blood pressure, and dyslipidemia. It should also be noted that metabolic dysfunction is associated with CVD and T2D, independent of obesity. With the rise in prevalence of obesity and chronic diseases such as T2D and CVD, it is imperative that areas for targeted intervention and prevention strategies are identified. Many modifiable lifestyle factors have been selected as potential means to help reduce the burden of chronic disease[114]. Because diet is a major factor in the etiology of both obesity and T2D, a major emphasis of research is on identifying modifiable dietary factors that affect energy and glucose homeostasis with the potential to prevent and treat obesity and T2D[114,115]. Dairy foods have been identified as one potential factor that may impact weight and metabolic health[116,117]. In fact, most dietary guidelines, including the Dietary Guidelines for Americans 2015, recommend the consumption of milk and dairy products as an important component of a healthy well balanced diet [325,326].

Both the observational and experimental literature mostly supports the inclusion of dairy foods in a diet that promotes cardiometabolic health. The observational literature indicates that dairy consumption as a whole is inversely associated with adiposity and T2D, but it remains unclear whether or not dairy consumption is inversely associated with CVD (Sections 3.1-3.3). However, the experimental data suggests that dairy consumption only improves adiposity measures in the context of energy restriction and dairy consumption in the context of an *ad libitum* diet either has no affect on adiposity or even leads to weight gain. There are no RCTs that have investigated the relationship between dairy consumption as a whole and incident T2D or CVD. However, there have been trials on the effect of dairy consumption on classic metabolic risk factors of T2D and CVD as indicated in section 3.5. The current evidence indicates that dairy consumption is not associated with and does not impact IR, β-cell function, liver fat, serum lipids, blood pressure, or inflammation (Section 3.5). However, it should be noted that there are many limitations, which should be addressed in future studies in order to better clarify the effect of dairy consumption on

metabolic health. With regards to the effect of dairy consumption on glucose homeostasis, these limitations include the fact that most studies tested non-fat or low-fat and mostly unfermented dairy products and the fact that most of the studies were not designed to test the impact of dairy foods on glucose homeostasis. Specifically, the majority of the studies relied on fasting measures of glucose and insulin, the participants were relatively healthy at baseline, there was no clear hierarchy of endpoints, most studies relied on a *per-protocol* analysis, and changes in weight or fat mass were not controlled for statistically.

Dairy fat is traditionally seen as a source of unnecessary calories and saturated fatty acids, leading to obesity and CVD. Therefore, the 2015 Dietary Guidelines for American currently recommends consuming three servings per day of low-fat or non-fat dairy products[119]. However, it remains unclear whether obesity and CVD actually manifest in high dairy fat consumers. Further, it remains unclear whether low-fat dairy foods confer a protective effect against the development of T2D as compared to their full-fat counterparts. The majority of the observational literature indicates that dairy fat consumption is inversely associated with adiposity, though the experimental data is mixed (Section 3.1.1). Additionally, the observational data indicates that dairy fat consumption is either inversely or not associated with the incidence of T2D or CVD (3.2.1 & 3.4.1). There are no RCTs that directly investigate the effect of full-fat dairy consumption on incident T2D or CVD, though there are a few trials the directly compare the effect of low-fat versus full-fat dairy foods on risk factors for T2D[159,160] and CVD[159,160,281,282]. Generally, these trials have found that there is no differential effect of full-fat dairy foods on risk factors for T2D and CVD. Though the current experimental evidence has many limitations including the fact that most studies tested predominantly unfermented dairy products, the participants were relatively healthy, most studies relied solely on a per-protocol analysis, and changes in weight or fat mass were not controlled for statistically. Further, with regards to glucose homeostasis related endpoints, most of the studies were not designed to test the impact of dairy foods on glucose homeostasis, with studies relying on fasting measures of glucose and insulin. Because of these limitations, it remains unclear whether full-fat dairy consumption is indeed worse for health outcomes when compared to non-fat or low-fat dairy. Overall, while dietary guidelines typically recommend consuming low-fat and non-fat dairy products, this recommendation is not supported by the current literature.

The clinical intervention trial underlying this dissertation, the DAIRY Study, fills a critical gap in the literature by clarifying the relationship between dairy consumption and metabolic health using a rigorously designed randomized dietary intervention trial that addresses many of the limitations of the current literature. The DAIRY Study directly compared low-fat versus full-fat dairy and included fermented dairy products in the form of yogurt and cheese. Further, it was designed to specifically test the impact of dairy foods on glucose homeostasis by using a FS-OGTT, enrolled individuals with MetS in order to be able to detect intervention effects in both directions, had a clear a priori-defined hierarchy of endpoints, reported results from both a per-protocol analysis and an ITT analysis, and controlled changes in weight and fat mass, both key determinants of glucose homeostasis, statistically. This study provides essential information on the impact of low-fat and full-fat dairy products on glucose homeostasis and additional endpoints relevant for risk of cardio-metabolic disease. Since dairy consumption is a modifiable dietary factor, strategies to alter dairy consumption in the US could play a role in reducing the incidences of cardiometabolic disease. Additionally, this study has the potential to help inform the current United States Department of Agriculture recommendations on the type of dairy products that should be consumed by Americans.

6.0 Objective and Specific Aims

The Dairy Study was a randomized controlled dietary intervention trial in parallel design that tested the effect of low-fat and full-fat dairy consumption on glucose homeostasis and its determinants. Seventy-two men and women with MetS and evidence of IR were enrolled into this study. Each participant completed a 4-week wash-in dietary period, which consisted of consuming 3 servings of non-fat milk per week (limited dairy diet). Baseline measurements were taken and participants were randomized to one of three dietary intervention arms: a diet almost free of dairy foods (limited dairy diet); a diet rich in non-fat milk, yogurt and low-fat cheese (low-fat dairy diet); a diet rich in full-fat milk, yogurt, and cheese (full-fat dairy diet). Clinic visit two took place at the end of the 12-week dietary intervention period. This study addressed the following **primary specific aim**:

> (**1) To compare the effects of the limited, low-fat, and full-fat dairy diets on glucose tolerance**. Glucose tolerance was assessed by measuring the glucose area-under-the-curve during a 3-hour FS-OGTT. We hypothesized that the full-fat dairy diet and the low-fat dairy diet would both improve glucose tolerance as compared to the limited dairy diet group.

We also addressed the following **secondary aims:**

> **(2) To compare the effects of the limited, low-fat, and full-fat dairy diets on major determinants of oral glucose tolerance, i.e. systemic insulin sensitivity and pancreatic β-cell function.** Systemic insulin sensitivity was assessed using the Matsuda-De Fronzo Insulin Sensitivity Index based on data from the FS-OGTT. Pancreatic β-cell function was assessed using the insulinogenic index, the oral disposition index (oral DI), and glucose sensitivity, all based on the FS-OGTT. We hypothesized that both dairy diets would similarly improve insulin sensitivity compared to the limited dairy group. Further, we hypothesized that the diets would not differ from one another with regards to the effects on pancreatic β-cell function.

(3) To compare the effects of the limited, low-fat, and full-fat dairy diets on major determinates of insulin sensitivity including liver fat content and low-grade chronic inflammation. Liver fat content was measured using abdominal a magnetic resonance imaging (MRI) scan. Chronic inflammation was assessed by measuring the concentration of the pro-inflammatory biomarkers c-reactive protein (CRP) and interleukin-6 (IL-6) in fasting plasma. We hypothesized that the diets would not differentially affect biomarkers of systemic inflammation, but that the full-fat dairy diet and the low-fat dairy diet would both reduce liver fat content as compared to the limited dairy diet.

We also addressed the following **exploratory aims**:

(4) To compare the effects of the limited, low-fat, and full-fat dairy diets on *ad libitum* energy intake. This was measured by comparing *ad libitum* energy intake during two five day controlled feeding periods, one in the third week of the wash-in diet period and a second during the second week of the dietary intervention period. During this controlled feeding period, all foods were provided to participants at 125% of the caloric needs in conjunction with their assigned dairy products. Participants consumed all study dairy products, but the other food provided was eaten *ad libitum*. The difference in the amount eaten per person in the first and the second 5-day controlled feeding period was compared among dietary groups. We hypothesized that there would be no difference in *ad libitum* energy intake among the dietary intervention arms.

(5) To compare the effects of the limited, low-fat, and full-fat dairy diets on body weight, body fat mass, body fat distribution. Body weight was measured at each clinic visit. Body fat mass and distribution was measured using a dual-energy x-ray absorptiometry (DEXA) scan. We hypothesized that there would not be a significant difference in body weight, fat mass, or fat distribution among the dietary intervention arms.

(6) To compare the effects of the limited, low-fat, and full-fat dairy diets on the fasting lipid profile, blood pressure, and fasting and diurnal glucose concentrations.

Fasting plasma was drawn at both sets of clinic visits for a comprehensive metabolic panel. Additionally, blood pressure was measured at each set of clinic visits. Average diurnal glucose concentrations were measured using HbA1c. We hypothesized that the limited, low-fat, and full-fat dairy diets would not differentially impact blood pressure or the fasting lipid profile. We hypothesized that the full-fat dairy diet and the low-fat dairy diets would both reduce fasting and diurnal glucose concentrations as compared to the limited dairy diet.

CHAPTER 2: THE IMPACT OF LOW-FAT AND FULL-FAT DAIRY CONSUMPTION ON GLUCOSE HOMEOSTASIS AND ITS DETERMINANTS

ABSTRACT:

Background: Dairy foods, particularly yogurt and full-fat dairy as measured by plasma biomarkers, are consistently associated with lower risk of type 2 diabetes, yet few trials assessing the impact of dairy on glucose homeostasis included fermented or full-fat dairy foods.

Objective: To compare the effects of diets rich in low-fat or full-fat milk, yogurt, and cheese on glucose tolerance and its determinants, compared to a limited dairy diet.

Design: In this parallel-design randomized-controlled-trial, 72 participants with metabolic syndrome completed a 4-week wash-in period, limiting dairy intake to ≤ 3 servings/week of nonfat milk. Participants were then randomized to either continue the limited dairy diet, or switch to a diet containing 3.3 servings/day of either low-fat or full-fat dairy for 12-weeks. Outcome measures included glucose tolerance (area-under-the-curve glucose during a standardized 180-minute oral glucose tolerance test, primary); insulin sensitivity, pancreatic β-cell function, systemic inflammation, liver-fat content, and body weight and composition.

Results: In the *per-protocol* analysis (n=67), we observed no intervention effect on glucose tolerance (p=0.340, time x intervention group interaction, repeated measures analysis of variance). However, both the low-fat and full-fat dairy diets decreased the Matsuda insulin sensitivity index (p=0.012), as compared to the limited dairy group. Dairy consumption also resulted in weight gain (p=0.006), with an increase in the full-fat dairy compared to the limited dairy diet, and the low-fat dairy diet falling in-between. Intervention effects on insulin sensitivity remained after adjusting for changes in adiposity. No intervention effects were detected for liver fat content or systemic inflammation. Findings in *intent-to-treat* analyses (n=72) were consistent.

Conclusions: Contrary to our hypothesis, neither low-fat nor full-fat dairy consumption improved glucose tolerance in individuals with metabolic syndrome. Both dairy diets decreased insulin sensitivity through mechanisms largely unrelated to changes in body weight, fat mass, liver fat content, or systemic inflammation.

1.0 Introduction

Type 2 diabetes (T2D) is a major global health issue. In 2017, it was estimated that 451 million people had diabetes worldwide, with the vast majority having T2D[8], costing 850 billion USD[8]. Identifying modifiable determinants of T2D risk is therefore a major public health focus.

Diet is a modifiable lifestyle factor that impacts glucose homeostasis and T2D risk[327,328]. Dairy is one food group that is inversely associated with T2D[116,161-165,167]. This is particularly the case for low-fat dairy and yogurt[166,167]. Although full-fat dairy intake as assessed by questionnaire is mostly not associated with T2D[169,329], biomarkers of dairy fat intake (i.e. phospholipid C15:0, C17:0, and *trans*-C16:1n-7) are consistently inversely associated with T2D[145,201]. These latter studies challenge the long-standing view that dairy fat provides excess calories and saturated fat, promoting weight gain and cardiometabolic disease.

The limited experimental literature is largely inconsistent with the results from observational studies. Randomized controlled trials (RCTs) consistently indicate that increasing dairy intake does not affect glucose tolerance[252,270,272,330]. Insulin sensitivity, a major determinant of glucose tolerance, also does not change in most RCTs[159,252-255,259,270-272,330-332]. Outcomes of existing RCTs may differ from observational findings because they commonly relied on fasting measures of glucose homeostasis, used participants with normal baseline glucose homeostasis, did not control for changes in weight, and predominantly included low-fat unfermented dairy products (i.e., skim milk). The latter leads to a particular gap in our understanding, as biomarkers of dairy fat intake and yogurt have most consistently been linked to improved metabolic health and a reduced T2D risk[166,169,276,329]. Only two studies have directly compared the impact of low-fat versus full-fat dairy on glucose homeostasis, one testing cheese[159] and the other milk[160]. No RCT has comprehensively evaluated the impact of a wide variety of low-fat versus high-fat dairy foods on glucose tolerance or its key determinants. As a result, it remains unclear whether dairy foods are protective against T2D, and whether this effect is dependent on the type of dairy consumed.

To address these gaps, we compared the effect of consuming 3.3 servings per day of low-fat or full-fat dairy foods versus a diet limited in dairy on glucose tolerance and its major determinants.

In contrast to previous studies, our trial included fermented dairy products in the form of both yogurt and cheese, in addition to milk. Our trial also included dynamic tests of glucose tolerance, insulin sensitivity, and pancreatic β-cell function. Assessment of major determinants of insulin sensitivity included liver fat content, biomarkers of systemic inflammation, and body weight and composition. We hypothesized that regularly consuming milk, yogurt, and cheese, particularly in their full-fat form, would improve glucose tolerance.

2.0 Methods

2.1 Trial Registration:

This trial was registered on clinicaltrials.gov on January 26, 2016 (registry number NCT02663544), prior to the enrollment of the first study participant. Changes were made after commencement of the study, but before the end of the trial and any laboratory or statistical analyses, to add several outcomes to broaden our ability to interpret trial effects on the primary endpoint. Specifically, we added glucose sensitivity, a measure of pancreatic β-cell function as an additional secondary outcome measure, and fasting insulin, the homeostasis model assessment index of insulin resistance (HOMA-IR), and several measures of adiposity (trunk fat mass, peripheral fat mass, visceral fat mass, waist circumference, hip circumference, and the waist-to-hip ratio) as secondary outcome measures.

2.2 Study Design:

This parallel-design randomized controlled dietary intervention trial was conducted at the University of Washington (UW) and the Fred Hutchinson Cancer Research Center (Fred Hutch) in Seattle, WA. All participants completed a 4-week wash-in diet period during which dairy food consumption was limited to no more than three servings of nonfat milk per week ("limited dairy diet"). After completing a baseline clinic visit in the last week of the wash-in period, participants were randomized to either continue the limited dairy diet or switch to a diet containing 3.3 servings per day of either low-fat or full-fat dairy foods in the form of milk, yogurt, and cheese, for 12 weeks. Subjects completed a follow-up clinic visit in the final week of the intervention period.

2.3 Subjects:

We enrolled 18-75 year old, weight stable participants with the metabolic syndrome[333]. Key exclusion criteria included regular recreational drug use; excessive alcohol consumption; recent use of anti-diabetic medications or insulin; uncontrolled diabetes (HbA1c >8.0%); history of bariatric surgery; or recent use of medications or diagnosis of any medical condition likely to interfere with study endpoints.

Our primary recruitment strategy was based on automated screens of the UW electronic medical record system. Potentially eligible individuals were contacted by mail, followed by a telephone

screening interview, and lastly an in-person screening visit at Fred Hutch. During this screening visit, eligibility was ascertained, the study design and procedures were discussed in detail, and participants sampled the intervention dairy foods. Informed consent was obtained from all participants prior to study initiation. The Fred Hutch institutional review board approved this study.

2.4 Study Diets:

During the wash-in period, participants were asked to not consume any dairy products other than a maximum of three servings (240 mL each) per week of nonfat milk ("limited dairy diet"). At the baseline clinic visit, participants were randomized to one of three diets: to continue the limited dairy diet, or switch to a diet rich in either low-fat or full-fat dairy foods. The randomization was performed using a random number generator by MK and SH using a block randomization procedure, with a block size of 3, stratified by gender and the screening visit HOMA-IR (<5.0 versus ≥ 5.0 or diagnosis of diabetes). Participants were enrolled and assigned to the intervention diets by KAS, GC, MSB, or JNK. Blinding subjects to their randomized diet was not possible since participants could easily discern which diet they had been assigned to based on the texture (i.e. full-fat vs. skim) or amount (dairy vs. limited dairy) of study dairy provided. Those randomized to stay on the limited dairy diet continued to not consume any dairy foods other than a maximum of three servings of nonfat milk per week. In the low-fat dairy diet, participants were asked to consume 3.3 servings per day of dairy in the form of nonfat milk and yogurt, and low-fat cheese. In the full-fat dairy diet arm, participants were asked to consume 3.3 servings per day of dairy in the form of whole milk (3.25% fat), full-fat yogurt (3.1% fat), and full-fat cheese. One serving was defined as 240 mL of milk, 170 g of yogurt, and 42.5 g of cheese. Nonfat and whole milk were produced by Darigold (Seattle, WA) and nonfat and full-fat yogurt by Mountain High (General Mills, Minneapolis, MN). Low-fat and full-fat cheeses were chosen to be identical in terms of manufacturer and manufacturing processes, other than fat content, and included low-fat and full-fat cheddar cheese (21.2% and 32.9% fat, respectively; Sargento, Plymouth, WI), gouda (18.0% and 32.2%, respectively; Beemster, Elizabeth, NJ), and mozzarella (10.6% and 21.2%, respectively; Frigo/Saputo, Lincolnshire, IL). The total amount of dairy fat in the administered dairy foods averaged 0 g per day in the limited dairy diet, 8 g per day in the low-fat dairy diet, and 29 g per day in the full-fat dairy diet. The Human Nutrition Laboratory at Fred Hutch provided all study dairy products. During all study diets, participants

were instructed to not consume any dairy foods other than those provided by the study, and to otherwise continue to consume their habitual diet *ad libitum*. They were specifically instructed to incorporate the administered dairy products into their regular meals and snacks, and to consume provided dairy foods daily. They were also asked to record their dairy consumption in a dairy log, and to return any leftover dairy foods for weigh backs.

2.5 Clinical Procedures and Data Collection:

At both clinic visits, we collected fasting blood; measured body weight and height, waist and hip circumference, and blood pressure; conducted a 3-hour frequently sampled oral glucose tolerance test (FS-OGTT) to assess glucose tolerance, insulin sensitivity, and pancreatic β-cell function; conducted a whole-body dual-energy x-ray absorptiometry (DEXA) scan on a Lunar iDXA scanner (GE Healthcare, Chicago, IL) to assess body composition; and conducted an abdominal MRI scan to assess liver fat content.

Participants also completed a physical activity questionnaire at baseline, clinic visit 1, and monthly during the 12-week intervention period to assess habitual physical activity throughout the study. Twice during the wash-in diet period and three times during the intervention period, participants completed an unannounced 24-hour dietary recall interview, administered by a staff member of the Fred Hutch Nutrition Assessment Shared Resource, who was otherwise not associated with the trial. Participants were told that their responses, including any indication of noncompliance, would not be shared with the study team prior to their completion of or drop out from the study.

Once during the last three weeks of the wash-in diet period and again within the first three weeks of the intervention period, participants completed a 5-day controlled feeding period during which they were provided with all of their food. These diets were standardized, based on the average American diet (other than dairy intake), and calibrated to provide 125% of each participant's estimated total energy expenditure. Participants were asked to consume all of the study dairy foods administered to them daily, to eat the rest of the administered diet *ad libitum*, and to return all leftover foods to the Human Nutrition Laboratory at Fred Hutch. Returned foods were weighed and subtracted from the weight of the administered foods to calculate total energy intake during these 5-day periods and to assess the degree to which participants compensated for

energy taken up from mandatory dairy products in the low-fat and full-fat dairy intervention arms.

2.6 Laboratory Procedures:

High-sensitivity C-reactive protein (CRP), glucose, insulin, C-peptide, and total adiponectin in fasting plasma and glycated hemoglobin (HbA1c) in fasting red blood cells were measured at Northwest Lipid Research Laboratories (NWLRL) in Seattle, WA. CRP was measured by immunonephelometry (Behring Diagnostics, Somerville, NJ), glucose on a Hitachi 917 autoanalyzer (Roche, Mannheim, Germany), and insulin and C-peptide on an AIA 600 II autoanalyzer (Tosoh Bioscience, San Francisco, CA). HbA1c analysis was performed using high performance liquid chromatography-based G7 and G8 autoanalyzers (Tosoh Bioscience, San Francisco, CA). Total adiponectin was measured in duplicate by a radioimmunoassay (EMD Millipore Inc., Saint Charles, MO). The interassay coefficient of variation at NWLRL for this assay is 8%. High-sensitivity interleukin-6 (IL-6) was measured in duplicate in the Kratz laboratory by a high-sensitivity enzyme-linked immunosorbent assay from R&D systems (Minneapolis, MN). The interassay coefficient of variation was 12%.

As one assessment of compliance with the dietary regimen, we measured the amounts of C15:0, C17:0, and *trans*-C16:1n-7 in plasma phospholipids (conducted in the Kraft Lab, Burlington, VT), as these are validated biomarkers of dairy fat intake [334,335]. Plasma phospholipids were extracted according to the method of Folch et al.[336]. Plasma phospholipids were isolated from total plasma lipids via solid-phase extraction using aminopropyl cartridges (Thermo Fisher Scientific, Waltham, MA), and transmethylated with boron trifluoride solution in methanol (Sigma-Aldrich, St. Louis, MO) to fatty acid methyl esters (FAME).[337] FAME were analyzed by gas-liquid chromatography[337].

2.7 Study Outcomes:

The *a priori*-defined primary study outcome was glucose tolerance, as assessed by measuring the area-under-the curve (AUC) glucose during a 3-hour FS-OGTT. Secondary outcomes included major determinants of oral glucose tolerance (i.e. systemic insulin sensitivity and pancreatic β-cell function) and major determinants of insulin sensitivity (i.e. liver fat content and low-grade chronic systemic inflammation). Systemic insulin sensitivity was assessed using the Matsuda-

DeFronzo insulin sensitivity index based on data from the FS-OGTT [262]. Pancreatic β-cell function was assessed using the insulinogenic index [338], the oral disposition index (oral DI), which is calculated as the product of the Matsuda-DeFronzo insulin sensitivity index (ISI) and the insulinogenic index, and modeled glucose sensitivity [339], all based on the FS-OGTT. Liver fat content was measured by an abdominal MRI scan in the UW Biomolecular Imaging Center on a Philips 3T Ingenia CX whole body scanner (Philips, Eindhoven, The Netherlands). Dixon MRI with multiple echo times was obtained, and fat and water MRI images as well as a quantitative liver proton density fat fraction map were produced. The entire liver was segmented on every 10th slice of the fat MRI images to estimate liver fat content. For each slice, the percent fat was weighted by the contour area as a percentage of the total contoured liver area (sum of area of all segmented slices), and the weighted fat percentages were summed. Low-grade chronic systemic inflammation was assessed using the concentration of CRP, and IL-6 in fasting plasma. Exploratory endpoints reported here to comprehensively assess intervention effects on glucose homeostasis included HbA1c, HOMA-IR [268], and fasting plasma glucose, insulin and total adiponectin concentrations as well as *ad libitum* energy intake during the two 5-day controlled feeding periods, body weight, body fat mass, trunk fat mass, peripheral fat mass, visceral fat mass, waist circumference, hip circumference, and the waist-to-hip ratio. We also assessed changes in the overall diet that resulted from the intervention.

2.8 Adverse Events:

No subject withdrew from the study due to adverse events. Of five adverse events reported during the trial, two were rated as unrelated to study procedures. Three adverse events were rated as related to study procedures and classified as mild-moderate in severity. One participant in the low-fat dairy group experienced hypoglycemia during both of their FS-OGTTs, with each incident being reported as a separate adverse event. A participant in the limited dairy intervention group experienced vertigo, nausea, and vomiting following the MRI scan.

2.9 Statistical Analyses:

We aimed to randomize 72 participants, with the goal of at least 60 participants in the primary *per-protocol* analysis, and at least 20 in each intervention group. Sample size was based on assumptions of baseline AUC glucose of 24,000 mg/dL x min and a standard deviation of the change of 2,300 mg/dL x min, which was estimated to provide 80% power to detect a differential

change in AUC glucose between any two intervention groups of 10% with a sample size of at least 20 per group and an adjusted α-error level of 1.67% (adjusted to account for three post hoc tests).

