Association between fatty acid biomarkers of dairy fat consumption and insulin sensitivity in humans

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Abstract

Background: Epidemiological studies have shown that greater consumption of dairy fat is associated with lower risk of type 2 diabetes (T2D). This inverse association is particularly strong when C15:0, C17:0, and tC16:1n-7 fatty acid (FA) proportions in circulation are used as biomarkers of dairy fat intake. Evidence for an association between plasma and serum levels of these biomarkers and insulin resistance, as measured by surrogate indices, remains inconclusive. Only two studies have assessed this relationship with biomarkers in adipose tissue which reflect long-term intake of FA. Branched-chain FA are emerging as potential markers of dairy fat intake and may help clarify the association between dairy intake and T2D.

Objective: To evaluate the association between established and potential biomarkers of dairy fat intake in adipose tissue and hyperinsulinemic clamp-based measures of insulin sensitivity.

Methods: Subcutaneous adipose tissue of 58 adults (mean age 47 y, 57% females, 45% with T2D) were analyzed. Fatty acids (n=57), including odd-chained FA, branched-chain FA and conjugated linoleic acids (CLA), were quantified using gas chromatography-mass spectrometry. Insulin sensitivity, expressed as insulin sensitivity index, glucose rate of appearance and rate of disposal, was assessed using the hyperinsulinemic-euglycemic clamp. Dietary intake was estimated from 3-day food diaries and a 24-h recall. Linear regression models examined the association between FA biomarkers of dairy fat intake and insulin sensitivity.

Results: A total of 57 FA were detected across all samples, with 37 present in 90% of samples and 19% being the targeted FA. The clamp-based insulin sensitivity index was found to range from 0.07 to 2.22 (mg/kg LBM·min)/(pmol/L). In a multivariable analysis adjusted for age, sex, BMI, and T2D status, FA C15:0 (β=4.01 [1.37, 6.64], p=0.004), anteiso-C15:0 (β= 2.51 [0.707, 4.31], p=0.007) and cis-9 trans-11 CLA (β= 5.30 [1.04, 9.57], p=0.02) in subcutaneous adipose tissue were positively and independently associated with peripheral insulin sensitivity. There was inverse association between C17:0 (β=-0.014 [-0.699, 0.671], p=0.97), and tC16:1n-7(β=-0.034 [-0.239,0.171], p=0.74), and whole-body insulin sensitivity. The method for FA detection was optimized with sufficient resolution and sensitivity for target FA.

However, difficulties in the analysis included distinguishing between endogenous and exogenous internal standards, co-elution of antioxidant with FA, the presence of contaminants and unidentifiable FA, and signal saturation for abundant FA.

Conclusion: Increased proportions of FA in adipose tissue considered as established and emerging biomarkers of dairy fat intake were positively associated with insulin sensitivity, independently of known factors. The findings imply that the consistent inverse association between dairy fat intake and T2D outcomes may be attributed to improved insulin sensitivity. Further optimization of the FA measurement method and additional studies are required to validate these findings.

Resumé

Contexte : Des études épidémiologiques ont démontré qu'une plus grande consommation de produits laitiers est associée à un risque réduit de diabète de type 2 (T2D). Cette relation inverse est surtout forte lorsque les proportions d'acides gras C15:0, C17:0 et tC16:1n-7 en circulation sont utilisées comme biomarqueurs de la consommation de graisses laitières. Les preuves d'une association entre les biomarqueurs plasmatiques/sériques et la résistance à l'insuline, telle que mesurée par des indices substituts, demeurent non concluantes. Seules deux études ont évalué cette relation avec les biomarqueurs du tissu adipeux, qui reflètent une consommation à long terme d'acides gras. Les acides gras à chaîne ramifiée émergent comme des marqueurs potentiels de la consommation de matières grasses laitières et pourraient aider à clarifier l'association entre la consommation de produits laitiers et la sensibilité à l'insuline.

Objectif : Nous avons pour objectif d'évaluer l'association entre les biomarqueurs établis de la consommation de matières grasses laitières dans le tissu adipeux et les mesures de sensibilité à l'insuline basées sur le clamp hyperinsulinique.

Méthodes : Le tissu adipeux sous-cutané de 58 adultes (âge moyen de 47 ans, 57 % de femmes, 45 % atteints de diabète de type 2) a été analysé. Les acides gras (n=57), y compris les acides gras à chaîne impaire, les acides gras à chaîne ramifiée et les acides linoléiques conjugués (CLA), ont été quantifiés à l'aide de la chromatographie en phase gazeuse/ spectrométrie de masse. La sensibilité à l'insuline, exprimée par l'indice de sensibilité à l'insuline, le taux d'apparition et le taux d'élimination du glucose, a été évaluée à l'aide du clamp hyperinsulinique-euglycémique. L'apport alimentaire a été estimé à partir de journaux alimentaires de 3 jours et d'un rappel alimentaire de 24 heures. Des modèles de régression linéaire ont été utilisés pour examiner l'association entre les biomarqueurs d'acides gras de la consommation de matières grasses laitières et la sensibilité à l'insuline.

Résultats: Au total, 57 acides gras ont été détectés dans tous les échantillons, 37 étant présents dans 90 % des échantillons et 19 % étant les acides gras ciblés. L'indice de sensibilité à l'insuline basé sur le clamp varie de 0,07 à 2,22 (mg/kg LBM·min)/(pmol/L).. Dans une analyse

multivariée ajustée pour l'âge, le sexe, l'IMC et la présence de diabète de type 2, les acides gras C15:0, (β=4.01 [1.37, 6.64], p=0.004), anteiso-C15:0 (β= 2.51 [0.707, 4.31], p=0.007) et le CLA cis-9, trans-11 (β= 5.30 [1.04, 9.57], p=0.02) dans le tissu adipeux sous-cutané étaient positivement et indépendamment associés à la sensibilité à l'insuline globale. Une association inverse a été observée entre C17:0 (β=-0.014 [-0.699, 0.671], p=0.97), et tC16:1n-7(β=-0.034 [-0.239,0.171], p=0.74), et la sensibilité à l'insuline globale. La méthode de détection des acides gras a été optimisée avec une résolution et une sensibilité suffisante pour les acides gras cibles. Cependant, des difficultés dans l'analyse comprenaient la distinction entre les standards internes endogènes et exogènes, la co-élution d'antioxydants avec les acides gras, la présence de contaminants et d'acides gras non identifiables, ainsi que la saturation du signal pour les acides gras abondants.

Conclusion: Les proportions accrues d'acides gras dans le tissu adipeux, considérées comme des biomarqueurs établis, et d'autres émergents, de la consommation de matières grasses laitières étaient positivement associés à la sensibilité à l'insuline et ce, indépendamment des facteurs connus. Les résultats suggèrent que l'association inverse entre la consommation de matières grasses laitières et les résultats du diabète de type 2 peuvent être attribués à une amélioration de la sensibilité à l'insuline. Une optimisation supplémentaire de la méthode de mesure des acides gras et des études supplémentaires sont nécessaires pour valider ces résultats.

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Contribution of Authors

The authors' responsibilities were: AG, SAB: designed the research; AG: conducted research; AG analyzed the data and performed the statistical analysis; AG: wrote the paper; AG and SAB: reviewed and edited the thesis; SAB and SC supervised research.

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List of Abbreviations

BCFA – Branched chain fatty acids

BMI – Body mass index

CE – Cholesterol ester

CFG – Canada's Food Guide

CLA – Conjugated linoleic acid

CVD – Cardiovascular disease

DR – Dietary recall

DXA - Dual-energy X-ray absorptiometry

FA – Fatty acids

FAME – Fatty acid methyl ester

FFA – Free fatty acids

FFQ – Food frequency questionnaire

FID – Flame ionization detection

HbA1C – Glycated hemoglobin

HEC – Hyperinsulinemic-euglycemic clamp

HOMA-IR – Homeostatic model of assessment insulin resistance

IS – Insulin sensitivity

ISI – Insulin sensitivity index

GC– Gas chromatography

LBM - Lean body mass

MS – Mass spectrometry

OCFA – Odd chained fatty acid

OGTT – Oral glucose tolerance test

PL – Phospholipid

Ra – Rate of glucose appearance

Rd – Rate of glucose disposal

SAT – Subcutaneous adipose tissue

SFA – Saturated fatty acids

TAG – Triacylglycerides

T2D – Type 2 diabetes

Introduction

Type 2 diabetes (T2D), accounting for 90% of diabetes cases, is a chronic metabolic condition characterized by insulin resistance and pancreatic β -cell dysfunction leading to persistent hyperglycemia [1]. Dietary habits are influential in both the management and prevention of T2D [2]. There is growing evidence that dairy intake is inversely associated with the incidence of T2D [3, 4]. Meta-analyses of prospective studies support a reduced risk of T2D with increased intakes of total dairy, low-fat dairy, and yogurt [5-7]. Despite this, randomized controlled trials evaluating the relationship between the underlying pathophysiological disorders of T2D and dairy intake remain inconclusive [8-13]. Glycemic outcomes, insulin resistance, and glucose tolerance show either no association [8-10, 13] or an inverse association [12] when comparing high (>3 servings/d) to low (<3 servings/d) dairy intake intervention groups.

Dairy intake is commonly measured using dietary assessment tools, such as dietary recalls, which are prone to recall bias and subjective reporting [14]. To overcome these limitations, circulating and tissue proportions of FAs have been used as objective biomarkers of dairy fat intake. The established biomarkers of dairy fat are the odd-chained FAs, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), and the natural trans-fat, trans-palmitoleic acid (t16:1n-7). Dietary sources of these FAs are principally obtained from ruminant products [15]. Concentrations of these biomarkers correlate with self-reported dairy fat intake as assessed by 24-h food recalls and multiple-day food diaries, in addition to reflecting habitual changes in dairy consumption as shown when comparing high-fat to low-fat dairy intakes [16-19]. These biomarkers can be useful to establish associations between dairy intake and T2D and its underlying mechanisms.

Branched-chain FAs (BCFA) may be a new biomarker of dairy fat consumption as they are synthesized from ruminal bacteria [20]. BCFAs are principally obtained from ruminant sources with minimal contributions from poultry, pork, seafood, and fermented foods [21]. Recent evidence suggests that BCFAs have a beneficial influence on cardiometabolic outcomes, including inflammation, obesity, and insulin metabolism [22].

Rationale

Epidemiological studies have consistently shown an inverse association between the incidence of diabetes and plasma and serum proportions of the established dairy fat biomarkers [23-27]. However, the association between these dairy fat biomarkers and the pathophysiological disorders underlying T2D, insulin resistance, and β-cell dysfunction remains inconclusive. This is partially due to the use of surrogate indices of insulin sensitivity rather than the gold standard hyperinsulinemic-euglycemic clamp (HEC). Most studies have measured FA biomarker levels in plasma and serum; however, evidence is inconsistent, and the methods used to assess insulin sensitivity differ [28-30]. Only two studies have assessed the relationship between insulin sensitivity and dairy fatty acid (FA) biomarker proportions in adipose tissue. Adipose tissue considered the gold standard for evaluating dietary FA, is preferred due to its prolonged turnover compared to other tissues. This characteristic renders it representative of the longer-term depot and FA intake [31, 32].

In addition, research has yet to explore new biomarkers of dairy fat intake to help clarify its relationship with IS. The origin of the established biomarkers of dairy fat in other dietary sources, preliminary evidence supporting endogenous synthesis, and their relatively low correlations with dairy fat intake warrant further investigation as well as identification of new biomarkers of dairy fat intake [15, 33]. BCFAs may help understand the association between dairy intake and insulin sensitivity.

Objectives and hypothesis

The objective is to determine the association between established and potential dairy fat intake biomarkers and hyperinsulinemic-euglycemic clamp-based insulin sensitivity measures. It is hypothesized that established FA biomarkers of dairy intake will be positively associated with insulin sensitivity. This study will clarify the association between biomarkers of dairy fat intake and human insulin sensitivity and provide a better understanding of the role of dairy consumption in preventing T2D.

Literature Review

1.1 Overview of Diabetes

1.1.1 Prediabetes and type 2 diabetes

a. Prevalence of type 2 diabetes

T2D is a chronic metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction leading to persistent hyperglycemia [1]. The prevalence of diabetes is a global health concern, with a projected increase to 578 million cases worldwide by 2030 [34]. In 2020, approximately 10% (3.7 million) of Canadians were diagnosed with diabetes, associated with 3.8 billion in direct healthcare costs [35]. In addition to its significant economic burden, the disease increases an individual's risk for a myriad of long-term complications, including neuropathy, nephropathy, cardiovascular disease, and premature death.

b. Diagnostic criteria of prediabetes and diabetes

Prediabetes is a term used to describe individuals at high risk of developing diabetes but do not yet meet the criteria for diagnosis of T2D [4]. According to the World Health Organization, this presymptomatic phase is diagnosed by any of the following: fasting glucose levels of 6.1-6.9 mmol/L (impaired fasting glucose), 2-hour plasma glucose in a 75 g-oral glucose tolerance test of 7.8-11.0 mmol/L (impaired glucose tolerance), or glycated hemoglobin (A1C) of 6.0% to 6.4%. The diagnostic criteria of T2D are fasting plasma glucose levels ≥7.0 mmol/L, 2-hour plasma glucose in a 75 g-OGTT ≥11.1 mmol/L, random plasma glucose ≥11.1 mmol/L or A1C ≥6.5%.