Statistical analyses were performed using SPSS (Version 26; IBM). The level of significance was set to $p<0.05$ for all analyses. We conducted both an *intent-to-treat* (ITT) and a *per-protocol* analysis. For the ITT analysis, we carried the baseline values forward for those time points where data were unavailable. For the *per-protocol* analysis for each endpoint, subjects were included if they (a) completed the dietary intervention and all clinic visits; (b) were compliant with the dietary regimen (defined as consuming at least 90% of the study dairy foods provided, and consuming 10 or fewer servings of non-study dairy foods during the intervention period); (c) had no changes in medications that may impact the respective study endpoint; and (d) remained free from any illness that may impact the respective study endpoint.

An unadjusted repeated measures ANOVA (RM-ANOVA) with time (clinic visit 1 vs. 2) as the within-subject variable and diet group (limited vs. low-fat vs. full-fat) as the between-subjects variable, with primary emphasis on the time by diet group interaction was considered model 1. Logarithmic transformations were performed on all outcome variables that were not normally distributed. Then, related baseline factors that differed by study arm as defined as a p-value <0.1 were included in the model as covariates, unless that baseline variable was already included in the model. This was considered the primary model, as it is thought to more accurately reflect intervention effects [340]. We also conducted sensitivity analyses adjusted for change in habitual physical activity and changes in dietary variables that were differentially changed by the intervention diets. For any glucose homeostasis-related variable for which we found differential changes by diet group, we conducted additional secondary analyses adjusting for changes in body fat mass and any body weight or composition measure that changed differentially to determine to which extent any difference observed between the groups may be attributable to a change in measures of adiposity. If the global RM-ANOVA indicated significant time by diet differences between the diet groups for an outcome variable, we conducted *post hoc* independent sample t-tests comparing the change in that variable in each of the three diet groups, or three RM-ANOVAs that included only two diet groups at a time for *post hoc* tests. In these analyses,

we adjusted for multiple testing according to Bonferroni. Again, *post hoc* tests were conducted on logarithmically-transformed data if the outcome variable failed the test for normality.

3.0 Results

3.1 Description of Participants

The trial was conducted between January 2016 and October 2018, when the recruitment goal was met. Recruitment letters were sent to 4,261 potentially eligible individuals identified by an automated screen and review of the UW electronic medical record system, and 17 self-referred volunteers (**Figure 2.1**). We conducted telephone screening interviews with 354 individuals and invited 130 potentially eligible and interested participants to attend an in-person screening visit, where 90 were deemed eligible for the trial. Excluding individuals unwilling or unable to participate, 76 subjects began the wash-in period. After excluding individuals who either dropped out or were non-compliant with study procedures during the wash-in diet period, a total of 72 adults were randomly assigned to one of the three diet groups: 24 each to the limited dairy, low-fat dairy, and full-fat dairy diet. All randomized participants were included in the ITT analyses. Five subjects were excluded from the *per-protocol* analyses of glucose tolerance and related endpoints (n=67): three dropped out prior to the final clinic visit and two were excluded for non-compliance. For body weight and body composition-related endpoints (n=66), one additional participant was excluded from the *per-protocol* analyses due to a change in thyroid medication. For the analysis of biomarkers of inflammation (n=59) an additional eight participants were excluded for acute illness or a change in medication or supplement intake. For the analysis of liver fat content (n=61), an additional six participants were excluded because they were unable to undergo an MRI scan. **Table 2.1** shows the baseline characteristics stratified by intervention group, for the *per-protocol* analysis. Baseline characteristics for those included in the ITT analyses can be found in **Table A5**.

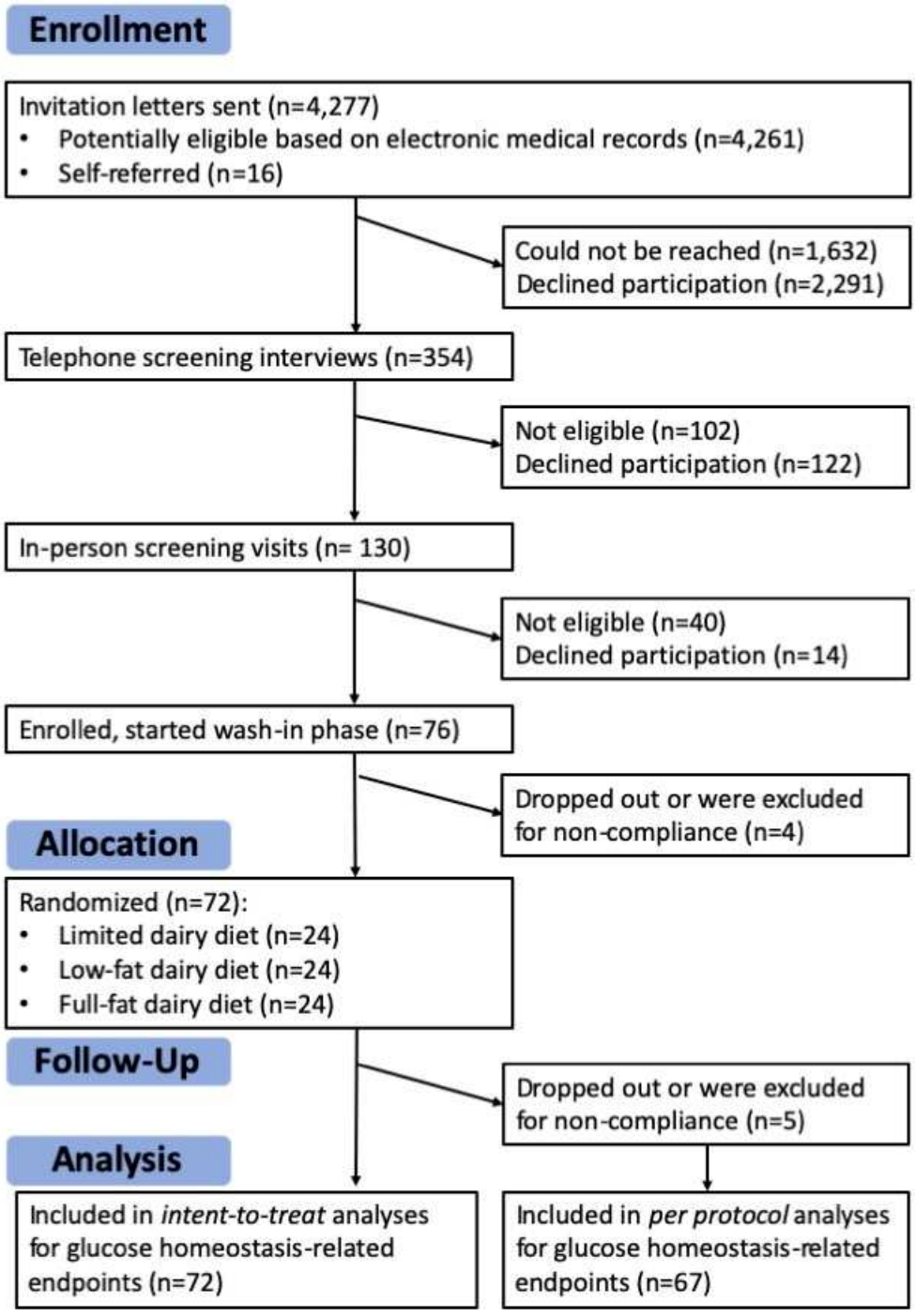

Figure 2.1 Consort Flow Diagram

Table 2.1 Baseline characteristics of study participants included in the primary *per-protocol* analyses for glucose tolerance and related endpoints (n=67).

Variable	**Limited dairy (n= 22)**	**Low-fat dairy (n=24)**	**Full-fat dairy (n=21)**	**P value**
Age (years)	56 (46; 68)	64 (58; 71)	65 (58; 68)	0.18
Male sex (%)	54.5	58.3	57.1	0.97
Caucasian race (%)	72.7	79.2	71.4	0.87
Body mass index (kg/m^2)	33.6 ± 5.9	32.6 ± 7.3	32.8 ± 6.4	0.85
Body weight (kg)	101 ± 16	96 ± 26	95 ± 17	0.58
Fat mass (kg)	37.9 (32.2; 45.1)	32.5 (23.6; 52.1)	36.7 (27.4; 48.8)	0.66
Lean mass (kg)	57.7 ± 10.3	55.6 ± 14.0	53.8 ± 8.8	0.54
Visceral adiposity ($inch^3$)*	148 (97; 202)	104 (71; 202)	124 (78; 177)	0.35
Physical activity (MET-h/week)	37.2 (23.4; 49.6)	41.0 (25.7; 89.7)	37.8 (19.6; 47.9)	0.29
2015 Healthy Eating Index (HEI)	71.9 ± 9.2	72.3 ± 9.7	72.2 ± 8.5	0.95
HOMA-IR	2.5 (1.9; 3.5)	3.3 (1.6; 4.4)	3.0 (1.7; 4.4)	0.98
Fasting glucose (mg/dL)	101 (93; 109)	110 (101; 119)	107 (102; 116)	0.09
Fasting insulin (μU/mL)	9.8 (7.1; 14.6)	12.3 (6.1; 15.6)	11.3 (7.9; 14.4)	0.86
Glycated hemoglobin (%)	5.4 (5.0; 5.5)	5.8 (5.5; 6.2)	5.7 (5.4; 5.9)	<0.001
Area under the curve glucose (mg/dL x min)	25,195 (23,445; 30,708)	29,895 (26,495; 32,849)	27,888 (24,831; 29,881)	0.07
Matsuda-insulin sensitivity index	2.7 (2.0; 3.8)	2.4 (1.8; 3.8)	2.3 (1.9; 3.4)	0.88
Insulinogenic index	1.0 (0.6; 1.5)	0.7 (0.4; 1.4)	1.2 (0.7; 1.9)	0.51
Glucose sensitivity (pmol x min^{-1} x m^{-2} x L x $mmol^{-1}$)	92 (64; 120)	74 (41; 103)	86 (68; 110)	0.47
Oral disposition index	2.3 (1.4; 4.5)	2.3 (1.4; 3.2)	2.8 (1.5; 4.8)	0.59
C-reactive protein (mg/L)	1.2 (0.9; 2.5)	0.9 (0.4; 2.1)	1.5 (0.9; 3.0)	0.52
Interleukin-6 (pg/mL)	3.5 (2.5; 4.2)	2.7 (1.8; 4.1)	2.9 (1.6; 4.2)	0.50
Total adiponectin (ng/mL)	5,150	6,425	5,900	0.86

	(3,838; 7,525)	(3,900; 9,300)	(3,925; 9,750)	
Liver fat content (% of total)ǂ	5.2 (1.0; 8.6)	4.4 (1.1; 10.1)	3.7 (2.1; 10.2)	0.60

Values are means ± standard deviations, or medians (25^{th}; 75^{th} percentiles) for non-normally distributed variables, or percentages (for categorical variables). P-values are based on an analysis of variance, except for gender and race, which were based on an independent samples Kruskal-Wallis test.

Abbreviations: HOMA-IR: homeostasis model assessment index of insulin resistance.

* Sample size for visceral adiposity: limited: n= 21, low-fat: n= 22, full-fat: n= 20

ǂ Sample size for percent liver fat: limited: n=20, low-fat: n=22, full-fat: n=19

3.2 Adherence to the Intervention and Dietary Intake

Based on data from the Human Nutrition Laboratory on administered and returned study dairy foods as well as participants' entries of consumption of non-study dairy foods on their dairy logs, *per-protocol* participants (n=67) consumed 98.2 ± 1.8% and 97.9 ± 2.8% (mean ± SD) of the study dairy foods provided to them during the low-fat and full-fat dairy intervention diet periods respectively (**Table 2.2**). During the limited dairy intervention period participants consumed an average of 74.9 ± 34.9% of the provided (non-mandatory) nonfat milk. Consumption of non-study dairy foods was 0.6 ± 1.0, 0.6 ± 0.9, and 1.3 ± 2.3 total servings during the 12 weeks of the limited, low-fat, and full-fat dairy diet periods respectively. Consistent data on total consumption of dairy foods were obtained from the average of three unannounced 24-hour dietary recall interviews conducted during the intervention period (**Table 2.2**). The percentage of two key biomarkers of dairy fat consumption, C15:0 and C17:0 in the plasma phospholipid fraction, increased in the full-fat dairy group compared to the limited dairy group and, in the case of C15:0, also compared to the low-fat dairy group (**Table 2.2**). No statistically significant differential changes were seen for the third established biomarker of dairy fat intake, the plasma phospholipid concentration of *trans*-C16:1 n-7.

The dietary intervention also led to some changes in the participants' habitual diet, as measured by repeated unannounced 24-hour dietary recall interviews (**Table A6**). Notable differential changes included the intake of saturated and monounsaturated fatty acids; the percentage of energy consumed as fat, carbohydrates, and protein; fiber (g/1,000 kcal); the healthy eating index; total energy intake; and calcium intake.

Table 2.2 Compliance with the dietary intervention of participants who were included in the *per-protocol* analysis for glucose tolerance and related endpoints (n=67).

	Limited dairy group (n=22)		Low-fat dairy diet (n=24)		Full-fat dairy diet (n=21)	
	Wash-in diet period	Intervention period	Wash-in diet period	Intervention period	Wash-in diet period	Intervention period
Consumed study dairy foods (administered study dairy foods – returned study dairy foods)						
Reduced-fat milk (servings/day)	0.41 (0.22; 0.42)	0.38 (0.17: 0.42)	0.42 (0.36; 0.42)	1.12 (1.08; 1.12)	0.42 (0.37; 0.42)	0.0 (0.0; 0.0)
Reduced-fat yogurt (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.00 (0.97; 1.01)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)
Reduced-fat cheese (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.14 (1.13; 1.14)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)
Whole milk (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.12 (1.10; 1.12)
Full-fat yogurt (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.01 (0.93; 1.01)
Full-fat cheese (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.14 (1.13; 1.14)
24-h dietary recall data [average of unannounced 24-hour dietary recall interviews in the wash-in (2 days) and intervention (3 days) periods]						
Reduced-fat milk (servings/day)	0.59 (0.15; 1.06)	0.53 (0.15; 1.29)	0.74 (0.33; 0.81)	1.11 (0.88; 1.75)	0.50 (0.31; 1.09)	0.0 (0.0; 0.42)
Reduced-fat yogurt (cups/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.14 (0.59; 1.63)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)
Reduced-fat cheese (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.25 (0.67; 1.54)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)
Whole milk (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.35)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.06)	1.25 (0.93; 1.92)
Full-fat yogurt (cups/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	0.0 (0.0; 0.0)	1.35 (0.84; 2.15)
Full-fat cheese (servings/day)	0.0 (0.0; 0.0)	0.0 (0.0; 0.01)	0.0 (0.0; 0.0)	0.0 (0.0; 0.04)	0.0 (0.0; 0.0)	1.19 (0.91; 1.50)

Plasma phospholipid biomarkers of dairy fat intake						
C15:0 (% of total)	0.25 (0.21; 0.32)	0.23 (0.16; 0.30)	0.20 (0.17; 0.22)	0.19 (0.15; 0.24)	0.24 (0.18; 0.27)	0.28 (0.22; 0.33)
C17:0 (% of total)	0.40 (0.29; 0.46)	0.36 (0.29; 0.42)	0.38 (0.31; 0.42)	0.38 (0.31; 0.45)	0.37 (0.30; 0.43)	0.46 (0.42; 0.50)
trans-C16:1 n-7 (% of total)	0.017 (0.008; 0.027)	0.013 (0.008; 0.022)	0.016 (0.007; 0.024)	0.015 (0.012; 0.019)	0.012 (0.000; 0.018)	0.024 (0.019; 0.027)

Values are means ± standard deviations or medians (25^{th}; 75^{th} percentiles) for non-normally distributed variables. One serving was defined as 240 mL for milk, 170 g for yogurt, and 42.5 g for cheese.

3.3 Glucose Tolerance and Its Determinants

Our primary endpoint, glucose tolerance as assessed by AUC glucose, was not differentially affected by the intervention diets (**Figures 2.2** and **2.3**). This was the case in the unadjusted analysis (model 1 in **Table A6**), as well as in model 2, which was adjusted for variables that differed or tended to differ across intervention groups at baseline (fasting glucose, and HbA1c).

Intervention effects were seen for insulin sensitivity, as assessed by the Matsuda insulin sensitivity index (p=0.012 for the overall time x diet interaction in model 2, **Figure 3** and **Table A7**). Specifically, the Matsuda insulin sensitivity index was significantly reduced in both dairy groups compared to the limited dairy group in *post hoc* tests, after adjustment for Bonferroni (**Figure 2.3**). Consistent with this reduction in insulin sensitivity, we observed a significant intervention effect for HOMA-IR and fasting insulin (overall time x diet interaction p=0.005 and p=0.010 respectively, **Figure 2.3** and **Table A7**). *Post hoc* t-tests showed that HOMA-IR significantly increased in both the low-fat and full-fat dairy groups compared to the limited dairy group (p=0.030 and p=0.003, respectively), with no difference between the two dairy intervention groups. There was also a significant increase in fasting insulin when comparing the low-fat dairy diet to the limited dairy diet (p=0.030). The reduction in insulin sensitivity is also evident when assessing the change in plasma insulin concentrations during the 3-hour FS-OGTT as compared to AUC glucose (**Figure 2.2**).

For measures of pancreatic β-cell function, we detected no overall intervention effect for the insulinogenic index and glucose sensitivity (**Figure 2.3** and **Table A7**). However, we detected an intervention effect for the oral DI (overall time x intervention interaction p=0.028), with a statistically significant decrease in the full-fat versus the limited dairy groups in *post hoc* testing (p=0.030).

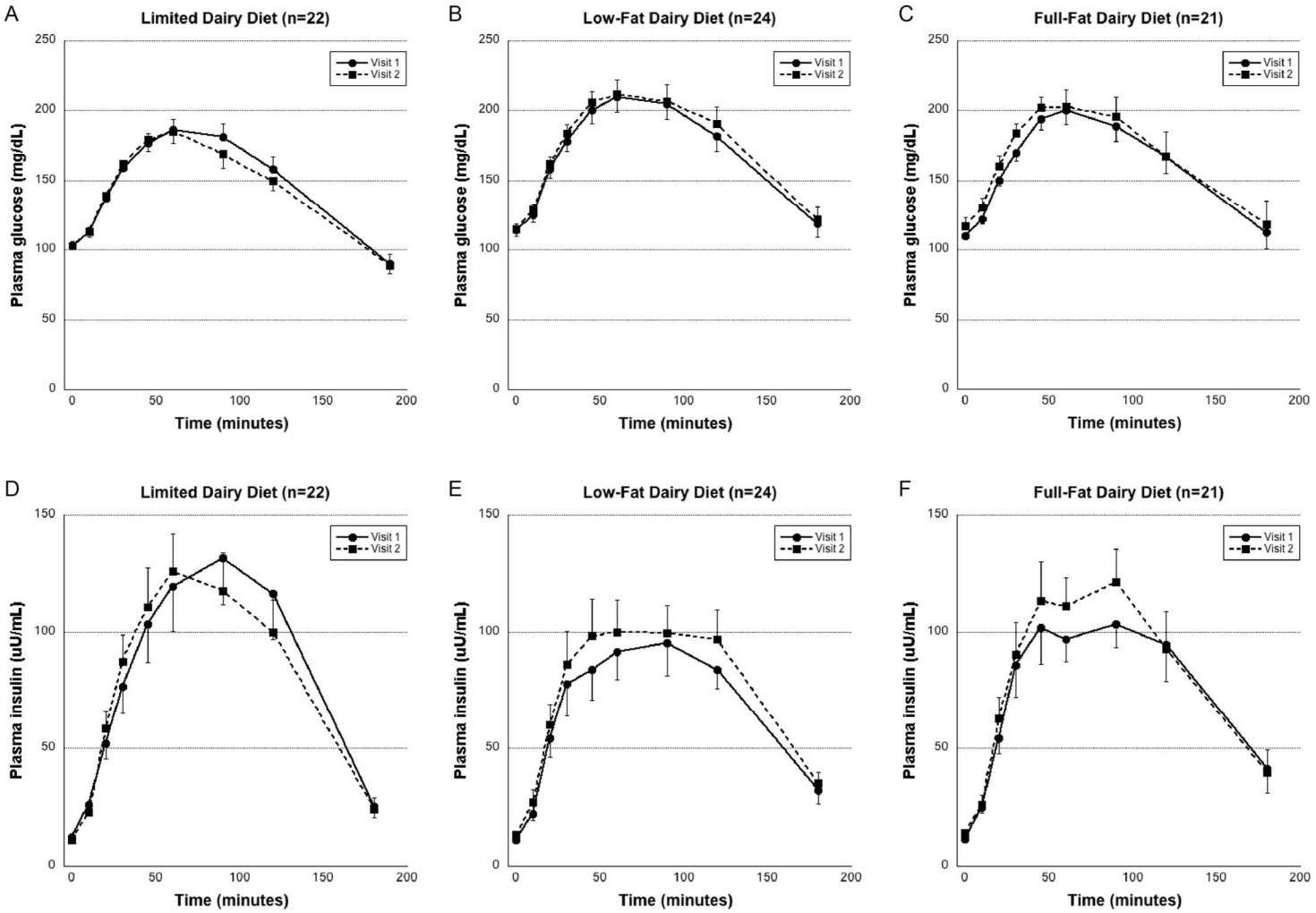

Figure 2.2 Impact of diets limited in dairy (a and d) or rich in low-fat dairy (b and e) or full-fat dairy (c and f) on plasma glucose (a-c) and insulin (d-f) concentrations in 3-hour frequently sampled oral glucose tolerance test. Circles and squares represent means at baseline (visit 1) and after the 12-week dietary intervention (visit 2), respectively. Error bars represent standard errors of the means.

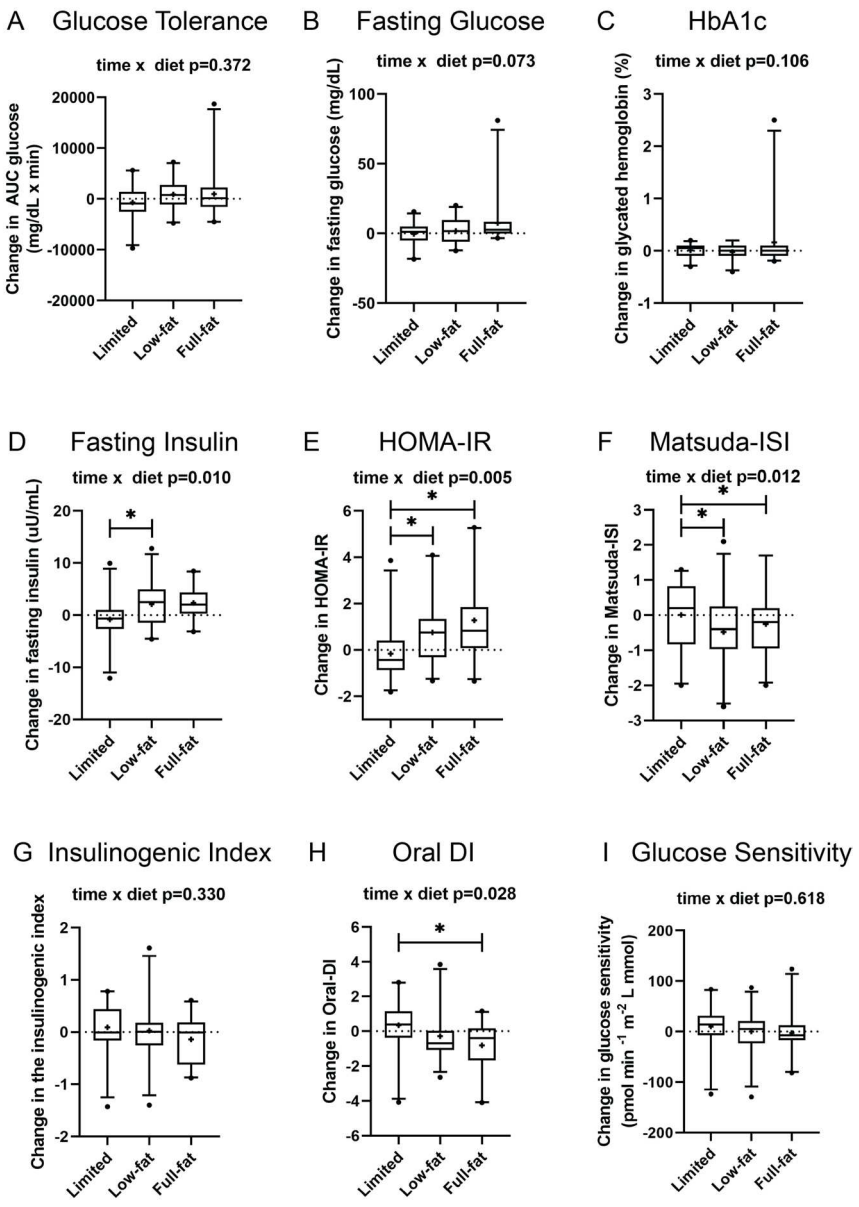

Figure 2.3 Changes in measures of glucose homeostasis, insulin sensitivity, and pancreatic β-cell function in the three dietary intervention groups (*per-protocol* analysis, n=67). Glucose homeostasis was assessed by (A) glucose tolerance, i.e., the area-under-the-curve glucose (AUC glucose), (B) fasting plasma glucose, and (C) glycated hemoglobin (HbA1c). Insulin sensitivity was assessed by (D) fasting plasma insulin, (E) the homeostasis model assessment insulin resistance (HOMA-IR) index, and (F) the Matsuda insulin sensitivity index (ISI). Pancreatic β-cell function was assessed by (G) the insulinogenic index, (H) the oral disposition index (DI), and (I) glucose sensitivity. Outcome variables are represented as the change variable calculated

as the value at follow-up minus the value at baseline. Boxes represent 25^{th}-75^{th} percentiles, and whiskers 5^{th} and 95^{th} percentiles, with outliers represented by a solid dot. The medians are represented by horizontal bars across the boxes and the means are represented by crosses. The p-values for the time by diet interactions from the overall repeated measures analysis of variance, adjusted for baseline area under the curve glucose, fasting glucose, and glycated hemoglobin, as appropriate are displayed at the top of each box-plot. Bars indicate significant differences between diet groups in *post hoc* testing, again adjusted for baseline area under the curve glucose, fasting glucose, and glycated hemoglobin, as appropriate (* $p<0.05$ after adjustment for multiple testing according to Bonferroni).