1.1.2 Pathogenesis of type 2 diabetes

Insulin resistance is a defect in insulin-mediated glucose metabolism and suppressing hepatic gluconeogenesis [36]. The emergence of and molecular responses to insulin resistance differ among the main insulin-sensitive tissues: muscle, adipose, and liver. Insulin resistance in skeletal muscle is a hallmark of T2D, being the predominant tissue for the body's glucose uptake in the prandial state [37]. Its etiology is a combination of lipotoxicity, glucotoxicity, and chronic inflammation due to factors such as overnutrition and genetics [37-40]. Prolonged and increased

circulating glucose and FA infiltrate tissues in excess, causing mitochondrial stress, endoplasmic reticulum dysfunction, proinflammatory cytokine recruitment, compromising lipid metabolism, and insulin signaling. A decreased response to insulin reduces lipolysis suppression, increasing circulating FA and lipid depots in tissues, perpetuating this cycle. Concurrently, pancreatic β cells compensate for elevated glucose levels and insulin resistance by increasing insulin secretion [41-43]. Chronic β cell overload reduces cell mass and mitochondrial function, ultimately impairing cell function to secrete insulin adequately. The development of insulin resistance and β cell dysfunction results in chronic hyperglycemia.

The HEC is the gold standard method to assess IS. In this method, recombinant human insulin is infused to reach prandial-like serum concentration and dextrose is infused at variable to maintain plasma glucose at 5.5 mmol/L [44]. The goal is to suppress hepatic glucose production such that the glucose infusion becomes equal to the tissue uptake from which insulin sensitivity can be assessed.

1.1.3 Risk factors for type 2 diabetes

The susceptibility to T2D is attributed to genetics, environment, and social determinants [35]. South Asians and Blacks are at increased risk of T2D compared to White Canadian adults [35, 44]. Having a first-degree relative with T2D is also associated with an inherited predisposition to T2D and its comorbidities [45]. Lifestyle risk factors, such as poor diet, alcohol intake, and physical inactivity, contribute substantially to T2D development and can be targeted to prevent its onset [46]. Individuals with T2D are typically overweight or obese; therefore, weight loss is a primary intervention [47]. Physical activity reduces fat mass and improves glucose metabolism, lipid profile, and insulin sensitivity in those with prediabetes and diabetes [47, 48]. Interventions commonly include nutritional therapy, as diet is a significant environmental contributor in developing, preventing, and managing T2D [49]. As detailed in the 2019 Canada's Food Guide (CFG), improving dietary habits by consuming fiber-rich and low saturated-fat foods can result in weight loss, glycemic control, and reduced risk of T2D and CVD in adults with prediabetes and diabetes [2]. A meta-analysis of randomized-controlled trials (n= 4090) including 4000 adults with impaired glucose tolerance showed a 47% reduced risk in T2D incidence following a lifestyle intervention focused on weight loss, increased physical activity,

and healthy dietary changes [50]. Dairy intake may also have a protective role in the risk of T2D due to its protein quality and diverse sources of FAs [2, 51].

1.2 Dairy intake and type 2 diabetes

1.2.1 Dairy intake and dietary guidance in Canada

The proportion of Canadians consuming milk and alternatives in 2015 was 87%, averaging 1.4 daily servings [52, 53]. The 2007 version of CFG recommended that adults consume 2-3 servings of lower-fat *milk and alternatives* [54]. However, the *milk and alternatives* and *meat and alternatives* food groups have been combined into protein foods in the 2019 CFG. This current CFG is a snapshot of a plate sectioned into vegetables and fruits, protein foods, and whole grains, where the concept of food groups and serving sizes have been removed [55]. The 2019 CFG recommends choosing low-fat dairy products and limiting the intake of SFA-rich foods, such as high-fat dairy products, to reduce CVD risk [56].

1.2.2 Saturated fats and cardiovascular disease risk

Dairy intake has often been scrutinized due to its substantial saturated fat content [57]. Dietary SFA has been shown to elevate plasma concentrations of total and low-density lipoprotein (LDL) cholesterol, which are risk factors for CVD [58]. The diet-heart hypothesis suggests that reducing dietary intake of SFA can improve cardiometabolic health. However, meta-analyses of prospective cohorts reported a neutral association between higher SFA intakes and risk of non-communicable diseases such as CVD, stroke, T2D, and all-cause mortality [3, 4]. A large prospective cohort of 18 countries (135 335 participants) supported the idea that total fat and SFA intake were inversely associated with all-cause mortality and had a neutral association with CVD [59]. Current literature does not support the idea that increased consumption of SFA from dairy and unprocessed red meat increases CVD incidence and mortality [60-62]. These findings have been used to support calls to re-evaluate the diet-heart hypothesis and, by extension, the public health guidelines on dietary SFA, specifically regarding dairy intake [60, 63].

1.2.3 Dairy matrices and fat content of dairy products

When evaluating the health impacts of dairy, the nutritional composition and food matrix should be considered rather than individual components[64]. Dairy provides high-quality protein, minerals (i.e., calcium, phosphorus, potassium), vitamins, and a rich FA profile. Fluid milk consists of solids, fat globules, and casein micelles emulsified in a nutrient-dense aqueous phase [65]. Milk fat, 1-5% of bovine milk, is the most complex natural fat, comprised of over 400 different FA. These FA vary from 4-24 carbons in length, mainly consisting of triacylglycerides. SFA compose 70% (palmitic acid, 22-35 wt%), followed by MUFA at 25% (oleic acid, 20-30wt%), while PUFAs and TFA compose 2-5% of total milk fat [65, 66]. Bacterial fermentation of milk produces a semi-fluid gel, or yogurt, where the aqueous phase is bound in a casein network due to decreased pH from lactic acid production [67, 68]. The FA profile is dependent on the fat content and type of yogurt, ranging from full fat (5%) to non-fat (<0.5%). Cheese is a casein-rich network of fat globules, minerals, and dissolved solutes resulting from the coagulation of milk [62]. Fat content varies among cheese types, around 30%, but typically contains a higher fat content than milk and yogurt [62, 69]. Fermented products, like cheese and yogurt, contain bioactive constituents such as probiotics, short-chain FA, and vitamins. The differences in processing, structure, and composition among dairy products are suggested to influence their digestibility, bioavailability, and health benefits.

1.2.4 Association between dairy intake and T2D risk

There is growing evidence that dairy intake is inversely associated with the incidence of T2D. Meta-analyses of prospective studies support a reduced risk of T2D with increased intakes of total dairy (RR= 0.85-0.89), low-fat dairy (RR= 0.81-0.83), and yogurt (RR= 0.82-0.86), summarized in **Table 1** [5-7]. Results from individual epidemiological studies vary among dairy fat content and across types of dairy products [70-78]. Some studies show an inverse association between total milk [71-73] and cheese [72] intake with T2D risk, while others show no association [70, 74-76]. Yogurt is consistently inversely associated with T2D [74, 75, 78], with a 14% (RR: 0.86) lower risk reported at intakes of 80 g/d [79]. Low-fat dairy products are typically inversely associated with T2D, with high-fat dairy showing no association. Despite this, randomized controlled trials evaluating the relationship between the underlying pathophysiological disorders of T2D and dairy intake remain inconclusive [8-13].

Glycemic outcomes, insulin resistance, and glucose tolerance show either no association [8-10, 13] or an inverse association [12] when comparing high (>3 servings/d) to low (<3 servings/d) dairy intake intervention groups. The few studies that assessed β-cell function showed no association with increased dairy intake [8, 10].