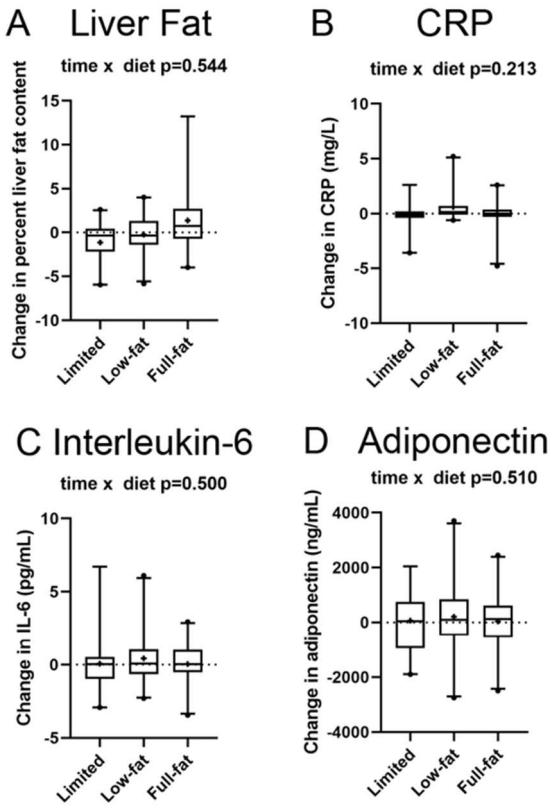

Figure 2.4 Changes in liver fat content (A) and measures of low-grade chronic systemic inflammation (B-D) in the three dietary intervention groups (*per-protocol* analysis, n=61 for liver fat, n=59 for biomarkers of inflammation). Outcome variables are represented as the change variable calculated as the value at follow-up minus the value at baseline. Boxes represent 25th-75th percentiles, and whiskers 5th and 95th percentiles, with outliers represented by a solid dot. The medians are represented by horizontal bars across the boxes and the means are represented by crosses. The p-values for the time by diet interactions from the overall repeated measures analysis of variance are displayed at the top of each box-plot. CRP: C-reactive protein.

No intervention effects were seen for any of the major determinants of insulin sensitivity (**Figure 2.4** and **Table A8**), including liver fat content (overall time x intervention interaction p=0.544) and biomarkers of systemic inflammation, including CRP (p=0.213), IL-6 (p=0.500), and total adiponectin (p=0.510).

We also assessed the impact of the intervention diets on other exploratory endpoints related to glucose homeostasis, fasting plasma glucose concentrations and HbA1c. While no intervention effect was seen for HbA1c (time x intervention interaction p=0.125, **Figure 2.3** and **Table A7**), we detected a trend for an intervention effect for fasting glucose (p=0.073).

In sensitivity analyses, adjustment of models that indicated an intervention effect (Matsuda ISI, fasting insulin, HOMA-IR) for changes in body fat mass, body weight, or waist circumference did not fundamentally change the results, even though in some cases the intervention effects were slightly attenuated ($p \leq 0.070$ for all adjusted variables). The sensitivity analyses for the oral DI adjusting for both the changes in waist circumference and weight led to a more substantial attenuation, becoming insignificant [p=0.099 and p=0.132 respectively (data not shown)]. We also ran extensive sensitivity analyses adjusting for changes in dietary variables that were differentially changed by the intervention diets (**Table A6**). The intervention effects for fasting insulin, HOMA-IR, the Matsuda insulin sensitivity index, and the oral DI tended to be very robust and remained significant even after adjustment for changes in percent energy from carbohydrates, added sugars, total fat, saturated fatty acids, monounsaturated fatty acids, and protein; the change in fiber intake (in g/1,000 kcal); and the change in the 2015 Healthy Eating Index. With the exception of the oral DI, all intervention effects also remained significant after adjustment for change in energy intake. Similarly, sensitivity analyses that excluded one outlier in the full-fat dairy group for several of the variables (**Figure 2.3**), or that included adjustment for changes in physical activity did not affect any of the results.

The ITT analysis (n=72) yielded results consistent with the *per-protocol* analyses for all endpoints .

3.4 Energy Intake, Body Weight, and Body Composition

During the 5-day controlled feeding period conducted during the intervention period as compared to the 5-day controlled feeding period during the wash-in period, participants received 281 kcal/d and 463 kcal/d more in the form of mandatory dairy foods in the low-fat and full-fat dairy groups, respectively. Comparing intakes from the 5-day controlled periods (intervention vs. wash-in), energy intake remained stable in individuals randomized to stay on the limited dairy diet (-21 ± 317 kcal/day), increased non-significantly in those who switched to the low-fat dairy diet (+166 ± 267 kcal/day), and increased significantly by 384 ± 175 kcal/day in participants who switched to the full-fat dairy diet ($p<0.001$ in the overall RM-ANOVA; adjusted $p<0.01$ in *post hoc* tests comparing full-fat dairy to both limited dairy and low-fat dairy). Similarly, total energy intake as measured by repeated 24-hour dietary recall interviews increased by 224 ± 375 kcal/d and 554 ± 467 kcal/d in the low-fat and full-fat dairy intervention groups, respectively, compared to the wash-in period, while it stayed relatively stable in the limited dairy group during the intervention period ($p=0.003$ in the overall RM-ANOVA; adjusted $p<0.05$ in *post hoc* tests comparing full-fat dairy to both limited dairy and low-fat dairy) (**Table A6**). Consistent with these increases in total energy intake, we observed an overall intervention effect for body weight (time x intervention interaction $p=0.005$), with a statistically significant increase in the full-fat dairy group compared to the limited dairy group (**Figure 2.5** and **Table A9**), with the low-fat dairy group in between the limited and the full-fat dairy groups (not significantly different from either after Bonferroni correction). Similarly, an overall intervention effect was seen for waist circumference (overall time x intervention interaction $p=0.015$), with a significant increase in both dairy groups compared to the limited dairy group in *post hoc* testing. There was a significant overall effect on fat mass (overall time x intervention interaction $p=0.024$), but no two diets differed from one another after adjustment for multiple testing in *post* hoc testing (**Figure 2.5** and **Table A9**). Further, there was a trend for a difference in lean mass (overall time x intervention interaction $p=0.082$) (Figure 2.5 and Table A9). No significant intervention effects were seen for measures of fat distribution including trunk fat, peripheral fat, or visceral adiposity (**Figure 2.5**). As with measures of glucose homeostasis, sensitivity analyses adjusted for changes in physical activity (model 2 in **Table A9**) or key dietary factors, as listed in **Table A6**, or excluding one major outlier in the full-fat dairy group yielded very similar results. The ITT

analysis (n=72) yielded results that were consistent with the *per-protocol* analyses for all endpoints.

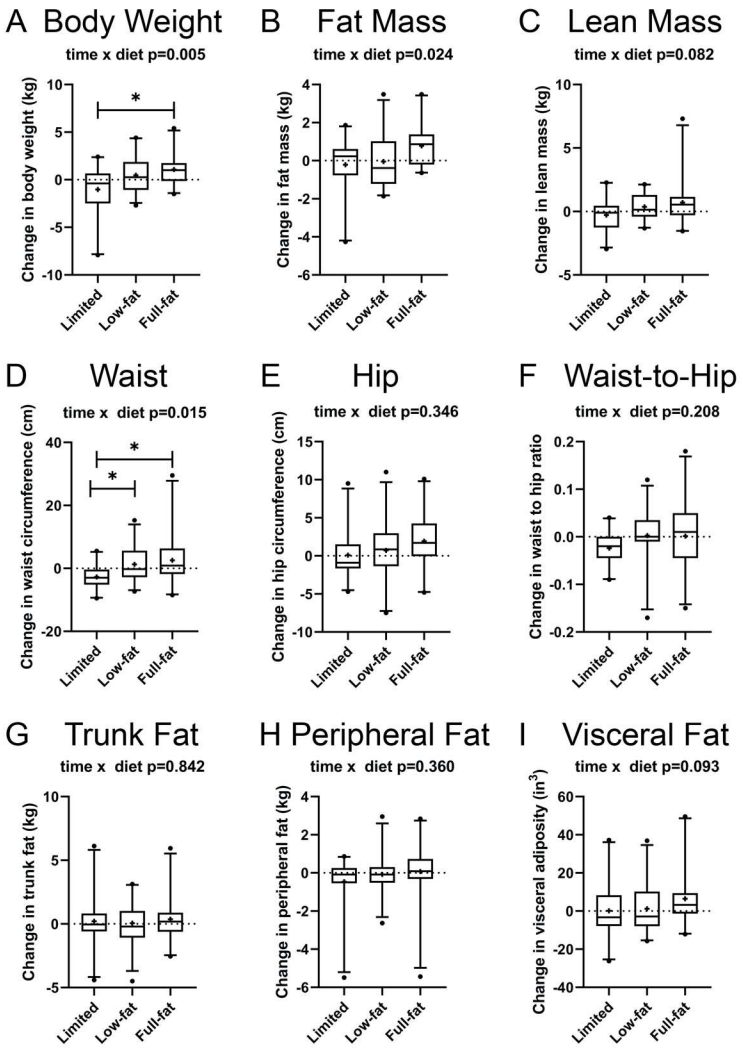

Figure 2.5 Changes in measures of body weight, anthropometrics, and body composition in the three dietary intervention groups (*per-protocol* analysis, n=66): (A) body weight, (B) total fat mass, (C) lean mass, (D) waist circumference, (E) hip circumference, (F) waist-to-hip ratio, (G) trunk fat mass, (H) peripheral fat mass, (I) visceral fat area. Outcome variables are represented as the change variable calculated as the value at follow-up minus the value at baseline. Boxes represent 25th-75th percentiles, and whiskers 5th and 95th percentiles, with outliers represented by a solid dot. The medians are represented by horizontal bars across the boxes and the means are represented by crosses. The p-values for the time by diet interaction from the overall repeated measures analysis of variance are displayed at the top of each box-plot. Bars indicate significant differences between diet groups in *post hoc* testing (* $p<0.05$ after adjustment for multiple testing according to Bonferroni).

4.0 Discussion

In contrast to our hypothesis, consuming three servings per day of low-fat or full-fat dairy in the form of milk, yogurt, and cheese did not improve glucose tolerance in males and females with metabolic syndrome. In fact, insulin sensitivity was reduced on both dairy diets compared to the control diet limited in dairy, and this effect was very robust in sensitivity analyses. This reduction in insulin sensitivity was also associated with a reduced oral DI in the full-fat dairy group, suggesting that pancreatic β-cell insulin secretion in response to the standardized glucose load did not increase sufficiently to fully compensate for the decrease in insulin sensitivity. It is possible that this reduction in insulin sensitivity could lead to a reduction in glucose tolerance over time, thereby increasing T2D risk. However, it is curious that the decrease in insulin sensitivity did not seem to be explained by changes in known determinants of insulin sensitivity (i.e., body weight, fat mass, liver fat content, or systemic inflammation), indicating that another mechanism is at play. One potential mechanism that was not explored in this study is that the observed reduction in insulin sensitivity in the dairy groups could plausibly be a physiological response to prevent hypoglycemia following dairy-rich meals. Dairy foods acutely trigger insulin responses far in excess of what would be expected based on their modest glycemic index[318]. This insulinotropic effect of dairy may be triggered by branched-chain amino acids found in dairy, which may act directly on the pancreatic β-cell and also promote the release of the incretin glucagon-like peptide-1 in intestinal cells, at least in vitro[319-321]. Sustained hyperinsulinemia causes insulin resistance[324,341]. Possibly, repeated postprandial hyperinsulinemia triggered by regular dairy consumption could similarly lead to insulin resistance. However, future investigations are needed to quantify and assess the impact of the hyperinsulinemia produced by dairy consumption and its potential to impact insulin sensitivity. It therefore remains unclear how the combination of dairy-induced insulin resistance coupled with the acute insulinotropic effect of dairy seen in previous studies affects glucose tolerance and T2D risk in the long term. Another potential mechanism not explored in this study are potential alterations in the gut microbial composition that may have resulted from following our dairy interventions, given that the gut microbiome is an emerging determinant of insulin sensitivity[342].

Another key finding from our trial was that there were no differential effects of our diets on liver fat content or biomarkers of systemic inflammation. The only additional endpoints with

intervention effects were body weight and waist circumference, and fat mass. Both body weight and waist circumference endpoints were more strongly increased in participants consuming full-fat dairy foods and had intermediate effects in participants consuming low-fat dairy foods. This increase in weight and waist circumference was associated with higher total energy intake, suggesting that participants did not reduce their *ad libitum* energy intake from non-dairy foods enough to fully compensate for the energy consumed in the form of mandatory dairy foods. This indicates that dairy consumption, and particularly full-fat dairy consumption, in the context of an ad libitum diet, increases energy intake resulting in increased adiposity. This conclusion is in alignment with the result of a recent meta-analysis that shows that dairy intake consistently increases weight in *ad libitum* studies [122]. Our study adds to this literature by providing evidence that full-fat dairy foods increase adiposity to a larger extent than their low-fat counterparts.

Our finding that neither the low-fat nor the full-fat dairy diet had an effect on glucose tolerance is consistent with previous RCTs[252,270,272,330]. Our data provide additional assurance that null findings in prior trials were not due to the fact that these trials included mostly low-fat unfermented dairy foods. Our finding of decreased insulin sensitivity in participants consuming dairy is also consistent with several prior trials [256-258]. At the same time, the majority of previous studies found no effect of dairy consumption on insulin resistance[159,252-255,259,260,270-272,330-332], and several trials found improvements in insulin sensitivity in participants increasing their dairy intake[251,260,261,343,344]. One factor that may explain the differential effects on insulin sensitivity is the duration of the interventions: all studies that showed a reduction in insulin sensitivity were 12 weeks in duration or shorter[256-258], while almost all studies that showed an improvement in insulin sensitivity were 12 weeks in duration or longer[251,260,261,343].

The results of our study are greatly at odds with data from observational cohort studies. Five meta-analyses evaluating the effect of dairy foods on T2D concluded that there is a significant inverse relationship between dairy consumption and risk of T2D[116,161-164], with particularly consistent data linking low-fat dairy, yogurt, and plasma biomarkers of dairy fat intake to reduced T2D incidence[145,166,201]. One possible explanation for the discrepancy between the observational data findings from trials is that residual confounding may have contributed to the associations in observational studies. It is also possible that effects of increasing dairy

consumption on glucose homeostasis were less beneficial in trials given a potential healthy participant bias. With regards to the data utilizing dairy-fat biomarkers, a potential explanation may be that early metabolic changes not commonly measured in observational studies that eventually lead to T2D, such as an increase in liver fat content, affect the concentration of dairy fat biomarkers in plasma phospholipids, thereby confounding the observed association.

This study had multiple strengths that increase our confidence in the results: it is the only study that directly compared a variety of low-fat vs. full-fat dairy foods on glucose tolerance; it assessed glucose homeostasis through dynamic testing; results were basically identical for *per-protocol* and ITT analyses; we controlled for changes in body weight and fat mass statistically; and participant compliance was excellent. Limitations of this study include a lack of generalizability of the results to populations other than those with metabolic syndrome, and the duration of our dairy intervention, which, even at 12 weeks, may have been insufficient to fully capture the long-term implications of habitual dairy consumption.

In conclusion, our study indicates that consuming three servings of milk, yogurt, and cheese per day, regardless of fat content, did not affect glucose tolerance in men and women with metabolic syndrome. However, both low-fat and full-fat dairy consumption resulted in a modest decrease in insulin sensitivity. It is unclear whether these changes would persist with prolonged exposure and would affect glucose tolerance and increase the risk of T2D over time. The effect of the dairy diets on insulin sensitivity was not explained by changes in systemic inflammation, liver fat content, body weight, or fat mass. Consuming three servings of dairy, particularly full-fat dairy, per day also resulted in an increase in *ad libitum* energy intake, body weight, and waist circumference. Future studies should investigate potential mechanisms by which dairy consumption may reduce insulin sensitivity and assess whether similar effects are seen in healthy populations. While results from a single study are insufficient to revise guidelines, the findings from the present study suggest that lower dairy intake may be beneficial in individuals with the metabolic syndrome.

CHAPTER 3: IMPACT OF LOW-FAT AND FULL-FAT DAIRY FOODS ON THE FASTING LIPID PROFILE AND BLOOD PRESSURE

ABSTRACT:

Background: Most dietary guidelines globally recommend low-fat dairy foods because dairy fat is thought to promote cardiovascular disease (CVD) due to its high saturated fat content. However, emerging evidence indicates that dairy fat may not negatively impact CVD risk factors when consumed in whole foods with a complex matrix. We aimed to compare the effects of diets rich in either low-fat or full-fat dairy foods, or a diet limited in dairy on CVD risk factors. We hypothesized that the full-fat dairy diet would not impact CVD risk factors differently than the low-fat or limited dairy diets.

Methods: Seventy-two participants with metabolic syndrome completed a 4-week wash-in period, limiting their dairy intake to ≤ 3 servings of skim milk per week. Participants were then randomized to either continue the limited dairy diet or switch to a diet containing 3.3 servings per day of either low-fat or full-fat milk, yogurt, and cheese for 12-weeks. A comprehensive fasting lipid profile and blood pressure were assessed before and after the intervention period.

Results: In the *per-protocol* repeated measures analysis of variance (n=66), there was no intervention effect on fasting serum total-, low-density lipoprotein-, and high-density lipoprotein-cholesterol; triglycerides; free fatty acids; or cholesterol content in 38 isolated plasma lipoprotein fractions ($p>0.1$ for all variables). There was also no intervention effect on diastolic blood pressure. There was a significant intervention effect for systolic blood pressure ($p=0.045$), with a trend for a decrease in the low-fat dairy diet compared to the limited dairy diet in *post hoc* testing after adjustment for multiple testing. *Intent-to-treat* results were consistent for all endpoints, except for the overall time x diet interaction for systolic blood pressure, which became non-significant ($p=0.80$).

Conclusions: In individuals with metabolic syndrome, consuming 3.3 servings of full-fat dairy per day did not negatively affect fasting blood lipids and blood pressure compared to consuming 3.3 servings of low-fat dairy or a diet limited in dairy. We conclude that dairy fat, when consumed as part of mixed complex whole foods, does not adversely impact the fasting lipid profile or blood pressure.

1.0 Introduction

Dairy has long been considered a part of a healthy diet and is recommended in dietary guidelines around the world[119,345,346]. Traditionally, low-fat dairy is recommended based on the hypothesis that consuming dairy fat promotes overconsumption of energy leading to weight gain, and increases the risk of cardiovascular disease (CVD) due to its high saturated fat content[347]. However, new evidence indicates that the association between saturated fatty acids (SFAs) and CVD is not as robust as previously believed[208]. A review by Calder emphasizes the substantial differences in the physiology and health effects of different SFAs[302].

In the observational literature, the consumption of full-fat dairy foods is consistently not associated with an increased risk of CVD[206,207]. Even consuming large amounts of butter is not associated with an increased risk for CVD, coronary heart disease, and stroke, based on a recent meta-analysis[348]. While many of these observational studies used food frequency questionnaires, a subjective assessment method subject to misreporting, there is also no association between dairy fat biomarkers in plasma phospholipids and CVD[349,350]. Therefore, the existing observational literature does not provide evidence to support the hypothesis that the consumption of full-fat dairy increases the risk for CVD.

This lack of an association between dairy fat intake and CVD risk may be unexpected, given that trials consistently indicate that isolated dairy fat in the form of butter, as compared to monounsaturated and polyunsaturated fatty acids, results in elevated fasting total and LDL-cholesterol concentrations[247]. However, recent trials suggest that a harmful impact of dairy fat on serum lipids is not observed when the same amount of dairy fat is consumed in the form of cheese[279,280]. These results suggest that dairy fat may differentially impact serum lipids when consumed as butter as compared to dairy foods with a complex matrix. Indeed, a review of randomized controlled trials (RCTs) that investigated the effect of dairy fat consumption in the form of whole dairy foods on the fasting serum lipid profile indicates that consumption of full-fat dairy products does not affect HDL- or LDL-cholesterol, or other CVD risk factors such as blood pressure[158]. However, most of these studies did not directly compare low-fat to full-fat dairy products and did not include the diverse range of dairy foods likely to be consumed by free-living individuals in their interventions. As a result, questions remain as to whether full-fat

versus low-fat dairy foods differentially affect the serum lipid profile and other CVD risk factors when dairy is consumed as a complex mixture of whole foods. Therefore, we compared the effect of consuming 3.3 servings of low-fat versus full-fat milk, yogurt, and cheese compared to a control group limited in dairy on a comprehensive fasting lipid profile and blood pressure in a 12-week parallel-design RCT.

2.0 Methods

2.1 Trial Registration:

This trial was registered on clinicaltrials.gov on January 26, 2016 (registry number NCT02663544), prior to enrolling the first participant. Changes were made after the commencement of the study, but before the end of the trial and any laboratory or statistical analyses, to add outcomes to broaden our ability to interpret trial effects on CVD risk. This includes adding the change in cholesterol concentrations in 38 plasma lipoprotein fractions and the change in the LDL relative flotation rate as exploratory endpoints.

2.2 Study Design:

This parallel-design RCT was carried out between January 2016 and October 2018 at the University of Washington (UW) and the Fred Hutchinson Cancer Research Center (Fred Hutch) located in Seattle, WA. All participants completed a 4-week wash-in diet phase during which their dairy consumption was limited to no more than three servings of nonfat milk per week ("limited dairy diet"). After completing a baseline clinic visit during the last week of the wash-in period, participants were randomized to either continue the limited dairy diet or switch to a diet containing 3.3 servings per day of low-fat dairy or full-fat dairy foods in the form of milk, yogurt, and cheese for 12-weeks. The randomization was performed using a random number generator by MK and SH using a block randomization procedure, with a block size of three, stratified by gender and the screening visit HOMA-IR (<5.0 versus ≥ 5.0 or diagnosis of diabetes). Participants were enrolled and assigned to the intervention diets by KAS, GC, MSB, or JNK. Subjects completed a follow-up clinic visit in the final week of the intervention period.

2.3 Participants:

We enrolled 18 to 75-year old, weight stable participants with metabolic syndrome (MetS)[333]. Key exclusion criteria included history of bariatric surgery; uncontrolled diabetes ($HbA_{1c} > 8\%$); the presence of major chronic inflammatory or autoimmune disease; fasting triglycerides >1,000mg/dL; or recent use of medications or diagnosis of any medical condition likely to interfere with study endpoints.

We identified potentially eligible participants predominately through automated screens of the UW electronic medical record system. After study staff reviewed medical records for key

exclusion criteria, the remaining potentially eligible participants were contacted by mail. This letter was followed by a telephone screening interview and then an in-person screening visit at Fred Hutch. During the screening visit, eligibility was ascertained, the study protocol was discussed, and participants sampled the intervention dairy foods. Informed consent was obtained from all participants prior to enrollment. The Fred Hutch institutional review board approved this study.

2.4 Study Diets:

During the wash-in period, participants were asked not to consume any dairy products other than a maximum of three servings per week of nonfat milk ("limited dairy diet"). At the baseline clinic visit, participants were randomized to one of three diets: to continue the limited dairy diet, or switch to a diet rich in either low-fat or full-fat dairy foods. In the low-fat dairy diet arm, participants were asked to consume 3.3 servings of dairy per day in the form of nonfat milk and yogurt, and low-fat cheese. In the full-fat dairy diet arm, participants were asked to consume 3.3 servings of dairy per day in the form of whole milk (3.25% fat), full-fat yogurt (3.1% fat), and full-fat cheese. One serving was defined as 240mL of milk, 170g of yogurt, and 42.5g of cheese. Darigold (Seattle, WA) produced the nonfat and whole milk and Mountain High (General Mills, Minneapolis, MN) produced the full-fat and nonfat yogurt. Low-fat and full-fat cheeses included cheddar cheese (21.2% and 32.9% fat, respectively; Sargento, Plymouth, WI), gouda (18.0% and 32.2% fat, respectively; Beemster, Elizabeth, NJ), and mozzarella (10.6% and 21.2% fat, respectively; Frigo/Saputo, Lincolnshire, IL). The total amount of dairy fat in the administered dairy foods averaged 0g per day, 8g per day, and 29g per day in the limited, low-fat, and full-fat dairy diets. The Human Nutrition Laboratory at Fred Hutch provided all study dairy products. During all study diets, participants were instructed not to consume any dairy foods other than those provided by the study, and to otherwise continue to consume their habitual diet *ad libitum*, incorporating the administered dairy products into their regular meals and snacks . They were also asked to record their dairy consumption in a daily log and to return any leftover dairy foods for weigh-backs.

2.5 Clinic Visits and Data Collection:

At both clinic visits, we collected fasting blood (after a 12-hour overnight fast); measured body weight, waist and hip circumference; conducted a whole-body dual-energy x-ray absorptiometry

(DEXA) scan on a Lunar iDXA scanner (GE Healthcare, Chicago, IL) to assess fat-mass; and measured blood pressure. Blood pressure was measured according to the guidelines outlined by the American Heart Association[351]. Participants sat comfortably for five minutes with legs uncrossed, with their back and upper arm supported; then, clothing that covered the arm was removed and the first blood pressure was taken. A minimum of two blood pressures was taken with an interval time of one minute. If there was a >5 mmHg difference between the first and second reading, a third reading was taken, and all readings were averaged together. Participants also completed physical activity questionnaires at baseline (clinic visit 1) and monthly during the 12-week intervention period. Twice during the wash-in diet period and three times during the intervention period, participants completed an unannounced 24-hour dietary recall administered by a staff member of the Fred Hutch Nutrition Assessment Shared Resource who was otherwise not associated with the trial.

2.6 Laboratory Procedures:

Serum lipids (triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol, free fatty acids, and LDL relative flotation rate), and cholesterol concentrations in 38 lipoprotein fractions in fasting plasma were measured at Northwest Lipid Research Laboratories (NWLRL) in Seattle, WA. Lipid profiling was done by enzymatic assays on a Roche Double Modular P Analytics automated analyzer. HDL-cholesterol was similarly assessed after precipitation of apo B-containing particles. The lipoprotein fractions were isolated using a non-equilibrium density gradient ultracentrifugation (DGUC) procedure to determine the cholesterol content of each of the 38 fractions and the flotation rate of LDL particles, as described previously[352]. This method is designed to optimize the resolution of apo B-containing lipoproteins. Briefly, the procedure involves the layering of density-adjusted plasma samples under KBr solution to form a discontinuous gradient. The plasma samples thus prepared are then subjected to ultracentrifugation and the resulting lipoprotein layers are drained from the bottom of each tube as 38 fractions. The measurement of cholesterol in each of the 38 fractions results in a lipoprotein profile where HDL, LDL, IDL, and VLDL are easily identified. The LDL relative flotation rate (Rf) is calculated as the fraction number of the major peak of LDL divided by the total number of fractions. The Rf of each plasma sample calculated by this procedure is highly reproducible with a CV consistently <2%. The LDL Rf indicates the mean density of the most

common LDL particles thus classifying individuals with small, dense LDL versus large, buoyant LDL.

As one assessment of compliance with the dietary regimen, we measured the amounts of C15:0, C17:0, and *trans*-C16:1n-7 in plasma phospholipids (conducted in the Kraft Lab, Burlington, VT), as these are validated biomarkers of dairy fat intake[334,335]. Plasma lipids were extracted according to the method of Folch et al.[336] Plasma phospholipids were isolated from total plasma lipids via solid-phase extraction using aminopropyl cartridges (Thermo Fisher Scientific, Waltham, MA), and transmethylated with boron trifluoride solution in methanol (Sigma-Aldrich, St. Louis, MO) to fatty acid methyl esters (FAME)[337]. FAME were analyzed by gas-liquid chromatography[337].

2.7 Study Outcomes:

The primary a-priori-defined study outcome was glucose tolerance. This paper reports results on all secondary and exploratory endpoints related to CVD risk, other than high sensitivity C-reactive protein, which is included in the primary paper. The exploratory endpoints in this manuscript include the fasting serum lipid profile including triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol, free fatty acids, and LDL relative flotation rate; the cholesterol content in 38 plasma lipoprotein fractions; and blood pressure.

2.8 Statistical Analyses:

We aimed to randomize 72 participants, with the goal of analyzing at least 20 in each of the three intervention groups, for a total of 60 participants in the primary *per-protocol* analysis. Sample size was calculated based on the primary aim of the trial, glucose tolerance.

Statistical analyses were performed using SPSS (Version 26; IBM). The level of significance was set to $p<0.05$ for all analyses. We conducted both an *intent-to-treat* (ITT) and a *per-protocol* analysis. For the ITT analysis, we carried the baseline values forward for those time points where data were unavailable. For the *per-protocol* analysis for each endpoint, subjects were included if they completed the dietary intervention and all clinic visits; were compliant with the dietary regimen (defined as consuming at least 90% of the study dairy foods provided, and consuming ten or fewer servings of non-study dairy foods during the intervention period); had no changes in

medications that may impact the respective study endpoint; and remained free from illness that may impact the respective study endpoint.

Logarithmic transformations were performed on all outcome variables that were not normally distributed prior to analyses. An unadjusted repeated measures analysis of variance (RM-ANOVA) with time (clinic visit 1 versus 2) as the within-subject variable and diet group (limited versus low-fat versus full-fat) as the between-subjects variable, with primary emphasis on the time by diet group interaction, was model 1. Then, baseline factors that differed by study arm, as defined as a p-value <0.1, were included in the model as covariates, as long as the variable was not included in the model already. This was considered our primary result as it is thought to better reflect intervention effects[340]. We also conducted sensitivity analyses adjusted for change in habitual physical activity. For any outcome variable for which we found differential change by diet group, we conducted additional secondary analyses adjusting for changes in body fat mass, body weight, and waist circumference to determine to which extent any difference observed between the groups may be attributable to a change in these variables. No sex-based or ethnicity/race-based analyses were conducted due to the limitation of sample size. If the global RM-ANOVA indicated significant time x diet differences between the diet groups for an outcome variable, we conducted *post hoc* independent sample *t*-tests comparing the change in that variable in each of the three diet groups, or three RM-ANOVAs that included only two diet groups at a time for *post hoc* tests on adjusted models. In these analyses, we adjusted for multiple testing according to Bonferroni.

3.0 Results

3.1 Description of Participants

A total of 9,424 individuals were identified from the UW electronic medical record system as potentially eligible. After reviewing medical records for key exclusion criteria, recruitment letters were sent to 4,277 individuals (**Figure 3.1**), including 16 self-referred individuals. We conducted telephone-screening interviews with 354 individuals; identifying 130 potentially eligible participants for the in-person screening visit, where 90 were deemed eligible for the trial. Excluding individuals not willing or able to participate, 76 individuals began the wash-in period. After excluding individuals who either dropped out or were excluded for noncompliance in the wash-in diet period, a total of 72 adults were randomly assigned to one of the three diet groups: 24 each to the limited, low-fat, and full-fat dairy diets. All randomized participants were included in the ITT analyses. A total of five subjects were excluded from the per-protocol analysis for changes in blood pressure, for a total of 67 participants. Three dropped out of the study prior to the final clinic visit, and two were excluded for non-compliance. An additional subject was excluded from the *per-protocol* analysis of serum lipids, for a total of 66 participants, due to a change in medication. **Table 3.1** shows the baseline characteristics stratified by intervention groups for participants included in the *per-protocol* analyses for fasting serum lipids. There was a trend for a difference between diet groups at baseline for HDL-cholesterol (p=0.10); otherwise, the randomization was successful for all variables. Per our statistical analysis plan, this variable was included in adjusted analyses of lipids (model 2) to account for this imbalance at baseline.

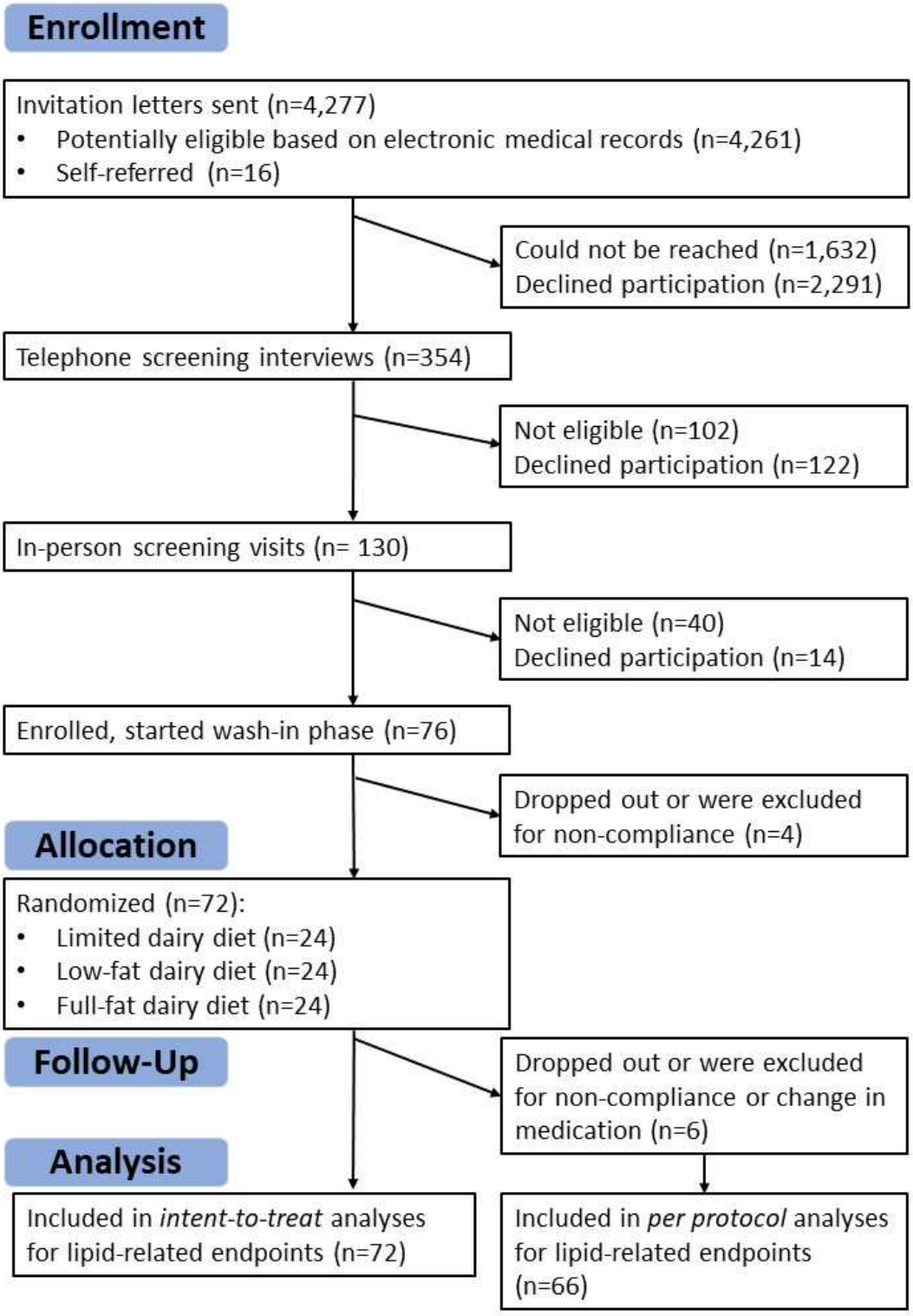

Figure 3.1 Flow sheet of study recruitment and enrollment.

Table 3.1 Baseline characteristics of study participants included in the *per-protocol* analyses for fasting serum lipids (n=66).

Variable	Limited	Low-fat	Full-fat	P value*
	N= 21	N=24	N=21	
Age (y)	56.0 (46; 69)	64.0 (58; 71)	65.0 (58; 68)	0.25
Male Sex (%)	52.4%	58.3%	57.1%	0.92
Caucasian Race (%)†	71.4%	82.6%	71.4%	0.61
Body weight (kg)	100.7 ± 16.5	96.5 ± 25.7	94.8 ± 17.4	0.64
Height (cm)	173.3 ± 9.2	171.6 ± 12.7	170.2 ± 6.3	0.61
Fat mass (kg)	38.4 (32.1; 47.3)	32.5 (23.6; 52.1)	36.7 (27.4; 48.8)	0.67
Lean body mass (kg)	57.2 ± 10.3	55.5 ± 14.0	53.8 ± 8.8	0.63
Visceral adiposity (in^3)†	143.7 (94.9; 207.3)	103.9 (71.4; 202.1)	123.5 (77.7; 176.6)	0.40
Diastolic blood pressure (mmHg)	76.1 ± 9.5	80.9 ± 10.6	78.0 ± 7.7	0.23
Systolic blood pressure (mmHg)	124	123	126	0.83
	(118; 135)	(119; 138)	(119; 136)	
LDL-cholesterol derived (md/dL)	88.4 ± 32.5	93.8 ± 33.6	105.2 ± 37.0	0.28
HDL-cholesterol (md/dL)	34.0 (29.5; 44.0)	36.5 (29.5; 48.5)	41.0 (37.0; 60.0)	0.10
Triglycerides (mg/dL)	142.0 (73.0; 184.5)	149.5 (92.5; 204.0)	122.0 (87.0; 191.5)	0.50
Total Cholesterol (mg/dL)	153.4 ± 43.8	168.3 ± 37.2	180.0 ± 43.6	0.12
Free Fatty Acids (mEq/L)	0.54 ± 0.19	0.59 ± 0.17	0.57 ± 0.16	0.61
LDL relative flotation rate	0.26 (0.24; 0.29)	0.26 (0.24; 0.29)	0.26 (0.25; 0.29)	0.20
CRP (mg/L)	1.3 (0.9; 2.9)	0.9 (0.4; 2.1)	1.5 (0.9; 3.0)	0.50
Interleukin-6 (pg/mL)	3.5 (2.3; 4.0)	2.6 (1.8; 4 .1)	2.9 (1.6; 4.2)	0.69
Adiponectin (ng/mL)	5,100	6,425	5,900	0.88
	(3,775; 7,650)	(3,900; 9,300)	(3,925; 9,750)	
Physical activity	36.7 (21.5; 52.7)	41.0 (25.7; 89.7)	37.8 (19.6; 47.9)	0.29
(MET hrs/week)				
Healthy Eating Index Score	72.1 ± 9.3	72.8 ± 9.7	72.2 ± 8.5	0.97

Data are means ± standard deviations or (for non-normally distributed variables) medians (25^{th}; 75^{th} percentile), or percentages

(for categorical variables).

Abbreviations: C-reactive protein (CRP), high-density lipoprotein (HDL), Low-density lipoprotein (LDL), metabolic equivalent of task (MET). * p-value based on an analysis of variance

†Sample size for Caucasian Race: limited n=21, low-fat n=23, full-fat n=21

† Sample size for visceral adiposity: limited n= 20, low-fat n= 22, full-fat n= 20

3.2 Adherence to Intervention and Dietary Intakes:

Based on the data from the Human Nutrition Laboratory on administered and returned study dairy foods as well as participants' entries of consumption of non-study dairy foods on their daily logs, the low-fat dairy group consumed 98.2 ± 1.8 % and 97.9 ± 2.8% (mean ± SD) of the dairy foods provided to them. During the limited dairy intervention period, participants consumed an average of 74.1 ± 35.3% of the provided (non-mandatory) nonfat milk. Consumption of non-study dairy foods was 0.6 ± 1.0, 0.6 ±0.9, and 1.3 ± 2.3 total servings during the 12-weeks of the limited, low-fat, and full-fat dairy diet periods. High compliance was confirmed through the evaluation of dairy-fat biomarker concentrations, including C15:0, C17:0, and *trans*-C16: 1n-7. The percentage of C15:0 and C17:0 in the plasma phospholipid fraction increased in the full-fat dairy group compared to the limited dairy group and, in the case of C15:0, also compared to the low-fat dairy group (data not shown). No statistically significant differential changes were seen for the plasma phospholipid concentration of *trans*-C16:1 n-7.

The dietary interventions also led to changes in the participants' habitual diet, as measured by repeated unannounced 24-hour dietary recalls. The intake of SFAs (%of energy intake) increased in the full-fat dairy group compared to both the limited and the low-fat dairy groups (adjusted $p<0.001$ in *post hoc* testing), and the intake of calcium increased in both dairy groups compared to the limited dairy diet (adjusted $p<0.05$ in *post hoc* testing). The intake of monounsaturated fatty acids decreased in the low-fat dairy diet compared to both the limited and full-fat dairy diets (adjusted $p<0.05$ in *post hoc* testing), but there was no effect on polyunsaturated fatty acid intake. The intake of total sugars (% of energy) increased in the low-fat dairy group as compared to the limited dairy diet (adjusted $p=0.006$, in post hoc testing), but there was no diet effect on the intake of added sugars. Nutrient density-adjusted fiber intake (in g/1,000 kcal) decreased in the full-fat dairy diet group as compared to the limited dairy diet group (adjusted $p=0.024$, in *post hoc* testing), but there was no diet effect on absolute fiber intake (in g/day). The differences in the fat content of the administered dairy foods affected the macronutrient composition of the overall diet, with an increase in carbohydrate intake (% of energy) in the low-fat dairy arm compared to the full-fat dairy arm (adjusted $p=0.015$ in *post hoc* testing), an increase in fat intake (% of total energy) in the full-fat dairy arm compared to the low-fat dairy arm (adjusted $p<0.001$ in *post hoc* testing), and a trend for an increase in protein intake in the low-fat dairy arm as

compared to the limited and full-fat dairy arms (adjusted p=0.087 and p=0.090 respectively in *post hoc* testing). Total energy intake increased in the full-fat dairy arm compared to both the limited and low-fat dairy arms (adjusted $p<0.05$ for both in *post hoc* testing), with no difference between the latter two. The 2015 Healthy Eating Index increased in the low-fat dairy group compared to the full-fat dairy group (adjusted $p<0.003$ in post hoc testing).

As reported in Chapter 2, there were overall intervention effects on change in body weight, waist circumference, and fat-mass. Both body weight and waist circumference were more strongly increased in participants consuming full-fat dairy foods and had an intermediate effect in participants consuming low-fat dairy foods. The increase in these adiposity-related outcome measures was largely attributed to the increase in *ad libitum* energy intake observed in the dairy consuming groups.

3.3 Fasting Blood Lipids

There was no differential diet intervention effect on total cholesterol (overall, *per-protocol*, model 2, p=0.328), triglycerides (p=0.446), LDL cholesterol (p=0.975), HDL cholesterol (p=0.788), or free fatty acids (p=0.825) in fasting serum, or the LDL relative flotation rate (p=0.118) (**Figure 3.2 and Table A10).** Similarly, there was no differential effect of the intervention diets on the cholesterol content in any of the 38 isolated plasma lipoprotein fractions (overall *per-protocol*, $p>0.1$ for all variables) (**Figure 3.3**). Sensitivity analyses adjusting for the change in physical activity did not affect any of the models.

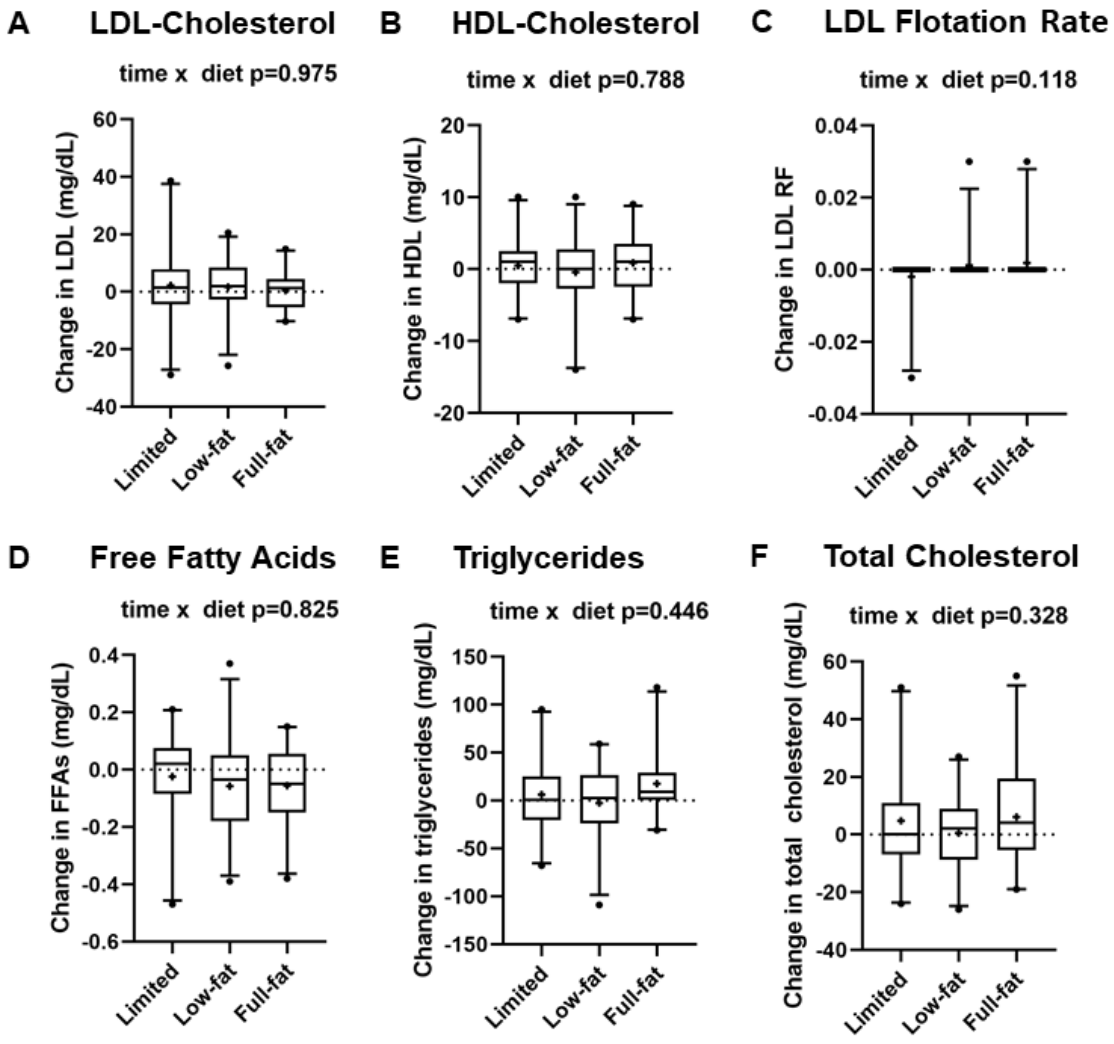

Figure 3.2 Changes in LDL-Cholesterol (A) HDL-Cholesterol (B) LDL flotation rate (C) Free fatty acids (D) Triglycerides (E) and total cholesterol (F) in the three dietary intervention groups (per-protocol analysis). Outcome variables are represented as the change variable calculated as the value at follow-up minus the value at baseline. Boxes represent 25th-75th percentiles, and whiskers 5th and 95th percentiles, with outliers represented by a solid dot. The medians are represented by horizontal bars across the boxes and the means are represented by crosses. The p-values for the time by diet interactions from the overall repeated measures analysis of variance, adjusted for baseline HDL-cholesterol (with the exception of change in HDL-cholesterol), are displayed at the top of each box-plot.