Table 1. Summary of meta-analyses of dairy intake on T2D

Author, y	Study, country, participants (T2D)	Dairy Intake	RR (95%CI)	RR (95%CI), per amount increase
Tong, 2011	7 prospective cohorts (US, UK, China, Japan),322 000	Total dairy intake	**0.86 (0.79–0.92)**	**0.94 (0.92-0.97), serving**
		Low fat dairy	**0.82 (0.74–0.90)**	**0.90 (95% CI, 0.85–0.95), serving**
		High fat dairy	1.00 (0.89–1.10)	NS
		Whole milk	0.95 (0.86–1.05)	NS
		Yogurt	0.83 (0.74–0.93)	NA
Gao, 2013	14 prospective cohorts, (US, Europe, Asia, Australia), 457893 (27095)	Total dairy intake	**0.89 (0.81–0.98)**	**0.95 (0.92-0.98), 200g/d**
		Low fat dairy	**0.81 (0.74–0.89)**	**0.88(0.84-0.93), 200g.d**
		Full fat dairy	0.95 (0.85–1.07)	0.95 (0.88–1.04)
		Milk (total)	0.89 (0.78–1.01)	0.89 (0.79–1.01)
		Low fat milk	**0.82 (0.69–0.97)**	NS
		Full fat milk	1.12 (0.99–1.27)	NS
		Yogurt	**0.85 (0.75–0.97)**	0.90 (0.82–1.00), 50/d
		Cheese	**0.82 (0.77–0.87)**	**0.80 (0.69–0.93), 30g/d**
Aune, 2013	17 cohorts (US, Asia, Europe, Australia), 426055 (26976)	Total dairy intake	**0.89 (0.82, 0.96)**	NA
		Low fat dairy	**0.83 (0.76, 0.90)**	
		High fat dairy	0.96 (0.87, 1.06)	
		Milk	0.87 (0.70, 1.07)	
		Yogurt	**0.86 (0.75, 0.98)**	
		Cheese	0.91 (0.84, 0.98)	
Chen, 2014	3,984,203 (15,156)	Total dairy intake	NA	0.98(0.96,1.01)
		Yogurt		**0.82(0.70,0.96), serving**
Gijsbers, 2016	22 prospective cohorts (US, Australia, Asia, Europe), 579832 (43118)	Total dairy intake	NA	0.97; (0.95,1), 200 g/d
		Low fat dairy		0.96 (0.92, 1.00), 200g.d
		High fat dairy		0.98(0.93, 1.04), 200 g/d
		Milk		0.97(0.93, 1.02), 200 g/d
		Yogurt		**0.86(0.83, 0.90), 80g/d**
		Cheese		1.05 (1.02, 1.09), 10g/d

Bold values are significant. Abbreviations: NA- not applicable; NS- not significant; RR- relative risk.

1.3 Fatty Acids Biomarkers of Dairy Intake and Type 2 Diabetes

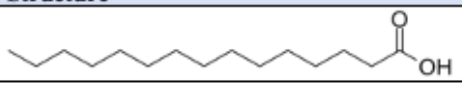
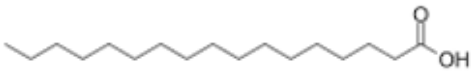
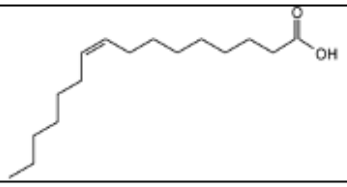
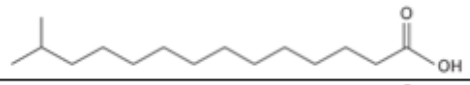
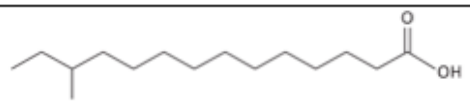
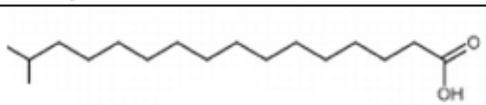
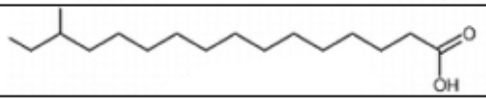
1.3.1 Biomarkers of dairy intake

a. Current dairy intake assessment methods

Current epidemiological studies largely rely on subjective dietary assessment tools (e.g., food frequency questionnaires (FFQ), 24-food recalls, and food records) to assess the association between food consumption and cardiometabolic disease risk [14]. 24-h recalls provide detailed short-term dietary intake with minimal participant burden, whereas FFQ represents longer-term intake. Both methods are time and cost-effective options suitable for larger-scale studies. Food records are useful to avoid memory reliance and capture habitual foods such as dairy. However, such dietary intake measures are prone to recall bias, subjective reporting, and misclassification and may not accurately capture dairy quantities from mixed meals [80]. To overcome these limitations, nutritional biomarkers are often used. These are objective measures of dietary constituents reflecting nutritional status and dietary exposure [81]. Selection of nutritional biomarkers is preferable to those not endogenously synthesized in order to accurately estimate a nutrient originating from diet.

To assess dairy intake, studies have measured the proportion of specific FA in blood lipid fractions and adipose tissue derived mainly from dairy foods as objective biomarkers of dairy fat intake. The established biomarkers of dairy fat intake reported in the literature are the odd-chained FA, pentadecanoic acid (15:0) and heptadecanoic acid (17:0), and the naturally occurring trans-fat, trans-palmitoleic acid (t16:1n-7). The chemical structure of these FA is shown in **Figure 1.** Proportions of these biomarkers correlated with self-reported dairy fat intake as assessed by 24-hour food recalls and multiple-day food diaries, in addition to reflecting habitual changes in dairy consumption as shown when comparing high-fat to low-fat dairy intakes [16-19]. Given this, these biomarkers may be useful to establish associations between dairy intake and T2D and its underlying mechanisms.

Figure 1. Structures of FA biomarkers of dairy fat intake

Fatty acid	Name	Structure
OCFA	C15:0	
	C17:0	
	t16:1, n-7	
BCFA	Iso C15:0	
	Anteiso C15:0	
	Iso C17:0	
	Anteiso C17:0	

Source: PubChem

b. Methods for assessing FAs in serum lipid fractions and adipose tissue

Fatty acid biomarkers are quantifiable in various biological media such as serum, plasma, and adipose tissue, albeit in low concentrations. Biomarkers measured in serum and plasma typically reflect shorter-term dietary intake, whereas in adipose tissue, they reflect longer-term intake [82]. In blood, FA are mostly contained in 49% triacylglycerides, 24% phospholipids, and 16% cholesterol esters, with the remainder as free FAs (FFA) [83, 84]. Almost all (99%) of FFA are in triacylglyceride in adipose tissue. Proper storage of samples is imperative to preserve the quality and ensure the reliability of subsequent analysis [84].

Current analytical methods used for dairy FA biomarker analysis are described in **Appendix 1**. The Folch method is the gold standard for lipid extraction from biological samples[85]. This is a highly efficient two-phase extraction to recover polar compounds and non-polar lipids in serum and adipose tissue [86]. This popular method is reproducible, quick, and provides high lipid fraction recovery [85, 87]. Gas chromatography is a powerful analytical technique to separate FA in lipids [88]. First, derivatization of FA to FA methyl esters (FAME)

is essential to stabilize and volatize the FA for effective GC separation. Acid-catalyzed transesterification is a technique in which a strong acid catalyzes the hydrolysis of complex lipids into glycerol and FA [89]. With the addition of alcohol, the latter are esterified into FAME, which can be easily recovered. The column is the main component of the GC. Columns range in length from 15-100 m, with longer columns providing greater separation of FA. The separation efficiency of FAME is based on its affinity to the column's stationary phase [90]. A polar and selective stationary phase is essential for analyzing isomeric mixtures FA found in dairy, allowing FAME to elute in order of increasing chain length and degree of saturation. The mobile phase, an inert gas, carries the volatilized FAME through the column to the detector for identification of FAME. The use of internal standards is essential to minimize systemic errors, ensure quality control and compensate for variability [84]. Particularly for the OCFA present in low concentrations, these tools are instrumental in validating FA identity and enhancing the reliability of quantitative results. The two main detectors used for FA analyses are the flame ionization detectors (FID) and mass spectrometer (MS). FID is the most common method used in FA detection as it is cost-effective and provides good selectivity and linearity. MS ionizes the FAME through a quadruple analyzer to generate a chromatograph [88]. The identity of the FA can be deduced based on the retention time in relation to reference standards and mass spectral libraries from databases such as the National Institute of Standards and Technology. GC-MS provides greater sensitivity, better selectivity, lower detection limit, and structural information of compounds compared to FID [83, 84].

c. Proportions of FA biomarkers in the general population

Proportions of FA in the general population are described in **Table 2**. The mean FA proportions varies, with oleic acid (43 mol%), palmitic acid (21.5 mol%), and linoleic acid (13.9 mol%) being the most abundant FA in subcutaneous adipose tissue [31]. Of biomarkers, levels of 15:0, 17:0, and t16:1n7 in these tissues generally range from 0.1% to 0.6% of total FA [25, 80, 91].

Table 2. Proportions of selected FA biomarkers in total plasma or serum fraction in the general population

FA	Mean [] umol/L (SD) in plasma total lipids[1]	Mean mol% (SD) in serum CE[2]	Mean serum mol% (SD) in serum PL[2]	Mean mol% (SD) in serum FFA[2]
14:0	63.6 (37.1)	1.05 (0.55)	0.52 (0.24)	3.62 (2.33)
15:0	17.8 (6.7)	0.29 (0.13)	0.29(0.10)	0.54 (0.34)
16:1 n-7	133.0 (67.2)	4.35 (2.33)	0.83 (0.45)	4.82 (2.28)
18:1 n-9	1285.5 (416.7)	19.36 (5.54)	10.00 (2.51)	34.36 (5.90)
18:2 n-6	2233.8(622.6)	49.58 (9.94)	19.01 (5.22)	14.15 (7.65)
t16:1 n-7	17.0 (9.1)			
c-9,t-11 18:2	14.4 (6.2)			

Abdelmagid et al. 2015 (n= 826; young healthy Canadian population)[1]; Bradbury et al., 2010 (n=2393; New Zealand National Nutrition Survey)[2]

d. Dietary sources of established fatty acid biomarkers

Dietary sources of the established dairy fat biomarkers are mainly obtained from ruminant products (**Appendix 2**) [15]. Odd-chained FAs are synthesized in the rumen and incorporated into bovine meat and milk [65]. Accordingly, the major contributors to 15:0 and 17:0 in the human diet are dairy and red meat. Of dairy products, the mean concentration of 15:0 and 17:0 in fluid retail milk are 1-2% and 0.5-1.5% of total FAs, respectively [92]. Other minor dietary sources include fish, some vegetables (cabbage, cucumber), seaweed, and lard [15]. Trans-fats are predominantly obtained from processed foods and oils, with only 20% provided from those naturally occurring in animal foods [93]. Ruminant trans-fat, including trans-palmitoleic acid and vaccenic acid, are exclusively derived from dairy and meat products [94].

Proportions of the established biomarkers are correlated (r = 0.2 to 0.6) to self-reported intake of total dairy and individual dairy products [80]. Of the established biomarkers of dairy intake, 15:0 in plasma/serum (total, phospholipids and cholesterol esters) and adipose tissue consistently show stronger associations with dairy compared to t16:1n7 and 17:0 [82]. These biomarkers reflect that dairy fat content has greater correlations with total dairy, followed by cheese and milk [95-97]. Similarly, prospective cohorts report higher plasma FA proportions with whole-fat dairy than low-fat dairy products [23]. Plasma 15:0 and 17:0 proportions among lacto-ovo vegetarians were similar to those of meat-eaters and significantly lower in vegans [98]. These FAs reflect dietary changes, both increases and decreases in dairy fat intake, supporting their sensitivity as biomarkers of dairy fat [18, 99]. As such, these biomarkers are considered characteristic of dairy fat intake.

1.3.2 Synthesis and metabolism of dairy FA in humans

a. Factors that influence FA proportions in serum lipid fractions and adipose tissue

The proportions of FAs in tissue and lipid fractions can be influenced by age, ethnicity, and genetics [100]. Older individuals experience altered lipid metabolism owing to reduced oxidative capacity and enzymatic activity, such as delta-6 desaturase, ultimately impacting tissue FA release and composition [101, 102]. The incorporation and concentrations of FA, shown largely for omega-3 FA, in serum and adipose tissue differ by age and sex [103-106]. Besides the influence of diet, genetics strongly regulate endogenous lipid metabolism, gene expression, and FA composition in tissues [107]. Lifestyle behaviors, including smoking, cholesterol status, and body weight, contribute to the variability of FA metabolism among individuals [108, 109]. Specific to OCFAs, adipose tissue and serum PL content were inversely associated with alcohol intake and positively correlated to increased physical activity [110].

b. Storage: Concentration and location in human tissue

Adipose tissue is the choice tissue for assessment of dietary FA due to its slower turnover than other tissues, which is representative of longer-term depot and intake of FA [31, 32]. However, common practice is to assess dairy fat biomarkers in plasma or serum as this is more accessible and affordable [105]. Triacylglycerides are the most variable of the lipid fractions as it rapidly responds to changes in dietary intakes reflecting short-term FA intake [84]. Cholesterol esters, influenced by diet, reflects intake of the past few days, and PL possesses greater stability as a biomarker of longer-term (weeks) circulating FA. The proportions of FAs and their correlations with dairy fat intake differ across all fractions, partially attributed to determinants, such as waist circumference, sex, and hypertriglyceridemia, that influence their incorporation into these fractions [28]. The correlation between biomarkers and dairy intake varies widely among cohorts, dietary assessment tools, and tissue assessment. A meta-analysis summarized the association between total dairy intake and circulating 15:0, 17:0, and t16:1n-7 in plasma/serum (assessed in CE, PL, FFA, or a combination) to be r=0.20, 0.10, and 0.08, respectively, with the highest correlations observed in adipose tissue; r = 0.34, 0.23, 0.51, respectively [82]. The correlations between dairy and biomarkers of dairy fat in adipose tissue are shown in **Table 3.**