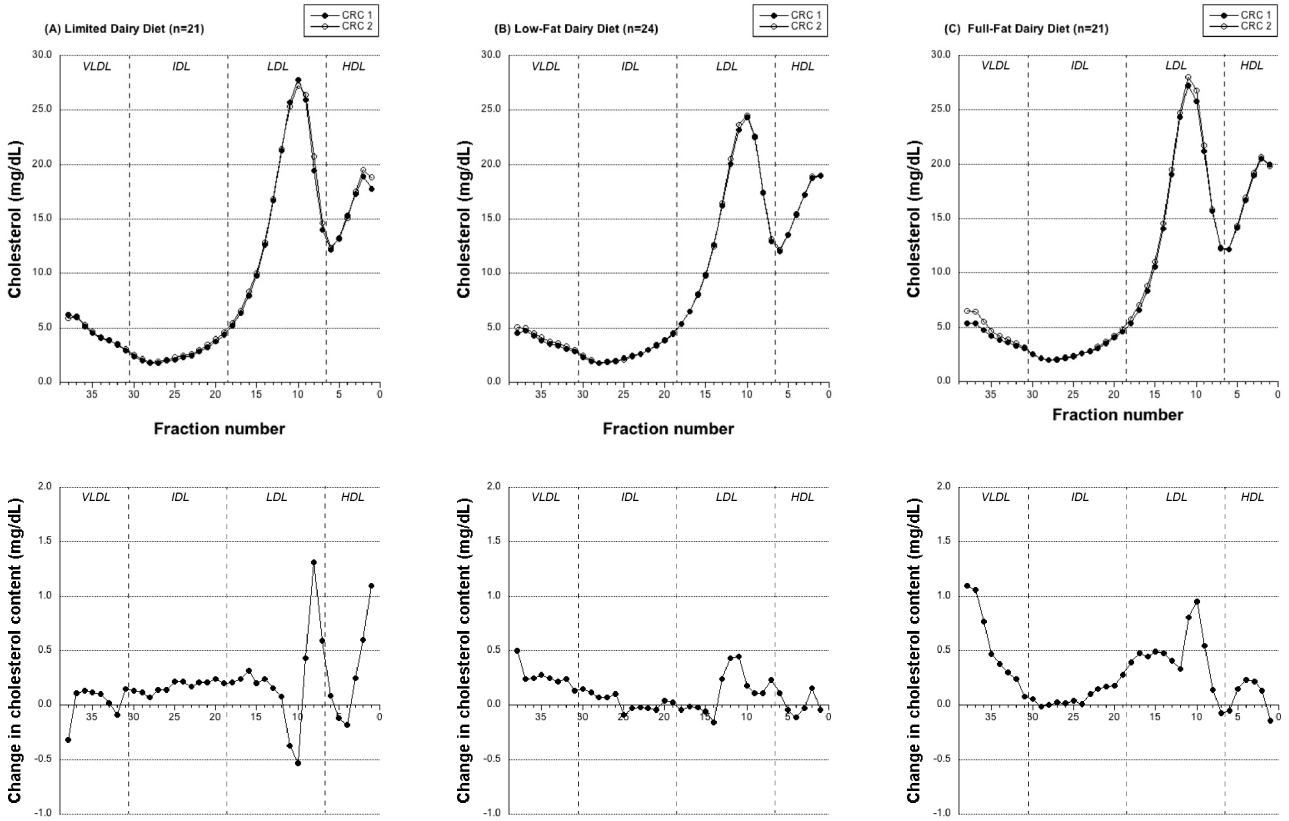

Figure 3.3 Cholesterol content in 38 fractions in participants consuming the limited dairy diet (A), the low-fat dairy diet (B), or the full-fat dairy diet (C). Upper panels show absolute concentrations from clinical research center (CRC) visit #1 (baseline) and visit #2 (follow-up), based on the *per-protocol* cohort for lipid analyses (n=66), and lower panels show changes in cholesterol content in each fraction during the interventions. VLDL: very low-density lipoprotein, IDL: intermediate-density lipoprotein, LDL: low-density lipoprotein, HDL: high-density lipoprotein

3.4 Blood Pressure

There was no effect of the dairy intervention on diastolic blood pressure. Yet, there was a significant difference among the diet interventions for systolic blood pressure (overall, *per-protocol,* p=0.045) (**Table 3.2**). However, after adjustment for multiple testing, no two intervention groups were significantly different from one another, though there was a trend for a decrease in the systolic blood pressure in the low-fat dairy group as compared to the limited dairy group. Further, a sensitivity analysis adjusting for change in fat mass attenuated the effect of diet on systolic blood pressure to a trend (overall, *per-protocol,* p=0.094) (**Table 3.2**). A sensitivity analysis adjusting for change in physical activity did not affect the results. We also ran extensive sensitivity analyses adjusting for changes in dietary variables that were differentially changed by the intervention diets. The intervention effects on systolic blood pressure remained significant after adjustment for changes in percent energy from carbohydrates, added sugar, total fat, saturated fatty acids, and monounsaturated fatty acids; the change in fiber intake (in g/1,000 kcal); and the change in the 2015 Healthy Eating Index. Both the change in total calories and percent of energy from protein attenuated the results (p=0.69 and p=0.51, respectively, after adjustment).

3.5 ITT Analysis

The distribution of baseline characteristics was similar for the ITT analysis (n=72) (**Table A11**). The significant effect of diet on systolic blood pressure observed in the unadjusted *per-protocol* analysis became a trend in the ITT analysis (overall, p=.080) (data not shown). Otherwise, the ITT analysis yielded results consistent with the *per-protocol* analyses for all other endpoints (p>0.05, data not shown).

Table 3.2 The effect of dairy consumption on blood pressure (*per-protocol* analysis) among the limited (n=22, low-fat (n=24) and full fat (n=21) dairy groups.

	*Baseline	*Follow-up	Delta	†RM-ANOVA (time x diet intervention)	
				Model 1	Model 2
Systolic blood pressure (mmHg)				0.045	0.094
Limited dairy	124 (119; 137)	131 (120; 137)	2.5 ± 8.2		
Low-fat dairy	123 (119; 138)	125 (116; 131)	-1.6 ± 8.6		
Full-fat dairy	126 (119; 136)	121 (110; 134)	-5.4 ± 16.1		
Diastolic blood pressure (mmHg)				0.512	
Limited dairy	76.8 ± 9.9	77.2 ± 8.9	0.5 ± 6.9		
Low-fat dairy	80.9 ± 10.6	78.3 ± 8.9	-2.6 ± 7.4		
Full-fat dairy	78.0 ± 7.7	77.0 ± 10.1	-0.9 ± 10.4		

*Values are mean ± SD or median (25^{th}; 75^{th} percentile) if non-normally distributed data.

†Reflects an overall comparison of the three dietary phases by repeated measures analysis of variance (RM-ANOVA)

Model 1: Unadjusted analysis

Model 2: Adjusted for change in fat mass

4.0 Discussion

In this 12-week intervention trial in men and women with MetS, consuming 3.3 servings of full-fat dairy per day in the form of milk, yogurt, and cheese did not significantly affect any measure of the fasting lipid profile compared to consuming identical amounts of low-fat milk, yogurt, and cheese, or compared to a diet strongly limited in dairy. This included no significant difference in the cholesterol content of the 38 isolated plasma lipoprotein fractions, in spite of substantial differences between the three diets in the consumption of total fat and SFA. There was also no intervention effect on diastolic blood pressure. There was a significant difference among the diet interventions for systolic blood pressure, with a trend for a decrease in the low-fat dairy diet compared to the limited dairy diet in *post hoc* testing after adjustment for multiple testing.

The results of this study challenge the hypothesis that the consumption of full-fat dairy products increases the risk of CVD due to elevated total and LDL-cholesterol concentrations resulting from its high content of SFA and cholesterol[347]. This hypothesis is predominantly based on short-term feeding trials demonstrating that isolated dairy fat in the form of butter results in dyslipidemia as compared to a variety of monounsaturated and polyunsaturated fatty acids[247]. There could be several reasons for this discrepancy, including the fact that most[353-356] but not all[357] intervention studies used a higher overall dose of dairy fat as butter than was consumed in the full-fat dairy arm of our study, and that most trials compared the impact of butter vs. specific plant oils rich in mono- or polyunsaturated fatty acids. Still, our trial suggests that even a fairly high daily dose of dairy fat consumed as milk, yogurt, and cheese, i.e. whole foods with a complex matrix, does not affect CVD risk factors. Therefore, the results of this study align with the observational literature, which predominantly indicates that full-fat dairy foods are not associated with CVD, CHD, or stroke[206,207].

Our finding that diets rich in low-fat or full-fat dairy did not differentially affect serum lipids is in alignment with two previous RCTs that directly compared low-fat and full-fat dairy interventions, one using milk[282] and the other cheese[159]. In a 12-week parallel-design RCT, Raziani et al. randomized individuals with MetS to a full-fat cheese, reduced-fat cheese, or a no-dairy carbohydrate control group. Similar to our data, there was no differential effect of the intervention diets on LDL-cholesterol, HDL-cholesterol, total cholesterol, or free fatty acids[159].

Steinmetz et al., in a 6-week randomized cross-over study of eight healthy men, found that whole milk did not lead to differential changes in HDL-cholesterol, LDL-cholesterol, total-cholesterol, or triglycerides as compared to skim milk[282]. In contrast to these studies, our trial included a larger variety and higher dose of dairy products, adding confidence that the null results in these prior trials were not due to the types or amounts of dairy products studied.

In contrast to our study, one previous RCT found that full-fat dairy consumption unfavorably affected LDL-cholesterol compared to low-fat dairy consumption[281]. In a 3-week randomized cross-over study by Nestel et al., 12 healthy overweight Australian adults followed three different isocaloric dairy diets: a low-fat dairy diet consisting of two daily servings of low-fat milk and yogurt; a full-fat fermented dairy diet consisting of cheese and yogurt; and a full-fat non-fermented dairy diet consisting of butter, cream, and ice cream. They found that both full-fat dairy diets resulted in higher LDL-cholesterol as compared to the low-fat dairy diet, with no difference between the fermented and non-fermented full-fat dairy diets[281]. There are a few potential explanations for why Nestel et al. observed an effect on LDL-cholesterol while we did not. One possibility is that LDL-cholesterol concentrations were affected by other dietary modifications that resulted from incorporating full-fat dairy into the diet. Specifically, the fermented dairy dietary phase led to significantly lower fiber intake as compared to the low-fat dairy diet, with the non-fermented dairy diet falling in-between. Another possible explanation is the heterogeneity in the types of dairy foods provided in each intervention diet beyond just the fat content. For example, it may be that components of the food matrix in cheese and ice cream as compared to milk and yogurt impact LDL-cholesterol differentially. In contrast, our study compared diets that had identical types of dairy foods that varied only in their fat content.

Also, in contrast to our results, two previous studies found that full-fat dairy consumption increased HDL-cholesterol as compared to a low-fat dairy intervention[160,281]. In the study by Nestel et al. described above, the authors found that HDL-cholesterol increased during the non-fermented dairy diet as compared to the low-fat dairy diet[281]. The other study, by Engel et al., was a randomized cross-over study in 18 healthy adults who followed a diet that included 0.5 L per day of whole milk or skimmed milk alongside an *ad libitum* diet for 3-weeks each. They found that HDL-cholesterol increased during the whole milk diet as compared to the skimmed

milk diet[160]. One possible explanation for the differential impact on HDL-cholesterol between these studies and ours is that both Engel et al. and Nestle et al. conducted their studies in healthy adult populations whereas our study was conducted in individuals with MetS. Additionally, given that Engel et al. was conducted in Denmark and Nestel et al. was conducted in Australia, it may be that full-fat dairy is more likely to increase HDL-cholesterol in the context of these dietary patterns as compared to the average American diet. Further, it may be that the fatty acid profile of the full-fat dairy foods consumed in the Engel et al. and Nestel et al. studies differed from those used in our intervention, potentially contributing to differential fasting lipid outcomes. To our knowledge, our study is the first to more comprehensively evaluate the effect of full-fat dairy consumption as compared to the low-fat dairy consumption on the lipid profile through reporting the LDL relative flotation rate and the cholesterol content in 38 plasma lipoprotein lipid fractions. This adds confidence in our conclusion that full-fat dairy consumption does not cause the development of an unfavorable lipid profile.

Blood pressure is another critical CVD risk factor investigated in our study. In alignment with two previous studies[159,284], we found that full-fat dairy consumption did not differ from low-fat dairy consumption in its effects on diastolic or systolic blood pressure. Given that these two studies investigated the impact of low-fat versus full-fat dairy using only cheese[25] or milk and yogurt[284], our study adds confidence that consuming a wide range of full-fat versus low-fat dairy foods does not differentially impact blood pressure. Similar to our study, Raziani et al. also compared their dairy diets to a control diet free of dairy. They found that neither their full-fat cheese nor their reduced-fat cheese interventions impacted diastolic or systolic blood pressure differentially compared to their control diet[159]. In contrast, our study found that low-fat dairy consumption tended to decrease systolic blood pressure as compared to a diet virtually free of dairy. However, this effect was attenuated in the ITT analysis. Therefore, it is likely that dairy consumption, regardless of fat content, does not affect blood pressure to a clinically significant degree.

There are a few potential explanations for the discrepancy between our findings and the historical view that dairy fat increases the risk of CVD. Dairy fat is complex and includes a diverse range of SFAs from C2:0 to C28:0 in addition to various mono- and polyunsaturated

fatty acids[300,301]. While SFAs have historically been viewed as an unhealthful fat, it is important to emphasize that the C12–C16 long-chain SFAs that have LDL-cholesterol-raising effects only make up around 40-50% of the total fatty acids in dairy[118]. The other short-, medium-, and branched-chain SFA in dairy have substantially different physiological and metabolic effects[302]. For example, butyric acid (C4:0), a short-chain fatty acid found in dairy, is also a key metabolic endproduct of dietary fiber fermentation in the gut that is thought to have substantial beneficial physiological effects[308]. Branched-chain SFAs found in dairy, such as phytanic acid, have been indicated to increase hepatic fat oxidation[305]. Given the heterogeneity in the health effects of various SFAs, an emerging view is that all SFAs should not be categorized together, and the health effects of SFA-rich foods are likely dependent on the composition of their SFAs. Dairy fat also includes a diverse range of mono- and polyunsaturated fatty acids, such as oleic acid and the potentially hormonally active fatty acids alpha-linolenic acid and palmitoleic acid[303-305]. The latter has been suggested to reduce *de novo* lipogenesis and stimulate fat oxidation in the liver, thereby reducing hepatic triglyceride content[304,313]. Therefore, it may be that the beneficial health effects of the short-chain SFAs, medium-chain SFAs, branched-chain SFAs, monounsaturated fatty acids, or polyunsaturated fatty acids attenuate the deleterious serum lipid effects of the long-chain SFAs found in full-fat dairy foods. Secondly, consuming dairy fat not in isolation, but as part of complex whole foods such as milk, yogurt, and cheese, is likely to modify its fasting serum lipid profile-related effects[358]. Specifically, the calcium, phosphorus, milk fat globule membrane, and starter cultures (fermented dairy) found in dairy foods have been indicated to modify the blood lipid response through either decreasing intestinal fat absorption, altering the gut flora, or by altering hepatic expression of genes involved in fatty acid synthesis and cholesterol metabolism[358,359].

There are some limitations of our study that should be accounted for when interpreting the results. This study was conducted in individuals with MetS and included predominantly older Caucasian participants, impacting the generalizability of the results. Providing all dairy foods to the study participants allowed better control over encouraging and assessing compliance. However, the dairy foods provided do not necessarily mimic the dairy foods that would be selected by free-living individuals following the Dietary Guidelines for Americans' recommendation to include 3-servings of dairy per day. Our study also has many strengths,

including the fact that it largely addressed the limitations of previous clinical trials in this area by including a wide range of dairy foods, controlling for changes in weight or fat-mass statistically, and assessing intervention effects on a more comprehensive lipid panel.

In conclusion, our study indicates that consuming 3.3 servings of milk, yogurt, and cheese per day, regardless of fat content, does not affect the fasting serum lipid profile compared to a diet virtually free of dairy in men and women with MetS. A diet rich in low-fat dairy may also have a moderate lowering effect on systolic blood pressure compared to the limited dairy diet. Even though future research should investigate whether similar outcomes are seen in healthy populations, our finding that consuming complex whole full-fat dairy foods including milk, yogurt, and cheese has virtually no negative effect on classic CVD risk factors has important implications for public policy.

CHAPTER 4: OVERALL SUMMARY AND CONCLUSIONS

Dairy foods are one potential modifiable dietary factor that may impact weight and metabolic health. Most dietary guidelines, including the Dietary Guidelines for Americans 2015, recommend the consumption of dairy products as an important component of a healthy well balanced diet[325,326]. Typically, low-fat dairy consumption is recommended based on the view that dairy fat is a source of unnecessary calories and saturated fat, leading to weight gain and metabolic disease. However, it remains unclear whether these outcomes actually manifest in high dairy fat consumers. There is particularly compelling evidence from observational studies utilizing dairy fat biomarkers, suggesting that dairy fat consumption is not only not deleterious, but also may be health promoting. However, trials directly comparing the consumption of low-fat to full-fat dairy foods have generally found that there was no differential effect of full-fat dairy foods on risk factors for T2D and CVD. This discrepancy between the observational data and experimental data may be due to several limitations of current RCTs. This includes the fact that most studies did not include a diverse range of dairy foods and tested mostly unfermented dairy products, the participants were relatively healthy, most studies relied solely on a per-protocol analysis, and changes in weight or fat mass were not controlled for statistically. Further, with regards to glucose homeostasis related endpoints, most of the studies were not designed to test the impact of dairy foods on glucose homeostasis, with the majority of the studies relying on fasting measures of glucose and insulin. Because of these limitations, questions remain as to whether dairy consumption is beneficial for cardiometabolic health, and whether low-fat versus full-fat dairy consumption differ in this regard.

The DAIRY Study filled a critical gap in the literature by clarifying the relationship between dairy consumption and metabolic health using a rigorously designed randomized dietary intervention trial that addressed many of the limitations of the current literature. The DAIRY Study directly compared low-fat versus full-fat dairy, included fermented dairy products in the form of yogurt and cheese in addition to milk, and included a control arm limited in dairy. .Further, it was designed to specifically test the impact of dairy foods on glucose homeostasis by using a FS-OGTT, using individuals with MetS as the study population, having a clear *a priori* hierarchy of endpoints, reporting results from both a *per-protocol* analysis and an ITT analysis,

and controlling for changes in weight and fat mass statistically. Therefore, this study helps us to gain a clearer picture as to whether the beneficial associations observed between dairy consumption, including full-fat dairy products, and T2D and CVD may be causal.

The primary endpoint for this study was glucose tolerance as measured by area under the curve glucose. In contrast to our hypothesis, we found that dairy consumption did not improve glucose tolerance as compared to a diet virtually free of dairy. However, we found that insulin sensitivity as measured by the Matsuda insulin sensitivity index was reduced and insulin resistance as measured by HOMA-IR was increased in the dairy groups. Further, there was also a reduction in oral DI in the full-fat dairy group, which indicates that insulin secretion by pancreatic β-cells did not increase sufficiently to compensate for the reduction in insulin sensitivity. All glucose-homeostasis-related effects of dairy were independent of the fat content of the dairy foods consumed. It is plausible that over time this reduction in insulin sensitivity could result in impaired glucose tolerance and increase one's risk for T2D. However, the diets also had no impact on liver-fat content or systemic inflammation, and the effect on insulin sensitivity remained even after adjustment for changes in adiposity. Therefore, given that the change in insulin sensitivity was not explained by known key determinants of insulin sensitivity, there may be an alternative explanation for this outcome. It is possible that the increase in insulin resistance is a compensatory response to dairy induced hyperinsulinemia resulting from regular consumption of dairy foods. If this were the case, regular dairy consumption would not necessarily lead to an increased risk of T2D. Therefore, it remains unclear how dairy induced changes in insulin sensitivity would impact long-term risk of T2D. Another potential mechanism not explored in this study are alterations in the gut microbial composition, which is an emerging determinant of insulin sensitivity[342]. Overall, our results do not provide any evidence indicating that T2D risk is reduced through regular consumption of three servings of dairy per day in the form of milk yogurt and cheese in individuals with the metabolic syndrome.

We also investigated the impact of the diets on energy homeostasis. We found that there was a significant increase in weight and waist circumference on the dairy diets as compared to a diet limited in dairy intake. Further, full-fat dairy increased these measures more than those consuming the low-fat dairy diets. However, there was no impact of the diets on other adiposity

related outcomes including total fat mass, lean mass, visceral adiposity, hip circumference, waist to hip ratio, trunk fat, or peripheral fat. The increase in weight and waist circumference was associated with higher total energy intake, suggesting that participants did not reduce their *ad libitum* energy intake from non-dairy foods enough to fully compensate for the energy consumed in the form of mandatory dairy foods. We hypothesize that this is a result of the request to consume all administered dairy foods daily rather than an inherent obesogenic nature of dairy products, given that a large body of observational and experimental literature, as addressed in Chapter 1 Section 3.1, do not indicate that dairy consumption is associated with or causes increases in adiposity. However, further investigations are needed to clarify whether or not full-fat dairy consumption promotes adiposity.

Another area of interest was the impact of dairy consumption on classic CVD risk factors. We found that consuming 3.3 servings of full-fat dairy per day did not significantly affect any measure of the fasting plasma lipid profile compared to consuming identical amounts of nonfat dairy or compared to a diet strongly limiting any dairy intake. This included no significant difference in the cholesterol content of any of the 38 isolated plasma lipoprotein fractions, in spite of substantial differences between the three diets in the consumption of total fat and SFA. There was also no intervention effect on diastolic blood pressure. There was a significant difference among the diet interventions for systolic blood pressure, with a trend for a decrease in the low-fat dairy diet compared to the limited dairy diet in *post hoc* testing after adjustment for multiple testing. Therefore, contrary to the traditional view that full-fat dairy products rich in SFAs promote CVD, our study is in alignment with recent evidence indicating that dairy fat, when consumed as part of whole foods with a complex matrix, does not affect CVD risk.

This study has multiple strengths that increase our confidence in the results: it is the only study that directly compared a variety of low-fat vs. full-fat dairy foods; it was designed to specifically test the impact of dairy foods on glucose homeostasis; results from *per-protocol* and ITT analyses were consistent with one another; we controlled for changes in body weight and fat mass statistically; and compliance by study participants was excellent. Limitations of this study include a lack of generalizability of the results to populations other than those with the metabolic syndrome, and the duration of our dairy intervention, which, even at 12 weeks, may have been

insufficient to fully capture the long-term health implications of habitual dairy consumption. Future studies should investigate potential mechanisms by which dairy consumption may reduce insulin sensitivity and assess whether similar effects are seen in healthy populations.

Overall, we drew the following conclusions from the results of our study: Consuming three servings of dairy per day, regardless of fat content, in the form of milk, yogurt, and cheese 1) does not affect glucose tolerance, but modestly increases insulin resistance, 2) increases body weight and waist circumference, and 3) does not significantly affect classic CVD risk factors including the fasting lipid profile or blood pressure in individuals with the metabolic syndrome.

Our findings that neither the low-fat nor the full-fat dairy diet affected glucose tolerance and increased insulin resistance challenge the consistent and compelling evidence from observational studies that indicate that dairy is inversely associated with T2D as outlined in Chapter 1 Section 3.2. This is in alignment with previous trials that similarly concluded that dairy does not affect Glucose Tolerance[252,270,272,330] and a few trials that indicate that dairy increases insulin resistance[256-258]. However, it must be mentioned that the majority of previous RCTs found no impact of dairy consumption on insulin sensitivity. Given that our study addressed critical limitations in the existing experimental literature, it provides additional confidence that similar outcomes in previous trials were not due to the fact that that these trials included mostly low-fat unfermented dairy foods (i.e., nonfat milk), and mostly relied on fasting measures of glucose and insulin only.

The results of this study also challenge the suggestion that the consumption of full-fat dairy products increase the risk of CVD due to its high SFA content[347]. In alignment with recent evidence, our trial suggests that dairy fat, when consumed as part of foods with a complex matrix, does not negatively impact the fasting lipid profile or blood pressure. In this way, the results of this study are in alignment with the observational literature, which predominantly indicates that full-fat dairy foods are not associated with CVD, CHD, or stroke[206,207]. Further, our conclusions are in alignment with two previous RCTs[159,284]. The DAIRY Study addressed several of the limitations of the existing RCTs, providing additional assurance in our conclusion that consuming full-fat dairy products does not promote the development of CVD.

In conclusion, our study, alongside the current experimental data, does not indicate that dairy as a whole is health promoting. In fact, our findings suggest that lower dairy intake may be beneficial in individuals with the metabolic syndrome. Therefore, while dairy can be included as part of a healthy diet, the effects of dairy on cardiometabolic disease risk seen here and in previous studies do not lend support for concerted efforts that encourage the consumption of three servings of dairy per day. Further, our study does not support the view that low-fat dairy foods should be selected over full-fat dairy foods to promote cardiometabolic health. Rather, it indicates that low-fat and full-fat dairy foods do not differ with regards to their impact on classic risk factors for T2D and CVD. Therefore, consumers should select the fat content of their dairy products based on personal preference, as the evidence does not support the view that low-fat dairy foods are more health promoting than their full-fat counterparts or vice versa. In this way, the conclusions of this study have implications for public policy as the recommendations around dairy consumption are reviewed and revised.

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APPENDIX:

Table A1. Observational studies investigating the association between full-fat dairy consumption and measures of adiposity.