Table 3. Correlations between dairy and biomarkers of dairy fat in adipose tissue

	Biomarkers of dairy fat intake		**Dietary Assessment**	**Author**
	C15:0	**C17:0**		
Dairy Intake	*r (95% CI)*	*r (95% CI)*		
Total dairy	0.34; (0.31, 0.36)	0.23 (0.08,0.37)	Meta-analysis	Pranger et al., 2018
	0.52 (0.37,0.65)	0.07(-0.12,0.26)	1-WR	Brevik et al.,2005
	0.31, p<0.01	0.31, p<0.01	FFQ	Baylin et al.,2002
	ρ= 0.34	ρ= 0.16	FFQ	Aslibekyan et al.,2012
	0.74 (P: < 0.001)		2 -WR; 14x 24h recalls	Wolk et al.,2001
	0.59	0.45	Dietary records, FFQ	Wolk et al.,1998
Dairy fat	0.51; (0.37, 0.63)	0.22 (0.12,0.32)	Meta-analysis	Pranger et al.,2018
Dairy Product				
Milk	0.16(-0.03,0.34)	-0.07 (0.25,0.13)	1-WR	Brevik et al.,2005
	0.55	0.42	Dietary records	Wolk et al.,1998
Cheese	0.39 (0.22,0.54)	0.06(0.14,0.24)	1-WR	Brevik et al.,2005

Abbreviations: FFQ, food frequency questionnaire, 1-WR, 1-week weighed food records.

c. Endogenous synthesis of FAs

Preliminary evidence suggests an endogenous synthesis of OCFA in humans. An indicator of this is the 2:1 ratio of 15:0/17:0 in milk fat; however, that ratio is consistently shown to be 1:2 in plasma [111]. One plausible mechanism is branched-chain amino acid (BCAA) catabolism that produces propionyl-CoA, a primer of OCFA synthesis, corresponding to increased 15:0 and 17:0 adipocyte levels observed in vitro [112, 113]. A crossover study proposed a similar mechanism for the positive association between plasma 17:0 concentration and inulin supplementation [33]. An increase in propionate concentration in serum, and by extension propionyl-CoA, is suggested through gut microbial fermentation of dietary fiber. Another potential mechanism is the alpha oxidation of straight-chain FAs in muscle and adipose tissue, which has been associated with an increase in 17:0, but not 15:0, in mice [114]. Cell models show potential bioconversion of 16:0 to 15:0 in differentiating adipocytes and propose the synthesis of 17:0 to 15:0 through elongase enzymatic activity [115, 116]. Trans-palmitoleic acid may also be endogenously synthesized through the partial β-oxidation of vaccenic acid, t18:1n7, with a 20% conversion rate [117].

1.3.3 Established fatty acid biomarkers and type 2 diabetes

Epidemiological studies have consistently shown an inverse association between the incidence of diabetes and plasma proportions of the established dairy fat biomarkers, summarized in **Table 4** [23-27]. A recent pooled analysis of prospective cohorts, including over 60 000 participants with an average follow-up of 9 years, concluded that increased individual and summed levels of 15:0, 17:0, and t16:1n-7 in circulating or adipose tissue are associated with a reduced risk of T2D, independent of major risk factors of T2D [80]. Specifically, increased levels of the sum of these biomarkers were associated with a 30% lower risk of developing T2D.

Only a few studies have used direct measures to assess IS. Based on intravenous glucose tolerance tests, insulin sensitivity was positively associated with serum 15:0, but not t16:1n7, in adults [118]. In 86 adults with metabolic syndrome, higher plasma PL 17:0, but neither 15:0 nor t16:1n-7, was associated with higher insulin sensitivity measured by intravenous glucose tolerance tests [29]. Only one cross-sectional study has assessed this relationship with the gold standard HEC and observed a direct association between PL t16:1n-7 and hepatic and systemic insulin sensitivity, but not β-cell function, in adults with non-alcoholic fatty liver disease [30]. However, these associations were no longer significant after adjustment for case-control status. Only one study has assessed this association with established biomarkers in adipose tissue. A cross-sectional analysis of 850 Swedish older men explored the association of dietary FA in SAT and insulin sensitivity assessed by gas-liquid chromatography and HEC, respectively [119]. 17:0 ($r=0.21$, $p<0.001$), but not 15:0, was correlated with insulin sensitivity in men with BMI>25 in a crude model.

Overall, the association between established dairy FA biomarkers and the pathophysiological disorders underlying T2D, insulin resistance, and β-cell dysfunction remains inconclusive. This gap in the literature may partially be attributed to the use of surrogate measures of insulin sensitivity and the lack of analysis in adipose tissue. The latter, along with their origin in other dietary sources, preliminary evidence supporting endogenous synthesis, and their relatively low correlations with dairy fat intake, warrants the identification of new biomarkers of dairy fat intake [15, 33].

Table 4. Summary of FA biomarkers on T2D

Author	Design	Population Characteristics, Participants (T2D), age	Parameters measures	FA Analysis Method	Lipid compartment	Association of FA on T2D risk			
						C14:0	C15:0	C17:0	C16:1n-7t
Krachler et al., 2008	Prospective study (5.4 y), Nested case control	291 (159), 45-60yo	Dietary intake (FFQ), incidence of diabetes	GC-FID	Erythrocyte	NS	0.65, (0.5-0.85),	0.47, (0.35-0.63)	NA
Yakoob et al., 2016	Prospective study (15y)	3333 (1364) 30-75 yo, no diabetes at baseline	Dietary intake (FFQ), incidence of diabetes	GC-FID	Plasma	NA	0.62 (0.46–0.85),	0.68, (0.50–0.91)	0.54, (0.40–0.73)
					Erythrocyte	NA	NS	0.73 (0.55–0.99)	0.54 (0.34–0.87)
Mozaffarian et al., 2010	Prospective cohort, Cardiovascular Health study (CHS)	3,736 (304), >67 yo	Metabolic risk markers, T2D incidence, Dietary intake (FFQ &24h recall)	GC-FID	Plasma phospholipids	NA	NS	NS	0.38 (0.24-0.62
Mozaffarian et al., 2013	Prospective cohort, Multi-Ethnic Study Atherosclerosis	2617 (205), 45-84 yo	Metabolic risk markers, Dietary intake (FFQ)	GC-FID	Plasma phospholipids	NS	NS	NA	0.52 (0.32–0.85)
Forouhi et al., 2014	Nested case cohort, EPIC and INTERACT studies	27296 (12403) ; Sub-cohort:1591 (755),40-60yo	Diet (FFQ or diet histories)		Plasma phospholipids	1.15 (1.09-1.22)	0.79 (0.73-0.85)	0.67 (0.63-0.71)	NA
Santaren et al., 2014	Prospective cohort (5y), US	659 (103), 55	IS via OGTT; Diet (FFQ, validated with 8 24 h DR and second FFQ	GC-FID	Serum	NA	0.73, (0.56-0.92)	NA	NS

Abbreviations: DR – dietary recall; FFQ- food frequency questionnaire; GC-FID – gas chromatography flame ionization detection; NA- not assessed; NS- not significant; OGTT- oral glucose tolerance test.