Study & Reference	Subjects	Exposure Assessment	Covariates	Outcome
Observational: Cross-Sectional Studies				
Barba et al.[128]	Country: Italy Age: 3-11 yr M and F N: 884	BL FFQ (1-year recall)	Age, sex, birth weight, parental overweight, PA, parental education, and intakes of fish, cereals, meat, F&V, SSBs, and snacks	Being overweight was (-) associated with the consumption of FF milk. When FF milk and skim milk consumption were pooled, the association was no longer statistically significant.
Beck et al.[137]	Country: US – Mexican Americans Age: 8-10 yr M and F N: 319	Adapted FFQ	Age and gender, PA, maternal BMI, maternal acculturation, maternal occupational status,	FF milk consumption was (-) associated with obesity, however this association was attenuated and did not meet statistical significance in the multivariate adjusted analysis. Skimmed-milk, 1% milk, and 2% milk were not associated with obesity.
Bergholdt et al.[154]	Country: Denmark Age: 20-100 yr M and F N: 97,811	Questionnaire	Sex, age, PA, smoking, alcohol intake, education, family history of diabetes, F&V intake, fish intake, intake of fast food, and intake of SSBs.	HF milk consumption (semi-skimmed to whole milk) was not associated with overweight/obesity. Fat-free milk consumption was (+) associated with risk of overweight-obesity.
Beydoun et al.[149]	Country: US Age: 18+ yr M and F N: 14,618	FFQ (24 h recall)	Age, sex, ethnicity, socioeconomic status, PA, and intakes of energy, F&V, grains, legumes, nuts/seeds, soy, meat/poultry/fish, eggs, added fats and oils, added sugars, alcohol, and caffeine	Dairy fat intake as a percentage of total fat was not associated with obesity or central obesity. FF milk intake was (-) associated with central obesity while LF milk was (+) associated with central obesity.
Crichton et al.[138]	Country: Luxemburg Age: 18-69 yr M and F N: 1,352	FFQ (3-month recall)	Age, sex, total energy intake, and education, and intake of remaining dairy products	Those with a BMI ≤ 25 ate significantly more FF dairy foods in the form of milk, yogurt, and cheese. LF dairy consumption was (+) associated with BMI.
Crichton et al.[139]	Country: Luxemburg Age: 18-69 yr M and F N: 1,352	FFQ (3-month recall)	Age, sex, education PA, smoking; total carbohydrate, protein, fat, and fiber intake; alcohol consumption, calcium intake, total energy intake, triglycerides, HDL cholesterol, LDL cholesterol, and systolic and diastolic BP	HF dairy intake was (-) associated with global and abdominal obesity. HF dairy consumption was (-) associated with BMI and WC. Consumption of LF dairy food was (+) associated with abdominal obesity, BMI, and WC.
da Silva et al.[152]	Country: Canada Age: Adult M and F N: 233	FFQ	Age and BMI	There was no association between HF dairy consumption and BMI or WC. Plasma fatty acid markers of dairy fat consumption were (-) correlated with BMI in M.
Murphy et al.[155]	Country: Australia Age: Adult M and F N: 720	FFQ	Age, gender, total energy intake, plus other dairy products (for individual dairy product analysis)	Dairy fat was (+) associated with BMI, %BF and abdominal fat. Dairy fat was also (+) associated with WC and hip circumference,

				but was only significant in the basic model. Dairy saturated fat was (+) associated with BMI and WC, but only in the basic model. LF milk intake was (-) associated with measures of adiposity.
Pranger et al.[146]	Country: Netherlands Age: 53.0 ± 15.5 M and F N: 864	Plasma triglyceride concentrations of C15:0, C17:0, and trans-C16:1(n-7)	Age and sex	C15:0 and C17:0 were (-) associated with weight, WC, and BMI. C16:1n-7 was not significantly associated with any measure of adiposity.
Rosell et al.[126]	Country: Sweden Age: 63 yr M N: 301	7-day food record; C15:0 and C17:0 in serum phospholipids and adipose tissue	PA, and intakes of alcohol and calcium	Dairy fat intake, C15:0, and C17:0 were (-) associated with abdominal obesity.
Smedman et al.[124]	Country: Sweden Age: 70 yr M N: 60	7-day dietary recall; plasma C15:0	PA, intakes of meat, beer, potatoes, vegetables, and root crops	Dairy fat intake, as assessed by dietary recall and C15:0, was (-) associated with BMI.
Snijder et al.[131]	Country: Netherlands Age: 50-75 yr M and F N: 2,064	FFQ	Age, sex, smoking, PA, education, antihypertensive medication use, and intakes of energy, fiber, and alcohol	WC was (-) associated with HF dairy intake; WC and BMI were (+) associated with LF dairy intake.
Vanderhout et al.[144]	Country: Canada Age: 12-72 month old M and F N: 2,745	Questionnaire	Age, sex, daily vitamin D supplementation, minutes of free play, screen time, milk volume consumed daily, volume of SSBs, maternal BMI, skin pigmentation, after-tax median neighborhood family income, maternal ethnicity, date of serum collection.	FF milk consumption was (-) associated BMI and had a reduced odds of being overweight or obese when compared to those drinking 1% milk.
Observational: Cohort or Case Control Studies				
Babio et al.[142] Prospective cohort	Country: Spain Age: 55-80 yr M and F N: 1,868	Annual FFQ	Sex, age, PA, BMI, smoking status, treatment for hypoglycemia, hyperlipidemia, hypertension, BL insulin, mean consumption during follow-up of V&F legumes, cereals, fish, red meat, cookies, olive oil, nuts, and alcohol, prevalence of MetS components at baseline	Consumption of FF yogurt was (-) associated with abdominal obesity. LF yogurt was not associated with abdominal obesity.
Berkey et al.[148]	Country: US Age: 9-14 yr M and F N: 12,829	BL FFQ (1-year recall)	Age, race, ethnicity, change in height during follow-up, BL BMI, menstrual history, and PA.	FF milk intake was not associated with BMI change; 1% and skim milk intake were (+) associated with BMI.
Bigornia et al.[151]	Country: England Age: 10-13 yr M and F N: 2,455	3-day dietary records at BL and 3-year follow up	Sex, dairy intake at follow-up, age at BL, height, adiposity, maternal education, maternal overweight status, PA, pubertal stage, dieting at follow-up, BL intake of cereal, total fat, total protein, fiber, 100%	Children in the highest quartile of FF dairy consumption versus those in the lowest quartile had a reduced risk of excess weight, a suggested reduction in prevalence of overweight, and smaller gains in BMI during

			fruit juice, F&V, and SSBs, and dietary reporting errors, dairy consumption at 3-year follow-up	follow-up. Associations between LF dairy consumption and adiposity related outcomes were not significant.
Duffey et al.[150]	Country: US Age: 18-30 yr M and F N: 2,774	BL and 7-year FFQ (one-month recall)	Age, sex, race, exam center, weight, smoking, PA, intakes of energy, fruit juice, SSBs, and alcohol	The incidence of elevated WC was not associated with FF or LF milk intake.
Holmberg and Thelin[136]	Country: Sweden Age: 39-62 yr M N: 1,322	15 item food questionnaire	Consumption of F&V smoking, alcohol consumption, PA, age, education, and profession	High intake of dairy fat in the form of milk, butter, and cream was (-) associated risk of central obesity.
Mozaffarian et al.[132]	Country: US Age: 65+ yr M and F N: 3,736	BL FFQ; plasma phospholipid trans palmitoleate	Age, sex, race, education, enrollment site, diabetes, CHD, smoking, PA, and intakes of alcohol, carbohydrates, protein, red meat, total fat, HF dairy, LF dairy, and energy	WC was (-) associated with trans palmitoleate. Prospective changes in WC were not reported.
Martinez-Gonzales et al.[140]	Country: Spain Age: M and F N: 8,516	FFQ (1-year recall)	Age, sex, BL weight, PA, hours of TV watching, hours spent sitting down, smoking status, marital status, years of university education, snacking between meals, following a special diet, total energy intake, and adherence to the Mediterranean diet.	Consumption of FF yogurt was (-) associated with a overweight/obese. No association was observed for LF yogurt consumption.
Noel et al[135]	Country: England Age: 10-13 yr M and F N: 2,245	3-day diet records at BL and end of study	Age, sex, height, pubertal status, maternal BMI, maternal education, and intakes of total fat, cereal, SSBs, fruit juice, calcium, and energy	At age 13, FF milk, but not LF milk consumption was (-) associated with %BF. In prospective analysis neither BL FF nor LF milk consumption assessed at age 10 was associated with %BF at age 13.
Pereira et al.[125]	Country: US Age: 18-30 yr M and F N: 3,157	BL FFQ (28 day recall)	Age, sex, race, BL BMI, energy intake, education, smoking, PA, supplement use, and intakes of alcohol, poly-unsaturated fat, caffeine, fiber, grains, meat, F&V, soda, magnesium, calcium, and vitamin D	HF dairy intake, but not LF dairy intake, was (-) associated with the risk of developing obesity among those overweight at BL.
Phillips et al.[147]	Country: US Age: 8-12 yr F N: 196	Annual FFQ (1-year recall)	Energy intake, parental overweight, and intakes of F&V, soda, and protein	Neither FF nor LF dairy consumption was associated with a change in BMI
Rajpathak et al.[129]	Country: US Age: 40-75 yr M N: 23,504	FFQ (1-year recall) at BL and every 4 years for 12 years	Age, BL weight, smoking, PA, glycemic load, and intakes of alcohol, calories, total fat, cereal fiber, whole grains, F&V, caffeine, *trans* fat, and soda.	BL HF dairy intake was (-) associated with weight gain, whereas LF dairy was not. An increase in HF dairy consumption over time was (+) associated with weight gain, while LF dairy was not.
Rautiainen et al.[143]	Country: US Age: ≥ 45 yr F N: 18,438	FFQ (1-year recall)	BL age, smoking status, PA, post-menopausal status, use of hormone replacement therapy, history of	Intake of HF dairy products was (-) associated with risk of becoming overweight or obese. Intake of HF dairy products was (-)

			hypertension, history of hypercholesterolemia, alcohol consumption, caloric intake, BL BMI.	associated with weight change during the follow-up period. No association was observed for LF dairy product consumption and changes in weight or incidence of overweight or obese.
Rosell et al.[130]	Country: Sweden Age: 40-55 yr W N: 19,352	BL and 10-year FFQ (6-month recall)	Age, BL height and weight, education, parity, BL intakes of energy fat, carbohydrate, protein, fiber, and alcohol, and change in intakes of energy, fat, carbohydrate, protein, fiber, and alcohol.	BL intake of FF milk and butter were (-) associated with BL BMI, while LF milk was not. Higher intake of FF milk was (-) associated with weight gain, while LF milk was not.
Santiago et al.[141]	Country: Finland Age: 55-80 yr M and F N: 4,545	Questionnaire (1 year recall)	Age, sex, PA, total energy intake, adherence to the Mediterranean diet pattern, smoking status, BL BMI, intervention group, and center.	FF yogurt consumption was (-) associated with WC and a higher probability for the reversal of abdominal obesity. There was no association between LF yogurt consumption and these parameters.
Te Velde et al.[134]	Country: Netherlands Age: 13-36 yr M and F N: 374	8 dietary history interviews over 23 years (1-month recall)	PA, smoking, and energy intake	Participants who were not overweight at 36 years of age had consume more FF dairy and less LF dairy 15 years earlier than participants who were overweight at 36
Wang et al.[153]	Country: US Age: 28-84 yr M and F N: 3,440	FFQ (1-year recall) at BL and 3 additional time points over 13 years	Weight or WC, age, smoking status, PA, BP, diabetic status, cholesterol-lowering medication use, levels of blood lipids at the beginning of each exam interval, average total energy intake within each exam interval, and sex	There was no association between HF dairy product consumption and weight gain over time. However, there was a trend to gain less WC for those who consumed ≥3 servings of HF dairy products per week compared to those who ate < 3 servings.
Warensjo et al.[127]	Country: Sweden Age: Adult M and F N: 234	BL C15:0 and C17:0 in serum lipids	None	The sum of C15:0 and C17:0 was (-) associated with BMI at BL. Prospective changes in BMI were not investigated.
Warensjo et al.[133]	Country: Sweden Age: 30-60 yr M and F N: 1,000	BL FFQ; C15:0 and C17:0 in serum phospholipids	None	C15:0 and C17:0 were (-) associated with BMI at BL.
Yakoob et al.[145] Prospective cohort	Country: US Age: 44-83 yr M and F N: 3,333	Plasma and erythrocyte concentrations of C15:0, C17:0, and t-16:1n-7	F&V, fish, meat, whole grains, SSBs, polyunsaturated fat, calcium, and glycemic load, biomarker levels of trans-18:1, and trans-18:2, palmitic acid, and stearic acid.	C15:0 was not associated with BMI, C17:0 and t-16:1n-7 were (-) associated with BMI.

Abbreviations:
%BF = percent body fat
BL = baseline
BMI = body mass index
BP = blood pressure
CHD = coronary heart disease
HDL = high density lipoprotein
HF= high-fat
LF= low-fat
LDL= low density lipoprotein
M= male
MetS = metabolic syndrome,
T2D = type 2 diabetes mellitus
US = United States
vs= versus
WC = waist circumference
WHR = waist to height ratio
yr= year

F= female FF= full-fat F&V= fruit and vegetable	PA = physical activity SSBs= sugar sweetened beverages	(-) = inversely (+) = positively

Table A2. Observational studies investigating the impact of full-fat dairy food consumption and incident T2D.

Study & Reference	Subjects	Assessment method	Covariates	Outcome
Observational: Cross-sectional				
Bergholdt et al.[154]	Country: Netherlands Age: 20-100 yr M and F N: 97,811	Questionnaire	Sex, age, PA, smoking, alcohol intake, education, family history of diabetes, F&V intake, fish intake, intake of fast food, and intake of SSBs.	Consumption of HF milk was not associated with T2D. However, consumption of LF milk was (+) associated with T2D.
Castro-Webb et al.[177]	Country: Costa Rica Age: M and F N: 1,744	cis-9, trans 11 CLA in adipose tissue	Age, sex, area of residence, PA, BMI, WC, income, current smoking status; and quintiles of fatty acid concentration in adipose tissue of trans-9 16:1 total trans, even-numbered saturated, odd-number saturated, n-3 PUFA, n-6 PUFA, and monounsaturated fats.	Higher amounts of cis-9 trans 11 CLA in adipose tissue was (-) associated with prevalence of T2D.
Eussen et al.[189]	Country: Netherlands Age: 40-75 yr M and F N: 2,391	FFQ (1 year recall)	Sex, age, education, BMI, PA, smoking, and intakes of energy, alcohol, F&V, meat and fish.	Consumption of FF dairy products was (+) associated with risk for T2D. LF dairy consumption was not associated with T2D.
Zong et al.[186]	Country: China Age: 50-70 yr M and F N: 2,091	Erythrocyte Trans-18:1 isomers	Age, sex, region, residence, smoking status, BMI, family history of diabetes, dietary fiber intake, trans-18:2n-6 9c12t and trans-18:2n-6 9t12c, and total dairy	There was an (-) association between erythrocyte Trans-18:1 isomers and T2D, though this association became insignificant after adjusting for total dairy intake.
Observational: Cohort and Case-Control				
Brouwer-Brolsma et al.[188]	Country: Netherlands Age: 55+ yr M and F N: 2,974	FFQ	Age, sex, family history of diabetes, following a diet regimen at the time of dietary assessment, alcohol consumption, smoking, education, PA, total energy intake, energy adjusted meat intake, energy-adjusted fish intake, and BMI.	There was no association between FF dairy or LF dairy consumption and T2D.
Chen et al.[184]	Country: US Age: 25-75 yr M and F N: 194,458	FFQ (1 year recall) at BL and biennially	Age, BMI, total energy intake, race, smoking, PA, alcohol consumption, menopausal status and menopausal hormone use, oral contraceptive use, family history of diabetes, diagnosed hypertension or hypercholesterolemia, trans-fat, glycemic load, and intakes of red and processed meat, nut, SSBs, and	Neither HF dairy nor LF dairy was associated with risk of T2D. In the analysis of individual types of dairy products, both skim milk and FF milk were (+) associated with T2D. Ice cream consumption was (-) associated with diabetes risk.

			coffee.	
Choi et al.[170]	Country: US Age: 40-75 yr M N: 41,254	BL FFQ (1-year recall)	Age, BMI, follow-up time, diabetes, smoking, hypercholesterolemia, hypertension, PA glycemic load, and intakes of energy, alcohol, cereal, fiber, trans fats, and polyunsaturated:saturated fat	Neither HF dairy nor FF milk intake was associated with diabetes incidence. LF dairy and skim/low-fat milk intake were (-) associated with diabetes.
Diaz-lopez et al.[187]	Country: Finland Age: 55-80 yr M and F N: 3,454	FFQ at BL and annually for approximately 4 years	Age, sex, BMI, intervention group, smoking status, PA, education level, hypertension or antihypertensive drug use, fasting glucose, HDL-cholesterol, triglycerides, and average consumption of F&V, legumes, meat, fish, olive oil, nuts, and alcohol.	Dairy fat consumption was not associated with T2D. LF dairy consumption was (-) associated with T2D.
Ericson et al.[197]	Country: Sweden Age: 50+ yr M and F N: 26,930	7-day diet record and recall at baseline	Age, sex, season, total energy intake, leisure-time physical activity, smoking, alcohol intake, education and BMI	Total high-fat dairy products, high-fat non-fermented dairy products, and high-fat fermented dairy products were all (-) associated with T2D when comparing the highest quintile to the lowest. Total low-fat and non-fermented low-fat dairy were (-) associated with T2D, but this association as not seen for low-fat fermented dairy foods when comparing the highest quintile to the lowest.
Forouhi et al.[183]	Country: Europe Age: M and F N: 27,296	BL plasma fatty acid profile concentrations of C15:0 and C17:0	Age, study center, sex, PA index, smoking status, education level, total energy intake, alcohol intake, and BMI.	Pentadecanoic acid and heptadecanoic acid concentrations at BL were associated with a reduced risk of incident T2D.
Grantham et al.[178]	Country: Australia Age: 25+ yr M and F N: 5,582	FFQ (1 year recall)	Age, sex, energy intake, family history of diabetes, education level, PA, smoking status, TAG, HDL cholesterol, systolic BP, WC, and hip circumference	HF milk intake was not associated with diabetes incidence. LF milk intake was (-) associated with risk of T2D.
Harris et al.[191]	Country: USA Age: 65- 80 yr F N: 6,379	Red Blood Cell trans-16:1	Age, race, WC, highest education, current smoking status, PA, weekly alcohol intake, glycemic load, and family history of diabetes	There was no association between trans-16:1 and incident T2D.
Hodge et al.[172]	Country: Australia Age: 36-72 yr M and F N: 3,737	BL FFQ and plasma phospholipid C15:0	Age, sex, BMI, WHR, country of birth, diabetes, PA, and alcohol intake	Plasma C15:0 was (-) associated with T2D. Dietary C15:0 was not associated with T2D across quintiles after adjustment.
Krachler et al.[173]	Country: Sweden Age: Middle-aged M and F N: 450	Erythrocyte membrane C15:0 and C17:0	BMI, HbA1c, and alcohol intake	C15:0 and C17:0 were (-) associated with T2D.

Kroger et al.[194]	Country: Germany Age: 35-65 yr M and W N: 2,724	Erythrocyte C15:0, C17:0 and trans-16:1n7	Age, sex, BMI, waist circumference, PA, education, smoking status, alcohol intake, occupational activity, coffee intake (energy adjusted), and fiber intake (energy adjusted).	Erythrocyte C15:0 and C17:0 were not associated with incident T2D, however trans-16:1n-7 was (+) associated with T2D.
Lankinen et al.[190]	Country: Finland Age: 55 ± 5.6 yr M N: 831	Plasma fatty acid C15:0, C17:0 and 16:1n-7	Age, BMI, smoking, and physical activity, fasting glucose at BL	C15:0, C17:0 and 16:1n-7 were not associated with incident T2D.
Liu et al.[171]	Country: US Age: Middle-aged F N: 37,183	BL FFQ (1-year recall)	Age, diabetes, smoking, BMI, hypercholesterolemia, hypertension, PA, hormone use, glycemic load, and intakes of energy, alcohol, fiber, total fat, calcium, vitamin D, and magnesium	HF dairy intake was not associated with T2D incidence. LF dairy intake was (-) associated with T2D.
Louie et al.[179]	Country: Australia Age: 49+ yr M and F N: 1,824	FFQ (1-year recall) at BL, 5 years, and 10 years	Age, sex, smoking status, PA, dietary glycemic load, fiber from vegetables, total energy intake, family history of T2D.	Neither HF dairy nor LF dairy was associated with risk of incident T2D.
Malik et al.[174]	Country: US Age: 34-53 yr F N: 37,083	Retrospective and BL FFQ; additional FFQ every 4 years	Age, BL BMI, PA, smoking, family history of diabetes, hormone use. Polyunsaturated:saturated fat, glycemic load, and intakes of BL energy intake, SSBs, alcohol, coffee, processed meat, trans-fat, and cereal fiber	Adolescent intake of HF, but not LF dairy was (-) associated with T2D incidence. This association became non-significant after adjustment for adult dairy intake and weight change. Adult intake of both HF and LF dairy was (-) associated with T2D.
Margolis et al.[175]	Country: US Age: 50-79 yr F N: 82,076	BL FFQ (3-month recall)	Age, race, ethnicity, income, education, smoking, family history of diabetes, hormone use, BP, BMI, PA, and intakes of energy, and alcohol	HF dairy intake was not associated with T2D incidence. LF dairy intake was (-) associated with T2D incidence.
Montonen et al.[198]	Country: Finland Age: 40-69 yr M a W N: 4,304	Diet interview at baseline	Age, sex, BMI, energy intake, smoking, family history of diabetes, and geographic area	Neither LF nor FF dairy products were not associated with T2D when comparing the highest quartile of dairy consumption to the lowest.
Mozaffarian[132]	Country: US Age: 65+ yr M and F N: 3,736	BL FFA; plasma phospholipid trans palmitoleic acid	Age, sex, race, education, enrollment site, smoking, BMI, WC, CHD, PA, and intakes of energy, alcohol, carbohydrates, protein, red meat, FF dairy, and LF dairy	Plasma phospholipid, trans palmitoleic acid, and FF dairy intake were (-) associated with T2D incidence
Mozaffarian et al.[180]	Country: US Age: 45-84 yr M and F N: 2,281	Fasting blood concentrations trans-16:1n-7	Age, sex, race-ethnicity, field center, education, smoking, alcohol, PA, BMI, and WC	Trans-16:1n7 were (-) associated with incident T2D.

O'Connor et al. [182]	Country: United Kingdome Age: 40-79 yr M and F N: 4,000	7 day dietary record at BL	Age, sex, BMI, family history of diabetes, smoking status, alcohol consumption, PA, social class, education level, total energy intake and intakes of fiber, F&V, red meat, processed meat, and coffee.	Neither HF dairy nor LF dairy was associated with risk of T2D.
Patel et al.[193]	Country: England Age: 40-79 yr M and W N: 199 cases, 184 controls	Plasma and Erythrocyte C15:0, C17:0 and trans-16:1n7	Age, sex, family history of diabetes, BMI, smoking status, PA, and alcohol intake	Plasma C15:0 and erythrocyte C15:0, C17:0 and trans-16:1n7 were not significantly associated with T2D. Plasma C17:0 and trans-16:1n7 were (-) associated with T2D.
Soedamah-Muthu et al.[176]	Country: England Age: 35-55 yr M and F N: 4,186	BL FFQ	Age, ethnicity, employment grade, smoking, BMI, PA, family history, and intakes of alcohol, F&V, bread, meat, fish, coffee, tea, and total energy	Neither HF nor LF dairy intake was significantly associated with incident T2D during 10 years of follow-up.
Santaren et al.[185]	Country: United States Age: 40-60 yr US M and F N: 659	FFQ (1 year recall)	Age, sex, ethnicity, center, PA, smoking status, alcohol intake, and education as well as total energy, F&V, red meat, soft drink, and fiber intakes	15:0 was associated with a 27% reduced risk for incident T2D. This remained significant even after further adjusting for BMI and WC. There was no association between trans-16:1n-7 and incident T2D. Though it should be noted that this biomarker was not associated with dairy consumption in this cohort.
Struijk et al.[181]	Country: Netherlands Age: 30-60 yr M and F N: 5,232	FFQ (1 month recall) at BL and 5 years	Age, gender, intervention group, education level, diabetes, family history, PA, smoking, alcohol, baseline intake of wholegrain cereal, meat, fish, coffee, tea, F&V, total energy intake, change in dietary quality score, WC, and BMI	Neither HF dairy nor LF dairy was associated with risk of T2D.
van Dam et al.[199]	Country: United States Age: W N: 41,186	68-item FFQ	Age, total energy intake, BMI, smoking status, PA, alcohol consumption, parental history of diabetes, education, coffee consumption, sugar-sweetened soft drink consumption, intake of processed meat and red meat.	HF dairy was not associated with T2D, but LF dairy was (-) associated with T2D when comparing those consuming at least one serving per day to those consuming less than one serving per week.
Wang et al.[192]	Country: USA Age: 45-64 yr M and W N: 2,909	Plasma cholesterol esters 16:1n7	Age, sex, baseline BMI, WHR, alcohol intake, cigarette-ears of smoking, PA, education, and parental history.	Those in the highest quintile of 16:1n-7 plasma cholesterol ester and phospholipid concentrations were at a significantly increased risk of incident T2D.