1.3.4 Putative dairy fat biomarkers

Branched-chain FAs (BCFA) may be new biomarkers of dairy fat consumption as they are synthesized from ruminal bacteria [20]. BCFAs are a class of saturated fats that contain one or more methyl group(s) and are categorized as mono-, di-, or multi-methyl BCFA. Dietary intake of BCFAs is principally obtained from ruminant sources with minimal contributions from fermented foods (1%wt in sauerkraut, 0.5%wt in miso, <0.01%wt in tempeh and tofu) and from poultry, pork, and seafood (each <0.05 %wt) [21]. Total BCFA concentrations are greatest in dairy and beef products ranging from 1.3-2.8 %wt and 1.6-1.9%wt, respectively. Daily intake of BCFAs in an American diet was estimated at 500 mg/day, originating mainly from beef and dairy foods. The intake of BCFAs from dairy at 300 mg/day is greater than combined intake from EPA and DHA at 100 mg/day. In particular, cow's milk is a significant source of BCFAs, comprising about 2% of the total FAs and is predominated by monomethyl BCFAs [120]. Monomethyl BCFAs contain an isopropyl or isobutyl group at the end of the carbon chain, denoted as *iso*- and *anteiso*- BCFAs, respectively. Of particular interest are the odd-chained monomethyl BCFAs, *iso*-15:0, *iso*-17:0, *anteiso*-15:0, and *anteiso*-17:0 (**Figure 1**), being the most abundant BCFAs in ruminant milk fat [21]. Recent evidence suggests that BCFAs may benefit cardiometabolic outcomes, including inflammation, obesity, and insulin metabolism [22]. The association between BCFAs and dairy fat intake has not been examined. However, their concentration in dairy products and minimal endogenous synthesis in humans make BCFAs an ideal candidate for biomarkers of dairy fat intake [20]. Although literature concerning BCFAs and T2D is limited, monomethyl BCFAs were indirectly associated with homeostasis model assessment (HOMA) index of insulin resistance (r= -0.28) and directly associated with skeletal muscle insulin sensitivity (ρ=0.59) assessed by HEC in obese individuals who underwent bariatric surgery [121, 122]. As such, monomethyl BCFAs may help clarify the association between dairy FAs biomarkers and IS.

1.3.5 Potential mechanisms by which dairy-derived FAs may influence T2D

The exact mechanism by which dairy-derived FAs influence T2D is unclear. OCFAs may elicit these biological effects by acting as nuclear ligands, precursors for various metabolites, and substrates for energy pathways [123]. The OCFAs, 15:0 and 17:0, may regulate lipid and glucose

metabolism and inflammatory processes by dual peroxisome proliferator-activated receptors agonist activity shown in vivo [124]. In mouse cells, 15:0 improved insulin-sensitizing and glucose uptake by increasing GLUT4 translocation to myotube membranes through AMPK signaling [125]. OCFA improves mitochondrial dysfunction, typical in T2D, by serving as anaplerotic intermediates of the citric acid cycle [126, 127]. Both 15:0 and 17:0 have anti-inflammatory and glucose regulatory action in humans attributed to the improved serum adipokine and chemokine profiles [124, 128-130]. Trans-palmitoleic acid may elicit its protective effect by inhibiting the lipotoxic effects of palmitate and mimicking cis-palmitoleic action—the latter insulin-sensitizing lipokine acts to regulate glucose-insulin metabolism and suppress hepatic de novo lipogenesis [131]. Accordingly, t16:1n7 was inversely associated with liver fat and improved glucose tolerance in NAFLD patients and case-controls [30].

Methods

2.1 Participants

This cross-sectional study assessed subcutaneous adipose tissue from a total of 58 participants with healthy weight, overweight, or obesity, without or with T2D. Participants were recruited from the Greater Montreal Area between January 2018 and November 2020. Characteristics of the participants are shown in **Table 5.** Volunteers were screened with a complete blood count, extensive biochemistry, serology, blood pressure, chest X-ray, electrocardiogram, urine analysis and a complete history and physical examination. The exclusion criteria were smoking, unstable weight during the preceding 6 months, any eating disorder, substance abuse, active medical conditions including any cancer other than skin within 5 years, abnormal electrocardiogram or chest X-ray, or medication known to affect glucose metabolism, serum creatinine > 120 µmol/L, hemoglobin < 130 g/L and positive viral serology. Ethics approval for conducting the primary study and use of data for secondary analysis was obtained from the Research Ethics Board of the McGill University Health Centre (Protocol no. 2018-3713) and McGill University (Protocol no. 22-09-049), respectively.

2.2 Experimental Procedures

Participants underwent an initial screening that assessed body weight, height, waist circumference, and blood pressure were measured. After an overnight fast, volunteers without diabetes underwent a 75-g OGTT with blood samples collected at -10, 0, 30, 60, 90, 120, and 180 minutes. Blood samples was used to measure insulin and C-peptide at 0- and 30-min. Body composition was determined by dual-energy X-ray absorptiometry (iDXA equipped with CoreScan software; GE Healthcare). Indirect calorimetry (True One system, Parvo Medics) was used to determine resting energy expenditure in the postabsorptive phase and during the clamp.

Hyperinsulinemic-euglycemic clamp procedure and adipose tissue biopsy

Insulin sensitivity was assessed by using the HEC procedure with stable isotopically-labeled glucose tracer infusion. Participants were admitted to the Center for Innovative Medicine at the RI-MUHC and in the morning, after an overnight fast, catheters were inserted into an antecubital vein for infusions and a contralateral dorsal hand vein retrograde for blood sampling. A primed

(22 µmol per kg of body weight), continuous (22 µmol per kg of body weight/minute) infusion of 6,6 ^{2}D-glucose was started and continued until the end of the clamp. After a 3-hour isotopic equilibration (basal) period, insulin (Humulin R; Eli Lilly) was infused at 40 mU per m^2/minute for 3 hours. During the hyperinsulinemic phase, a 20% glucose solution was infused at variable rates to maintain constant concentrations of plasma glucose at 5.5 mM based on measurements at 5-minute intervals. Hyperglycemic participants did not receive the glucose until reaching 5.5 mM. Blood samples for substrates, hormones and isotopic glucose enrichment (mole percent excess) were collected at 30-minute intervals starting at 90 minutes in the basal period and at 30 minutes in the hyperinsulinemic state, and at 10-minute intervals during the last 60 minutes of both states. Percutaneous muscle and fat biopsies were taken under local anesthesia using aseptic technique immediately before the start of insulin infusion and at the end of the hyperinsulinemic clamp period from the lateral portion of the thigh. The tissue was snap-frozen in liquid N_2 and stored at -80°C.

Assessment of insulin sensitivity

Glucose rates of appearance and disappearance during the basal and hyperinsulinemic states were calculated from steady-state equations, using tracer infusion rate and measured plasma isotopic enrichment, obtained at each steady-state period according to previously published methods [132]. The glucose kinetics (rate of glucose appearance, Ra, and disposal, Rd) of participants before and during HEC were calculated.