Wang et al.[196]	Country: United States Age: 74 ± 5 M and W N: 2,919	Plasma trans16:1n-7	Age, sex, race, education, enrollment site, smoking status, alcohol consumption, PA, BMI, WC, prevalence of CVD and hypertension at baseline.	Plasma trans-16:1n-7 was (-) associated with T2D.
Yakoob et al.[145]	Country: US Age: 44-83 yr M and F N: 3,333	Plasma and erythrocyte concentrations of C15:0, C17:0, and t-16:1n-7	F&V, fish, meat, whole grains, SSBs, polyunsaturated fat, calcium, and glycemic load, biomarker levels of trans-18:1, and trans-18:2, plamitic acid, and stearic acid.	Plasma C15:0, C17:0 and t-16:1n-7 were all (-) associated with T2D risk. Similar results were observed for the association between erythrocyte concentrations of these fatty acids and T2D risk.
Zong et al.[195]	Country: China Age: 50-70 yr M and W N: 2,066	Erythrocyte 16:1n-7	Age, sex, region, residence, PA, educational attainment, current smoking, current drinking, family history of diabetes, total energy intake, percentage of energy intake from carbohydrate, and energy-adjusted dietary glycemic index, and BMI	Erythrocyte 16:1n-7 was not associated with T2D after adjustment.

Abbreviations:

%BF = percent body
BL = baseline
BMI= body mass index
BP = blood pressure
CHD = coronary heart disease
CVD = cardiovascular disease
F= female
FF= full-fat
F&V= fruit and vegetable
HF= high-fat
HDL = high density lipoprotein
LF= low-fat,
M= male, fat
MetS = metabolic syndrome
overweight = overweight
PA = physical activity
SSBs= sugar sweetened beverages
T2D = type 2 diabetes mellitus
US = United States
vs= versus,
WC = waist circumference
WHR = waist to height ratio
yr= year
(+) = positively
(-) = inversely

Table A3. Observational studies investigating the relationship between dairy fat biomarkers C15:0, C17:0, and C16:1n-7 and incident type 2 diabetes.

Reference	Lipid compartment	Sample Size	Multivariable Adjusted Hazard Ratio, OR, or RR (95% CI)		
			C15:0	C17:0	16:1n-7
Castro-Webb et al.[177]	Adipose tissue	n= 1,744 (232 cases)			0.42 (0.26-0.68)
Forouhi et al.[183]	Plasma	n=27,296 (12,132 cases)	0.80 (0.74-0.86)	0.67 (0.62-0.72)	
Harris et al.[191]	Red Blood Cells	N=6,379 (703 cases)			0.95 (0.87-1.04)
Hodge. et al.[172]	Plasma	n=3,737 (364 cases)	0.40 (0.26-0.63)		3.55 (2.27-5.54)
Krachler et al.[173]	Erythrocytes	n=450 (159 cases)	0.71 (0.52-0.97)	0.54 (0.35-0.83)	1.32 (0.91-1.89)
Kroger et al.[194]	Erythrocyte	N=2,724 (673 cases)	0.79 (0.54-1.16)	0.74 (0.52-1.06)	2.11 (1.46-3.05)
Lankinen et al.[190]	Plasma	N= 831 (71 cases)	0.85 (0.66-1.09)	0.83 (0.65-1.06)	1.08 (0.87-1.33)
Mozaffarian et al.[200]	Plasma	n=3,736 (304 cases)			0.38 (.24-0.62)
Mozaffarian et al.[132]	Plasma	n=2281 (205 cases)- MESA n=5266 (509 cases) – MESA + CHS			0.52 (0.32,0.85) –MESA Only 0.38 (0.24-0.62) – MESA +CHS
Patel et al.[193]	Plasma and Erythrocyte	N=199 cases and 184 controls	1.20 (0.66-2.16) Plasma 0.90 (0.50-1.62) Erythrocyte	0.43 (0.24-0.79) Plasma (0.39-1.24) Erythrocyte	0.40 (0.22-0.72) Plasma 0.55 (0.31-0.99) Erythrocyte
Wang et al.[192]	Plasma	N=2,909 (252 cases)			1.86 (1.15-3.01)
Wang et al.[196]	Plasma	N=2,919 (287 cases)			0.63 (0.44-0.91)
Yakoob et al.[145]	Plasma	N=3333 (278 cases)	0.64 (0.47-0.89)	0.73 (0.55-0.99)	0.54(0.41-0.73)
Zong et al.[186]	Serum	N=659 (103 cases)	0.76 (0.58-0.99)		NS- data not provided
Zong et al.[195]	Erythrocyte	N=2,066 (cases not specified)			1.23 (1.00-1.53)

Table A4. Observational studies investigating the association between full-fat dairy consumption and incident CVD, CHD, and stroke.

Study & Reference	Subjects	Assessment Methods	Covariates	Outcome
Observational: Cross-Sectional				
Crichton et al.[139]	Country: Luxembourg Age: 18-69 yr M and F N: 1,432	FFQ	Age, education, sex, total energy intake, intake of remaining dairy products, LF dairy food intake (for FF analysis), FF dairy food intake (for LF dairy analysis)	High intakes of FF dairy were (+) associated with cardiovascular health. LF dairy intake was not associated with cardiovascular health.
Observational: Cohort and Case-Control				
Aslibekyan et al.[229]	Country: Costa Rica Age: Adult M and F N: 3,630	C15:0 and C:17:0 in adipose tissue at follow-up	Age, sex, area of residence, income, smoking, total energy intake, PA, WHR, alcohol intake, self-reported history of hypertension, T2D, and hypercholesterolemia, adipose tissue CLA and trans fatty acids, intake of calcium, and saturated fats	Neither C:15:0 nor C17:0 in adipose tissue was associated with risk of non-fatal MI.
Avalos et al.[228]	Country: US Age: 50-93 yr M and F N: 1,759	FFQ BL	Age, BMI, diabetes, hypertension, LDL-cholesterol and current oestrogen use in F.	There was no association between FF milk, cream, butter, ice-cream, or cheese and CHD morbidity and mortality. There was an (+) association with risk of CHD morbidity and mortality in F who consumed LF cheese and non-fat milk.
Biong et al.[209]	Country: Norway Age: 45-75 yr M and F N: 197	C14:0, C14:1, C15:0, C17:0, and C17:1 in adipose tissue	Age, sex, WHR, smoking, family history of CHD, and education	Adipose tissue C14:1, C15:0, and C17:1 were each (-) associated with risk of a first MI
Bonthuis et al.[217]	Country: Australia Age: 25-78 yr M and F N: 1,529	Repeated FFQ	Age, energy intake, BMI, school education, PA, smoking, supplement use, beta-carotene treatment, medication use, and intakes of alcohol and calcium	Dairy intake was not assessed with all-cause mortality. FF dairy intake was (-) associated with CVD mortality.
Chen et al.[184,231] Prospective cohort	Country: US Age: 25-75 yr M and F N: 241,601	FFQ biennially	Age, calendar time, BMI, total energy intake, ethnicity, smoking, PA, alcohol consumption, menopausal status, menopausal hormone use, BL diagnosed hypertension and hypercholesterolemia, dietary intakes of F&V, coffee, protein, vegetable fat, and other animal fat.	Dairy fat consumption was not associated with CVD, CHD, or stroke
Dalmeijer et al.[220]	Country: Netherlands Age: 21-64 yr M and F N: 33,625	BLFFQ	Gender, age, PA, smoking, education, BMI, intakes of energy, alcohol, coffee, F&V, fish, meat and bread	Neither HF nor LF dairy intake was significantly associated with incident CHD or stroke during 13 years follow-up.
Dehghan et al.[240]	Country: 21 countries (PURE cohort) Age: 50.1 (9.9) N: 136,384	FFQ	Age, sex, education, urban or rural location, smoking status, PA, history of T2D, family history of CVD, family history of cancer (All); Quintiles of F&V, red meat, starchy foods, total energy intake (analysis of dairy); Energy, protein intake (analysis of saturated	1-2 servings per day and >2 servings per day of FF dairy was (-) associated with major CVD as compared to <0.5 servings of FF dairy per day. Increasing saturated fat intake from dairy sources was not associated with major CVD.

			fat).	
de Oliveira Otto et al.[226]	Country: US Age: 45-84 yr M and F N: 5,209	FFQ BL	Age, sex, race-ethnicity, energy intake, field center, education level, active leisure, sedentary leisure, alcohol intake, smoking, BMI, dietary supplement use, cholesterol lowering medication, intakes of F&V, energy-adjusted intakes of dietary fiber, dietary vitamin E, and PUFA.	Dairy saturated fatty acid intake was () associated with CVD risk. However, there was no association with between saturated fatty acid intake from butter and risk of CVD.
de Oliveira Otto et al.[227] Prospective cohort	Country: US Age: 45-84 yr M and F N: 2,837	BLC15:0, C14:0, and trans-16:1n-17 in plasma	Age, sex, race/ethnicity, field center, education, cigarette smoking, alcohol, PA, FF dairy, processed and unprocessed meat, totally energy intake, F&V, BMI, diabetes, hypertensive medication use, lipid-lowering medication use, LDL cholesterol	Concentrations of C15:0 in plasma was (-) associated with CVD and CHD whereas C14:0 and trans-16:1n-17 had no association.
De Oliveira[234]	Country: US Age: 74.8 ± 5.2 N: 2,907	Plasma concentrations of C15:0, C17:0, trans 16:1n-7	Age, sex, race, education, enrollment site, smoking status, T2D, hypertension, atrial fibrillation, PA, BMI, WC, and alcohol intake.	C17:0 was (-) associated with CVD mortality, CHD, and stroke. C15:0 and 16:1n-7 were not associated with CVD, CHD or stroke. None of the biomarkers were associated with CVD events.
Ding et al.[236]	Country: US Age: Adult M and F N: 168,153 F and 49,602 M	116-item FFQ 1984-1985 131-item FFA 1986-on completed every 4 years	Family history of CVD and cancer, PA, HEI 2010, total energy intake, smoking status, alcohol consumption, menopausal status, and postmenopausal hormone use, BMI, hypertension, hypercholesterolemia.	Skimmed or low-fat milk was not associated with cardiovascular mortality. FF milk was (+) associated with cardiovascular mortality.
Goldbohm et al.[219]	Country: England Age: 55-69 yr M and F N: 120,852	BL FFQ	Age, education, smoking, PA, BMI, multivitamin use, and intakes of alcohol, energy, mono- and polyunsaturated fatty acids, F&V	Dairy fat intake was (-) associated with the risk of death from ischemic heart disease in M, but (+) in F. Dairy fat intake was not associated with the risk of death due to stroke in M or F.
Haring et al.[242] Prospective cohort	Country: US Age: 45-64 yr old N: 11,601	66-item FFQ at baseline and follow-up	Age, sex, race, study center, total energy intake, smoking, cigarette years, education, systolic blood pressure, use of antihypertensive medication, HDL cholesterol, total cholesterol, use of lipid lowering medication, BMI, WHR, alcohol intake, sports-related PA, leisure-related PA, carbohydrate intake, fiber intake, fat intake, and magnesium intake	Neither LF nor HF dairy was not associated with incident of stroke
Hu et al.[213]	Country: US Age: Adult US W N: 80,082	Repeated FFQ	Age, BMI, smoking, menopausal status, hormone use, family history, vitamin E supplement use, hypertension aspirin use, vigorous exercise and intakes of energy and alcohol	Neither HF dairy intake nor LF dairy products was associated with CHD risk.
Khaw et al.[225]	Country: England Age: 40-49 yr M and F N: 7,301	BL C15:0 and C17:0 in plasma	Age, sex, BMI, PA, smoking, alcohol intake, social class, education, plasma vitamin C, personal history of T2D, systolic BP, and blood cholesterol.	Odd chain fatty acid levels were (-) associated with incidence of CHD.

Larson et al.[216]	Country: Finland Age: 50-69 yr smokers N: 26,556	FFQ	Age, supplementation group, education, smoking, BMI, serum cholesterol, T2D, CHD, PA, intakes of total energy, caffeine, sugar, red meat, poultry, fish, F&V, fruit juices, potatoes, whole grains, and refined grains	FF milk intake, but not LF milk intake, was (+) associated with risk for cerebral infarction and intracerebral hemorrhage. However, cheese and cream were (-) associated with cerebral infarction, while butter intake did not show an association with any type of stroke.
Larsson et al.[224]	Country: Sweden Age: 45-83 yr M and F N: 74,961	FFQ BL and follow-up	Age, sex, smoking, education, BMI, PA, aspirin use, history of hypertension, history of T2D, family history of MI, and quintiles of alcohol, coffee, fresh red meat, processed meat, fish, F&V.	FF dairy consumption was not associated with risk of total stroke, cerebral infarction, or hemorrhagic stroke. LF dairy consumption was (-) associated with total stroke and cerebral infarction.
Laursen et al.[235]	Country: Denmark Age: 50-64 yr M and W N: 55,211	192-item FFQ	Total energy intake, age, BMI, WC, education, smoking status, PA, alcohol intake, F&V intake, red meat intake, processed red meat intake, fish intake, hypertension, hypercholesterolemia, T2D, and MI.	Substituting LF milk for FF milk was not associated with risk of stroke. Substituting semi-skimmed fermented milk for FF fermented milk or FF milk was not associated with risk of stroke or ischemic stroke. Substituting FF fermented milk for LF milk was not associated with risk for stroke or ischemic stroke.
Laursen et al.[238]	Country: Denmark Age: 51.5 (31.1-63.4) N: 36,886	79-item FFQ	Intake of individual subgroups of dairy other than the product being substituted, total servings of dairy, and total energy intake. Education, BMI-adjusted WC, smoking, PA, alcohol intake, Hypertension, hypercholesterolemia, T2D and MI at BL	Substituting one serving of LF yogurt for FF yogurt was not associated with ischemic stroke. Substituting LF yogurt of whole-fat yogurt was not associated with risk of hemorrhagic stroke. Substituting LF milk for FF milk was not associated with a reduced risk of ischemic stroke or hemorrhagic stroke.
Locheart et al.[215]	Country: Norway Age: 45-75 yr M and F N: 211	FFQ	Age, marital status, education, family history, smoking, and energy intake	No association between the risk of MI and the following food groups: cheese and yogurt, LF dairy, and HF milk
Louie et al.[223]	Country: Australia Age: 49+ yr M and F N: 2,900	FFQ BL and follow-up	Age, sex, BMI, change in weight, previous acute MI, previous stroke, smoking status, stage II hypertension at BL, T2D at baseline, use of hypertensive medications and statins, change in dairy intake	Neither FF nor LF dairy consumption was associated with CVD mortality, stroke mortality, or CHD mortality.
Matthan et al.[244] Nested Case Control	Country: US Age: postmenopausal W N: 2,448	Plasma fatty acid 15:0 and 16:1n-7	Age, enrollment date, race/ethnicity, hysterectomy status, BMI, systolic BP, smoking, education, medication use, family history of CVD /stroke/MI and T2D, and PA, intake of carbohydrate protein add alcohol intake as a percent of energy.	Neither 16:1n-7 nor 15:0 was associated with CHD risk.

Ness et al.[214]	Country: Scotland Age: 35-64 yr M N: 5,765	BL FFQ and a second FFQ in approximately half the study population	Age, smoking, BP, serum cholesterol, BMI, social class, education, forced expiratory volume, angina, ischemia, bronchitis, alcohol consumption and social factors	The consumption of milk was (-) associated with death from CVD (and all causes). Although the fat content of the milk consumed was not assessed, the authors note that during the study period (1970-1995), the majority of milk consumed in Scotland was FF milk)
Pala et al.[237]	Country: Italy Age: 50.1 ± 0.037 N: 45,009	FFQ	Energy intake, body weight, height, and WHR, alcohol consumption, smoking, PA socioeconomic status, intakes of dietary fiber, Italian Mediterranean index.	Consumption of FF milk was not associated with cardiovascular mortality. Consumption of reduced-fat milk was (-) associated with cardiovascular mortality.
Patterson et al.[221]	Country: Sweden Age: 48-83 yr F N: 33,636	BL FFQ (1-year recall)	Smoking status, PA, WHR, alcohol consumption, diagnosis of hypertension, diagnosis of high cholesterol, family history of early MI, postsecondary education, use of aspirin, use of postmenopausal hormone therapy, energy intake, intakes of F&V, and whole grains as well as intakes of LF dairy foods were mutually adjusted for the corresponding FF intake and vice versa	Neither FF nor LF milk, yogurt, or cheese was associated with incidence of MI. There was a trend for a decreased risk of MI with FF cheese consumption, though this trend became non-significant after adjustment for calcium.
Praagman et al.[230] Prospective cohort	Country: Netherlands Age: 55+ yr M and F N: 4,235	BL FFQ	Age, sex, total energy intake, BMI, smoking, educational level, alcohol use, intakes of F&V, meat, fish, bread, coffee, and tea	HF dairy intake was (+) associated with fatal stroke, but not incident stroke. LF dairy intake was no associated with either incident or fatal stroke. Neither HF dairy nor LF dairy was associated with CHD incidence or mortality.
Smit et al.[212]	Country: Costa Rica Age: Adult M and F N: 3,626	9-cis, 11-trans conjugated linoleic acid in adipose tissue	Age, sex, area of residence, PA, income, smoking, WHR, family history, adipose tissue content of alpha-linoleic acid and trans fatty acids, and intakes of alcohol and saturated fatty acids	Adipose tissue content of 9-cis, 11-trans conjugated linoleic acid was (-) associated with risk of MI
Soedamah-Muthu et al.[176]	Country: England Age: 35-55 yr M and F N: 4,255	BL FFQ	Age, ethnicity, employment grade, smoking, BMI, PA, family history, and intakes of alcohol, F&V, bread, meat, fish, coffee, tea, and total energy	Neither HF nor LF dairy intake was significantly associated with incident CHD.
Sonestedt et al.[218]	Country: Sweden Age: 44-74 yr M and F N: 26,445	BL diet history questionnaire	Age, sex, season, BMI, smoking, PA, education, and intakes of energy, alcohol, V&F, berries, fish, shellfish, meat, coffee, and whole grains	Neither LF or HF milk, nor cheese, butter, nor cream consumption was associated with CVD. Intake of fermented milk products was (-) associated with CVD.
Sun et al.[210]	Country: US Age: Adult N: 493	C15:0, trans C16:1, and C17:0 in plasma and erythrocytes	Age, smoking, fasting status, BMI, postmenopausal status hormone use, PA, aspirin intake, family history, hypertension, hypercholesterolemia, T2D, plasma/erythrocyte C18:2 and trans fatty acid content, and alcohol intake	Plasma C15:0 was (+) associated with the risk of ischemic heart disease. None of the other fatty acids were significantly associated with ischemic heart disease.

Talaei et al.[233]	Country: Iran Age: 18+ yr M and F N=5,432	48-item FFQ	Age, sex, educational level, BMI, PA< smoking status, dietary intake of red meat, poultry, fish, F&V, legumes, tea, coffee, and no-diet cola, BL T2D, and hypertension	Compared to non-consumers, less than daily drinkers of FF milk had a significantly (-) risk of CVD and CHD. Daily FF milk consumption was not associated with CHD, CVD, or stroke as compared to non-consumers, but was (+) associated with all cause mortality.
Um et al.[239]	County: Us Age: 55-69 yr W N: 35,221	127-item FFQ	Age, family history of colorectal cancer, BMI, smoking, alcohol, PA, hormone replacement therapy use, total energy intake, vitamin D, F&V intake, red and processed meat intake, dietary oxidative balance score, and supplemental calcium	Neither LF or HF dairy consumption were associated with CHD mortality. Neither LF or FF milk were associated with CHD mortality.
Um et al.[241]	Country US Age: >45 yr M and F N: 21,427	107-item FFQ	Age, sex, race, region, BMI, smoking, alcohol, PA, non-steroidal anti-inflammatory drug and aspirin use, hormone replacement therapy use, education, annual income, supplemental calcium, total energy intake, F&V intake, processed and red meat intake, dietary oxidative balance score.	Both LF milk and FF milk intake were not associated with CVD. Increasing LF milk intake (-) associated with risk of CVD.
Van Aerde et al.[222] Prospective cohort	Country: Netherlands Age: 50-75 yr N: 1,956	BL FFQ	Age, sex, BMI, smoking, education, total energy intake, alcohol consumption, PA, intakes of meat, fish, bread, F&V, coffee, and tea	For every 1 standard deviation increase in HF dairy consumption, there was a 32% higher risk of CVD mortality. LF dairy consumption was not associated with CVD mortality.
Warensjo et al.[211]	Country: Sweden Age: Adult M and F N: 386	C15:0 and C17:0 in plasma phospholipids	BMI, serum cholesterol, tobacco use, and BP	Sum of C15:0 and C17:0 was (-) associated with the risk of stroke. Adjusting for other foods (Assessed by FFQ) including fish, F&V and alcohol intake had little effect on the model.
Warensjo[127]	Country: Sweden Age: Adult M and F N: 234	BL C15:0 and C17:0 in serum lipids	Serum cholesterol, smoking, BP, serum triglycerides, serum fasting insulin and leptin, BMI, plasminogen activator inhibitor-1, tissue-type plasminogen activator, and von Willebrandt factor	The sum of C15:0 and C17:0 was (-) associated with the risk of acute MI. Adjustment for "metabolic" risk factors (serum triglycerides, insulin, leptin, BMI), eliminated the association.
Warensjo et al.[133]	Country: Sweden Age: Adult M and F N: 1,000	C15:0 and C17:0 in plasma phospholipids	PA, BMI, smoking, ratio of apolipoprotein B to A-I, systolic BP, diabetes, and intakes of F&V	In F, C17:0 and C15:0 + C17:0 were (-) associated with the risk for MI. This association became not significant after adjusting for classic risk factors for CVD. Among M, the trend was similar without reaching statistical significance.
Yaemsir et al.[245] Case Control	Country: US Age: 50-79 N: 964 cases 964 controls	Serum fatty acid C15:0, C17:0 and 16:1n-7	Age, race, time to follow-up, BMI, smoking, T2D, aspiring use, systolic blood pressure, antihypertensive medication use, total cholesterol, HDL-Cholesterol ratio and normalized-triglycerides	C15:0, C17:0 and 16:1n-7 were all not associated with ischemic stroke.

Yakoob et al.[232]	Country: US Age: M and F N: 1,188	Red blood cell and plasma concentrations of C14:0, C15:0, C17:0, trans 16:1n-7	Age, race, month of blood collection, smoking status, PA, alcohol, family history of T2D, parental history of MI, menopausal status in the NHS, postmenopausal hormone use in the NHS, consumption of fish, processed meats, unprocessed meats, F&V, whole grains, coffee, SSBs, glycemic load, dietary calcium, polyunsaturated fat, and total energy, plasma total trans18:1, plasma total trans 18:2, plasma 16:0, and plasma 18:0.	None of the plasma or red blood cell fatty acid biomarkers was associated with stroke.
Yamagishi et al.[243] Prospective Cohort	Country: United States Age: 45-64 yr M and W N: 3,870	Plasma fatty acid and cholesterol ester 15:0 and 16:1n-7	Age, sex, smoking, alcohol intake.	15:0 in both the plasma fatty acid and cholesterol ester were not associated with ischemic stroke. However, 16:1n-7 was (+) associated with stroke in both the plasma fatty acids and cholesterol esters.

Abbreviations:

%BF = percent body fat
BL = baseline
BMI= body mass index
BP = blood pressure
CHD = coronary heart disease
CVD = cardiovascular disease
F= female
FF= full-fat
F&V= fruit and vegetable
HDL = high density lipoprotein
HEI= Healthy eating index
HF= high-fat
LF= low-fat
LDL= low density lipoprotein
M= male
MetS = metabolic syndrome
MI = Myocardial Infarction
overweight = overweigh
PA = physical activity
PUFA= poly-unsaturated fatty acids
SSBs= sugar sweetened beverages
T2D = type 2 diabetes mellitus
US = United States
vs= versus
WC = waist circumference
WHR = waist to height ratio
yr= year
(-) = inversely
(+) = positively

Table A5. Baseline characteristics of study participants included in the intent to treat analyses for glucose tolerance and related endpoints (n=72).

Variable	Limited N= 24	Low-fat N=24	Full-fat N=24	P value
Age (y)	56. (46; 69)	64 (58; 71)	63 (53; 67)	0.36
Male Sex (%)	54.2	58.3	58.3	0.95
Caucasian Race (%)[#]	75.0	79.2	66.7	0.68
Body Mass index (kg/m^2)	33.2 (28.7; 36.2)	30.9 (27.0; 39.1)	32.0 (27.8; 37.1)	0.95
Body weight (kg)	100.3 ± 15.8	96.5 ± 25.7	95.5 ± 16.5	0.68
Fat mass (kg)	37.9 (32.0; 42.6)	32.5 (23.6; 52.1)	36.2 (29.3; 45.1)	0.69
Lean body mass (kg)	57.3 ± 10.5	55.6 ± 14.0	54.4 ± 9.2	0.68
Visceral adiposity (inch^3)*	153 (100; 190)	104 (71; 202)	127 (86; 171)	0.26
Physical activity (MET-h/week)	38.0 (26.0; 55.8)	41.0 (25.7; 89.7)	36.3 (20.7; 48.5)	0.27
2015 Healthy Eating Index Score (HEI)	72.0 ± 8.9	72.8 ± 9.7	69.9 ± 10.9	0.72
HOMA-IR	2.6 (2.0; 3.7)	3.3 (1.6; 4.4)	3.1 (1.8; 4.4)	0.97
Fasting glucose (mg/dL)	102 (93; 108)	110(101; 119)	107 (102; 138)	0.07
Fasting Insulin (μU/mL)	10.2 (7.3; 15.7)	12.3 (6.1; 15.6)	12.0 (8.1; 14.9)	0.69
Glycosylated hemoglobin (%)	5.3 (4.9; 5.5)	5.8 (5.5; 6.2)	5.8 (5.4; 5.9)	<0.001
Area under the curve glucose (mg/dL x min)	25,195 (23,300; 30,417)	29,895 (26,494; 32,848)	27,905 (25,535; 30,494)	0.04
Matsuda-insulin sensitivity index	2.4 (1.9; 3.7)	2.4 (1.8; 3.8)	2.1 (1.7; 3.4)	0.65
Insulinogenic index	1.1 (0.6; 1.7)	0.7 (0.4; 1.4)	1.2 (0.7; 1.8)	0.35
Glucose sensitivity (pmol x min^{-1} x m^{-2}x L x mmol^{-1})	98 (66; 140)	74 (41; 103)	85 (66; 105)	0.33
Oral disposition index	2.3 (1.5; 4.7)	2.3 (1.4; 3.2)	2.8 (1.5; 4.2)	0.51

C-reactive protein (mg/L)	1.3 (0.9; 2.0)	0.9 (0.4; 2.1)	1.6 (1.0; 3.2)	0.38
Interleukin-6 (pg/mL)	3.5 (2.2; 4.1)	2.7 (1.8; 4.1)	2.8 (1.6; 4.2)	0.61
Total Adiponectin (ng/mL)	5,150 (3,713; 7,775)	6,425 (3,900; 9,300)	6,050 (3,813; 10,338)	0.82
Liver fat content (%)ǂ	5.2 (0.9; 8.0)	4.4 (1.1; 10.1)	4.9 (2.4; 11.8)	0.32

Values are means ± standard deviations, or medians (25th; 75th percentiles) for non-normally distributed variables, or percentages (for categorical variables). P-values are based on an analysis of variance, except for gender and race, which were based on an independent samples Kruskal-Wallis test.

Abbreviations: HOMA-IR: homeostasis model assessment index of insulin resistance.

* Sample size for visceral adiposity: limited n= 23, low-fat n= 22, full-fat n= 23

ǂ Sample size for percent liver fat: limited n= 22, low-fat n= 22, full-fat n= 21

ǂǂ Sample size for Caucasian Race: limited n=24, low-fat n=23, full-fat n=24

Table A6. Dietary intakes during wash-in and intervention phases, based on unannounced 24-hour dietary recalls, for the participants included in the *per-protocol* analysis for glucose tolerance and related endpoints (n=67).

	Limited dairy group (n=22)		Low-fat dairy diet (n=24)		Full-fat dairy diet (n=21)		*RM-ANOVA
	Wash-in diet period	Change during intervention period	Wash-in diet period	Change during intervention period	Wash-in diet period	Change during intervention period	(time x diet interaction)
Energy intake (kcal/day)	1,998 (1,624; 2,307)	81 ± 544[a]	2,041 (1,526; 2,625)	224 ± 375[a]	1,712 (1,364; 2,098)	554 ± 467[b]	0.003
Carbohydrates (%E)	46.1 ± 11.4	-0.7 (-4.4; 0.9)[a]	47.7 ± 8.6	1.6 (-4.3; 6.2)[a,b]	46.3 ± 7.1	-4.1 (-7.6; -0.8)[b]	0.020
Total sugars (%E)	18.0 ± 7.0	-1.9 ± 5.6[a]	18.5 ± 6.6	3.4 ± 5.0[b]	17.1 ± 6.2	0.9 ± 4.7[a,b]	0.004
Added sugars (%E)	9.3 ± 4.5	-0.5 ± 3.3	8.5 ± 4.1	0.7 ± 4.3	9.1 ± 6.1	-0.9 ± 4.1	0.349
Fiber (g/day)	23.9 ± 8.9	1.5 ± 7.9	25.7 ± 9.0	-0.3 ± 6.2	21.5 ± 9.0	0.2 ± 5.8	0.650
Fiber (g/1,000 kcal)	12.3 ± 5.0	0.3 ± 3.8[a]	12.6 ± 3.4	-1.4 ± 2.5[a,b]	12.2 ± 4.4	-2.8 ± 3.3[b]	0.011
Fat (%E)	34.0 ± 8.1	1.3 (-2.7; 5.7)[a]	34.2 ± 7.7	-2.5 (-8.2; 0.0)[b]	35.0 ± 9.0	4.4 (0.6; 7.3)[a]	<0.001
Saturated fatty acids (%E)	8.0 ± 2.0	0.7 (-0.3; 3.8)[a]	8.2 ± 2.1	0.6 (-1.5; 2.1)[a]	8.7 ± 2.6	5.2 (3.4;6.9)[b]	<0.001
Monounsaturated fatty acids (E%)	14.1 ± 4.3	-0.3 ± 3.8[a]	14.0 ± 3.9	-3.1 ± 2.5[b]	13.7 ± 4.1	-0.2 ± 4.6[a]	0.014
Polyunsaturated fatty acids (%E)	9.2 ± 2.8	0.3 ± 2.3	9.3 ± 3.1	-1.8 ± 3.0	9.7 ± 3.5	-1.1 ± 3.4	0.058
Protein (%E)	15.4 (12.5; 18.1)	0.0 ± 0.1	16.2 (13.7;17.7)	0.1 ± 0.1	15.3 (14.1; 18.6)	0.0 ± 0.1	0.024
2015 Healthy Eating Index (HEI)	71.9 ± 9.2	-2.5 ± 10.0[a,b]	72.8 ± 9.7	2.9 ± 8.7[b]	72.2 ± 8.5	-5.6 ± 7.6[a]	0.008

Calcium (mg/1,000 kcal)	307 (222; 440)	-9 ± 150[a]	298 (252; 358)	401 ± 167[b]	338 (276; 426)	277 ± 194[b]	<0.001

Values are means ± standard deviations or medians (25^{th}; 75^{th} percentiles) for non-normally distributed variables. Abbreviations: %E: percent of total energy intake.

*Reflects an overall comparison of the three dietary phases by RM-ANOVA. Data with different superscript letters are statistically significantly different in Bonferroni-adjusted *post hoc* testing ($p<0.017$).

Table A7. The effect of consuming a control diet limited in dairy vs. diets rich in either low-fat or full-fat dairy foods on glucose homeostasis (*per-protocol* analysis, n=67).

	[+]Baseline	[+]Follow-up	[+]Delta	[*]RM-ANOVA (time x diet interaction)		
				Model 1	**Model 2**	**Model 3**
Glucose tolerance (area under the curve glucose, mg / dL x min)				0.340	0.372	
Limited dairy	25,195	25,820	-919			
	(23,445; 30,708)	(23,451; 29,356)	(-2,549; 1,371)			
Low-fat dairy	29,895	31,060	+746			
	(26,495; 32,849)	(25,908; 36,372)	(-1,133; 2,764)			
Full-fat dairy	27,888	27,718	+130			
	(24,831; 29,881)	(24,561; 32,756)	(-1,579; 2,260)			
Matsuda insulin sensitivity Index				0.096	0.012	0.013
Limited dairy	2.7 (2.0; 3.8)	2.8 (2.0; 4.3)	0.00 ± 0.92[a]			
Low-fat dairy	2.4 (1.8; 3.8)	2.3 (1.5; 3.5)	-0.47 ± 1.07[b]			
Full-fat dairy	2.3 (1.9; 3.4)	1.9 (1.5; 2.8)	-0.25 ± 0.91[b]			
Insulinogenic index				0.324	0.330	
Limited dairy	1.0 (0.6; 1.5)	1.2 (0.7; 1.9)	-0.01 (-0.16; 0.44)			
Low-fat dairy	0.7 (0.4; 1.4)	0.8 (0.4; 1.4)	+0.12 (-0.26; 0.18)			
Full-fat dairy	1.2 (0.7; 1.9)	1.0 (0.7; 1.4)	-0.01 (-0.62; 0.19)			
Oral disposition index				0.060	0.028	0.056
Limited dairy	2.3 (1.4; 4.5)	3.1 (1.9; 5.1)	+0.4 (-0.4; 1.1)[a]			
Low-fat dairy	2.3 (1.4; 3.2)	2.0 (0.8; 3.8)	-0.7 (-1.1; 0.0)[a,b]			
Full-fat dairy	2.8 (1.5; 4.8)	1.9 (1.4; 3.6)	-0.4 (-1.7; 0.2)[b]			
Glucose sensitivity (pmol x min^{-1} x m^{-2} x L x $mmol^{-1}$)				0.598	0.618	
Limited dairy	92 (64; 120)	92 (70; 138)	+14 (-7; 31)			
Low-fat dairy	74 (41; 103)	73 (48; 100)	+5 (-23; 21)			
Full-fat dairy	86 (68; 110)	89 (62; 122)	-7 (-18; 13)			

Fasting glucose (mg/dL)				0.084	0.0473	
Limited dairy	101 (93; 109)	101 (94; 108)	+1.00 (-5.3; 4.9)			
Low-fat dairy	110 (101; 119)	110 (108; 116)	+1.5 (-6.1; 9.5)			
Full-fat dairy	107 (102; 116)	110 (106; 119)	+2.5 (-0.25; 8.25)			
Fasting insulin (µU/mL)				0.025	0.010	0.009
Limited dairy	9.8 (7.1; 14.6)	9.7 (6.4; 14.1)	-0.5 ± 7.8[a]			
Low-fat dairy	12.3 (6.1; 15.6)	11.0 (8.2; 18.7)	+2.1 ± 4.1[b]			
Full-fat dairy	11.3 (7.9; 14.4)	14.7 (9.4; 19.0)	+2.4 ± 17.6[a,b]			
Homeostasis model assessment (HOMA) index of insulin resistance				0.004	0.005	0.003
Limited dairy	2.5 (1.9; 3.5)	2.5 (1.6; 3.9)	-0.44 (-0.86; 0.41)[a]			
Low-fat dairy	3.3 (1.6; 4.4)	3.1 (2.3; 6.1)	+0.75 (-0.32; 1.33)[b]			
Full-fat dairy	3.0 (1.7; 4.4)	3.9 (2.4; 5.6)	+0.82 (0.07; 1.84)[b]			
Glycated hemoglobin (%)				0.156	0.106	
Limited dairy	5.4 (5.0; 5.5)	5.3 (5.1; 5.6)	+0.05 (-0.10; 0.10)			
Low-fat dairy	5.8 (5.5; 6.2)	5.8 (5.4; 6.2)	0.00 (-0.10; 0.10)			
Full-fat dairy	5.7 (5.4; 5.9)	5.7 (5.5; 5.9)	0.00 (-0.10; 0.10)			

[+]Values are means ± standard deviations or medians (25th; 75th percentiles) for non-normally distributed variables.

[*]Reflects an overall comparison of the three dietary phases by RM-ANOVA. Data with different superscript letters are statistically significantly different in Bonferroni-adjusted *post hoc* testing ($p<0.017$).

Model 1: Unadjusted analysis

Model 2: Adjusted for those variables that tended to differ at baseline ($p<0.10$): area under the curve glucose, fasting glucose, and glycated hemoglobin at baseline, as applicable.

Model 3: Model 2 with additional adjustment for change in fat mass (only performed on those variables with a significant intervention effect)

Table A8. The effects of consuming a control diet limited in dairy vs. diets rich in low-fat or full-fat dairy foods on liver fat content and biomarkers of inflammation (*per-protocol* analysis).

	[+]Baseline	[+]Follow-up	[+]Delta	*RM-ANOVA (time x diet intervention)
Liver fat content (% of total)				0.544
Limited (n=20)	5.2 (1.0; 8.6)	3.3 (1.5; 7.8)	-0.3 (-2.2; 0.5)	
Low-fat (n=22)	4.4 (1.1; 10.1)	3.9 (1.7; 11.0)	-0.4 (-1.4; 1.3)	
Full-fat (n=19)	3.7 (2.1; 10.2)	3.9 (1.8; 13.1)	+0.7 (-0.8; 2.7)	
C-reactive protein (high sensitivity, mg/L)				0.213
Limited (n= 19)	1.2 (0.7; 2.0)	1.0 (0.5; 1.6)	-0.07 (-0.42; 0.15)	
Low-fat (n=20)	1.1 (0.4; 2.7)	1.2 (0.5; 2.4)	+0.16 (-0.12; 0.71)	
Full-fat (n=20)	1.6 (0.9; 3.2)	2.1 (0.7; 3.0)	+0.03 (-0.26; 0.37)	
Interleukin-6 (high sensitivity, pg/mL)				0.500
Limited (n=19)	3.46 (2.07; 4.52)	3.55 (1.92; 4.04)	+0.03 (-0.98; 0.52)	
Low-fat (n= 20)	2.44 (1.82; 4.21)	2.28 (1.85; 4.98)	+0.09 (-0.64; 1.04)	
Full-fat (n=20)	2.79 (1.56; 4.23)	2.71 (1.83; 5.01)	+0.02 (-0.53; 1.02)	
Total adiponectin (ng/mL)				0.510
Limited (n=19)	5,100 (3,650; 7,400)	5,600 (3,650; 7,800)	+50 (-950; 750)	
Low-fat (n=20)	7,100 (4,362; 9,300)	7,625 (4,925; 9,375)	+100 (-500; 850)	
Full-fat (n=20)	5,825 (3,913; 10,050)	5,725 (3,900; 10,950)	+120 (-550; 625)	

[+]Values are means ± standard deviations, or medians (25^{th}; 75^{th} percentiles) for non-normally distributed variables.

[*]Reflects an overall comparison of the three dietary phases by RM-ANOVA, at $p<0.05$ after adjustment for multiple testing.

Table A9. The effect of consuming a control diet limited in dairy vs. diets rich in either low-fat or full-fat dairy foods on body weight, body composition, and anthropometric measurements (*per-protocol* analysis, n=66).

	[+]Baseline	[+]Follow-up	[+]Delta	[*]RM-ANOVA (time x diet intervention)	
				Model 1	**Model 2**
Weight (kg)				0.005	0.006
Limited dairy	101.1 ± 16.6	100.1 ± 15.8	-0.4 (-2.5; 0.7)[a]		
Low-fat dairy	96.5 ± 25.7	97.0 ± 24.9	0.3 (-1.1; 1.9)[a,b]		
Full-fat dairy	94.8 ± 17.4	95.9 ± 17.3	1.0 (-0.2; 1.8)[b]		
Hip circumference (cm)				0.346	0.491
Limited dairy	116.0 (106.4; 119.4)	115.5 (110.0; 119.0)	0.1 ± 2.9		
Low-fat dairy	110.0 (101.9; 122.5)	110.6 (104.6; 123.1)	0.7 ± 4.2		
Full-fat dairy	111.8 (103.2; 123.4)	115.0 (106.0; 125.0)	1.9 ± 3.7		
Waist circumference (cm)				0.015	0.011
Limited dairy	112.6 ± 11.1	109.9 ± 9.0	-3.0 (-5.2; -0.4)[a]		
Low-fat dairy	109.9 ± 16.4	111.2 ± 17.3	-0.3 (-2.8; 5.7)[b]		
Full-fat dairy	111.2 ± 12.7	113.7 ± 15.2	0.9 (-1.8; 6.3)[b]		
Waist to hip ratio				0.208	0.092
Limited dairy	0.98 ± 0.05	0.95 ± 0.05	-0.02 ± 0.03		
Low-fat dairy	0.97 ± 0.06	0.98 ± 0.06	0.00 ± 0.06		
Full-fat dairy	0.97 ± 0.08	0.97 ± 0.05	0.00 ± 0.07		
Fat mass (kg)				0.024	0.030
Limited dairy	37.5 (32.1; 46.7)	37.7 (31.0; 46.5)	-0.2 ± 1.5		
Low-fat dairy	32.5 (23.6; 52.1)	33.7 (24.0; 52.9)	0.0 ± 1.5		
Full-fat dairy	36.7 (27.4; 48.8)	37.1 (27.4; 49.6)	+0.8 ± 1.1		

Lean mass (kg)				0.085	0.082
Limited dairy	57.8 ± 10.5	57.6 ± 10.3	-0.1 (-1.3; 0.5)		
Low-fat dairy	55.6 ± 14.0	55.9 ± 13.6	+0.1 (-0.4; 1.3)		
Full-fat dairy	53.5 ± 8.7	54.2 ± 9.1	+0.5 (-03; 1.2)		
Trunk fat (kg)				0.842	0.842
Limited dairy	22.9 ± 5.2	23.1 ± 5.1	0.0 (-0.6; 0.8)		
Low-fat dairy	22.4 ± 9.8	22.4 ± 9.6	-0.2 (-1.0; 1.0)		
Full-fat dairy	22.8 ± 8.5	23.1 ± 8.6	0.2 (-0.6; 0.9)		
Peripheral fat (kg)				0.360	0.483
Limited dairy	13.3 (10.8; 18.8)	12.7 (10.0; 19.1)	-0.1 (-0.5; 0.2)		
Low-fat dairy	12.0 (8.9; 19.2)	11.0 (9.0; 18.7)	-0.1 (-0.5; 0.3)		
Full-fat dairy	14.6 (9.6; 18.0)	14.0 (9.6; 17.7)	0.1 (-0.3; 0.7)		
Visceral adiposity (in^3)				0.093	0.107
Limited dairy	150.3 (94.9; 207.3)	145.2 (92.0; 205.8)	-3.3 (-7.8; 8.3)		
Low-fat dairy	103.9 (71.4; 202.1)	100.3 (69.3; 199.4)	-2.9 (-8.0; 10.2)		
Full-fat dairy	123.5 (77.7; 176.6)	133.6 (87.8; 174.2)	3.2 (-1.4; 9.4)		

[+]Values are means ± standard deviations or medians (25^{th}; 75^{th} percentiles) for non-normally distributed variables.

[*]Reflects an overall comparison of the three dietary phases by RM-ANOVA. Data with different superscript letters are statistically significantly different in Bonferroni-adjusted *post hoc* testing ($p<0.0167$).

Model 1: unadjusted

Model 2: adjusted for change in physical activity

Table A10. The effect of dairy consumption on blood lipids (*per-protocol* analysis) among the limited (n=21), low-fat (n=24) and full-fat (n=21) dairy groups.

	*Baseline	*Follow-up	Delta	†RM-ANOVA (time x diet intervention)	
				Model 1	**Model 2**
Total Cholesterol (mg/dL)				0.485	0.328
Limited dairy	153.4 ± 43.8	158.2 ± 42.2	0.0 (-7.0; 11.0)		
Low-fat dairy	168.3 ± 37.2	168.8 ± 39.2	2.0 (-8.8; 9.0)		
Full-fat dairy	180.0 ± 42.6	186.1 ± 42.6	4.0 (-5.5; 19.5)		
Triglycerides (mg/dL)				0.480	0.446
Limited dairy	142.0 (73.0; 184.5)	130.0 (82.0; 188.0)	5.9 ± 40.1		
Low-fat dairy	149.5 (92.5; 204.0)	140.0 (96.0; 216.5)	-2.5 ± 40.6		
Full-fat dairy	122 (87.0; 191.5)	133.0 (93.0; 186.5)	17.6 ± 35.0		
LDL-cholesterol (mg/L)				0.854	0.975
Limited dairy	75.4 ± 28.4	77.6 ± 27.5	1.4 (-4.4; 7.9)		
Low-fat dairy	82.1 ± 27.3	83.8 ± 27.1	2.0 (-2.8; 8.5)		
Full-fat dairy	89.6 ± 32.7	90.0 ± 30.1	1.2 (-5.4; 4.5)		
HDL-cholesterol (mg/L)				0.788	Not applicable

Limited dairy	34.0 (29.5;44.0)	34.0 (31.0;44.0)	0.5 ± 3.9		
Low-fat dairy	36.5 (29.5;48.5)	38.5 (30.0;50.0)	-0.5 ± 5.5		
Full-fat dairy	41.0 (37.0;60.0)	42.0 (36.5;61.5)	0.9 ± 4.3		
Free Fatty Acids (mg/dL)				0.736	0.825
Limited dairy	0.54 ± 0.19	0.52 ± 0.14	-0.02 ± 0.17		
Low-fat dairy	0.59 ± 0.17	0.54 ± 0.17	-0.06 ± 0.17		
Full-fat dairy	0.57 ± 0.16	0.52 ± 0.14	-0.06 ± 0.13		
LDL Relative Flotation Rate				0.105	0.118
Limited dairy	0.26 (0.24;0.29)	0.26 (0.24;0.29)	0.00 (0.00; 0.00)		
Low-fat dairy	0.26 (0.24;0.29)	0.26 (0.24;0.29)	0.00 (0.000; 0.00)		
Full-fat dairy	0.26 (0.25;0.29)	0.28 (0.26;0.29)	0.00 (0.00; 0.00)		

*Values are mean ± SD or median (25^{th};75^{th} percentile) if non-normally distributed data.

†Reflects an overall comparison of the three dietary phases by RM-ANOVA

Model 1: Unadjusted analysis

Model 2: Adjusted for those variables that significantly differed at baseline (P<0.10) including HDL-cholesterol

Table A11. Baseline characteristics of study participants included in the *intent to treat* analyses of fasting serum lipids.

Variable	Limited	Low-fat	Full-fat	P value*
	N= 24	N=24	N=24	
Age (y)	56 (46; 69)	64 (58; 71)	63 (53; 67)	0.36
Male Sex (%)	54.2	58.3	58.3	0.95
Caucasian Race (%))†	75.0	82.6	66.7	0.46
Body weight (kg)	100 ± 16	97± 26	96 ± 17	0.68
Height (cm)	174 ± 10	172 ± 13	171 ± 8	0.54
Fat mass (kg)	37.9 (32.0; 42.6)	32.5 (23.6; 52.1)	36.2 (29.3; 45.2)	0.69
Lean body mass (kg)	57.3 ± 10.5	55.5 ± 14.0	54.4 ± 9.2	0.68
68Visceral adiposity (in^3)‡	153 (100; 190)	104 (71; 202)	127 (86; 171)	0.26
Diastolic blood pressure (mmHg)	76.0 ± 9.8	80.9 ± 10.6	78.7 ± 8.1	0.21
Systolic blood pressure (mmHg)	123 (115; 135)	123 (119; 138)	127 (120; 137)	0.66
LDL-cholesterol (md/dL)	88.1 ± 30.8	93.8 ± 33.6	102.8 ± 36.9	0.32
HDL-cholesterol (md/dL)	35.0 (30.0; 44.0)	36.5 (29.5; 48.5)	44.0 (37.3; 63.0)	0.02
Triglycerides (mg/dL)	139.5 (69.5; 174.8)	149.5 (92.5; 204.0)	121.0 (87.0; 183.8)	0.38
Total Cholesterol (mg/dL)	152.5 ± 41.1	168.3 ± 37.2	178.5 ± 43.1	0.09
Free Fatty Acids (mEq/L)	0.53 ± 0.19	0.59 ± 0.17	0.57 ± 0.16	0.40
LDL relative flotation rate	0.26 (0.24; 0.29)	0.26 (0.24; 0.29)	0.28 (0.24; 0.29)	0.18
CRP (mg/L)	1.3 (0.9; 2.0)	0.9 (0.4; 2.1)	1.6 (1.0; 3.2)	0.38
Interleukin-6 (pg/mL)	3.5 (2.2; 4.1)	2.6 (1.8; 4.1)	2.8 (1.6; 4.2)	0.61
Adiponectin (ng/mL)	5,150 (3,713; 7,775)	6,425 (3,900; 9,300)	6,050 (3,913; 10,338)	0.82
Physical activity (MET hrs/week)	38.0 (26.0; 55.8)	41.0 (25.7; 89.7)	36.3 (20.7; 48.5)	0.27
Healthy Eating Index Score	72.0 ± 8.9	72.8 ± 9.7	69.9 ± 10.9	0.59

Data are means ± standard deviations or (for non-normally distributed variables) medians (25^{th}; 75^{th} percentile), or

percentages (for categorical variables).

Abbreviations C-reactive protein (CRP), high-density lipoprotein (HDL), Low-density lipoprotein (LDL), metabolic equivalent of task (MET).

*P-values are based on an analysis of variance, except for gender and race, which were based on an independent samples Kruskal-Wallis test.

† Sample size for Caucasian Race: limited n=24, low-fat n=23, full-fat n=24

ǂ Sample size for visceral adiposity: limited n=23, low-fat n=22, full-fat n=23