2.3 Dietary Assessment

Participants completed a 24-hour dietary recall at screening. Participants also completed a 3-day food record detailing all foods and beverages consumed in the three days preceding the study. Dietary data from both assessments were combined for analysis. Intake of dairy products and their fat content (fluid milk, cheese, yogurt, cream, butter, frozen dairy, evaporated milk, and kefir) consumed alone and in home-made mixed dishes was recorded. Energy and nutrient intakes were analyzed using Food Processor software with 80% foods classified according to the Canadian Nutrient File 2015 and the remaining according to the USDA dataset. Intake of food groups will be estimated as previously described [53, 133].

2.4 Adipose Tissue Fatty Acid analysis

Total lipids were extracted from subcutaneous adipose tissue by using the method of Folch [85]. Frozen SAT samples were weighed under liquid N_2 vapor in duplicate (10 mg per replicate). Briefly, 10 mg of adipose tissue and an internal standard solution (19:0, 15:0-d3, 17:0-d3, and 0.01% butylated hydroxytoluene) were added to 2:1 chloroform-methanol solution. SAT was homogenized by use of an ultrasonic processor and lipids were separated, twice extracted, and washed by use of 0.003N $MgCl_2$, 86:14 (v/v) $CHCl_3$/MeOH and 48:47:3 (v/v/v) MeOH/H_2O/$CHCl_3$, respectively. FAME were prepared by acid-based transesterification with 14% BF3-MeOH incubated for 60 min at 60°C [90]. FAME were extracted with a 1:1 (v/v) n-hexane/purified water and re-extracted with n-hexane. FAME were separated carried out with GC-MS (Agilent 8890 x Gas Chromatograph with 5977B Series MS detector and OpenLab software), equipped with a 100-m Select FAME fused-silica capillary column CP-7420, 0.25mm, 0.25µm in. Sample (1 µL) was injected once in split mode 5:1 into an inlet held at 250°C. The two participant replicates were run consecutively with n-hexane blanks run between participants. The oven program was as follows: 50°C for 2 min; followed by 15°C/min to 170°C for 11 min, hold time 1 min; 1°C/min to 210°C for 60 min, hold time 9 min. Post run was set at 250°C for 15 min. Helium was the carrier gas, and the column flow rate was 0.8 ml/min. A routine bake-out was set at 250°C for 10 hours. A FAME reference standard was performed each run and prepared by combining the GLC reference standard (Nu-Chek Prep, 674), BCFA FAME (Larodan, 90-1053), CLA FAME (Sigma, O5632) and 19:0 FAME (Cayman Chemicals, 20607). FA identification was confirmed by comparing mass spectra and retention times to National Institute of Standard spectra libraries and the FAME reference standard known prepared weight. This assay generated data on a total of 57 FAs detected across all samples, with 37 FA present in 90% (equivalent to 53 participants) of samples.

2.5 Statistical analysis

All variables were tested for normality according to the Shapiro-Wilks criteria. Baseline characteristics were analyzed by group, and dietary intake and FA composition will be analyzed by group using ANOVA as a parametric test or a Kruskal-Wallis test as a nonparametric test. Differences between two groups were compared using Mann Whitney-U Test. Multivariable linear regression models were used to examine the association of FA biomarkers of dairy fat

intake and measures of insulin sensitivity. The dependent variables were clamp-based measures of hepatic and peripheral insulin sensitivity, expressed as glucose Ra and Rd respectively. The independent variables were OCFA (15:0 and 17:0), BCFA (anteiso15:0, anteiso16:0, iso11:0, iso13:0, iso16:0, and iso17:0), and TFA (t16:1n7) in adipose tissue. Model 1 is a crude model; model 2 is adjusted for age, sex, and BMI; and model 3 includes covariates from model 2 plus T2D status. We also conducted group analyses by group. We considered $P < 0.05$ as the minimum acceptable statistical significance.

Results

3.1 Participant Characteristics

Table 5 summarizes characteristics of the 58 participants categorized into 3 groups: healthy weight (n=20), overweight and obese (n=12) and T2D (n=26). Data was further stratified by sex described in **Appendix 3**. The mean age was 46.5 years, 57% (n=33) were women, and 48% (n=28) were overweight or obese. Blood lipids were within normal ranges for sex and groups, except for increased LDL cholesterol in participants with overweight and obesity, and females who are of healthy weight.

Glycemic measures differed among groups. Impaired fasting glucose was observed among individuals with T2D and men in the healthy weight, and overweight and obese groups. Females with overweight and obesity exhibited impaired glucose tolerance. Fasting glucose and HbA1C levels were normal among groups without T2D. For clamp-based measures, the rate of glucose disposal during the clamp (Rd), the rate of glucose appearance during the clamp (Ra), and insulin sensitivity index (ISI) differed between groups. Individuals of healthy weight exhibited the greatest median ISI, with a 1.5-fold increase compared to those with overweight and obesity and 2.5-fold greater than those with T2D. Similarly, glucose Rd in the healthy weight group was 2-fold and 3-fold greater than in the overweight and obesity, and T2D groups, respectively. Sex differences were not performed due to an uneven ratio among groups.

Table 5. Participant Characteristics

	Healthy Weight N=20	**Ow/Ob** N=12	**T2D** N=26	p[1]
Age (years)	31 [24, 54][a]	49 [31, 55][a,b]	55 [48, 61][a]	0.002
Sex (F/M)	11/9	10/2	12/14	0.097[≠]
BMI (kg/m^2)	23.0 [21.6, 25.0] [a]	28.5 [26.6, 31.9][b]	29.4 [27.7, 34.9] [b]	< 0.001
Waist (cm)	79.1 [72.4, 82.6] [a]	94.3 [85.9, 101.1] [b]	103.7 [91.8, 111.5] [b]	< 0.001
LBM (kg)	47.7 (12.7)	48.2 (10.1)	52.5 (11.7)	0.339
Triglycerides (mmol/L)	0.85 [0.69, 1.12] [a]	1.07 [0.87, 1.70] [a,b]	1.42 [0.87, 2.00] [b]	0.008
Total cholesterol (mmol/L)	4.26 (0.80)	4.89 (1.04)	4.45 (1.11)	0.224
LDL cholesterol (mmol/L)	2.44 [1.77, 2.80]	2.69 [2.31, 3.59]	2.28 [1.92, 3.61]	0.204
HDL cholesterol (mmol/L)	1.38 [1.21, 1.68] [a]	1.27 [1.17, 1.45] [a,b]	1.12 [0.92, 1.46] [b]	0.016
non-HDL cholesterol (mmol/L)	2.85 [2.08, 3.40]	3.15 [2.85, 4.33]	2.95 [2.60, 3.93]	0.174
Systolic pressure (mm Hg)	120.5 (15.5) [a]	126.2 (13.6) [a,b]	135.0 (16.3) [b]	0.012
Diastolic pressure (mm Hg)	66.6 (10.5)	70.0 (10.0)	72.1 (9.5)	0.200
Insulin (pmol/L)	21.6 [17.4, 32.3] [a]	30.1 [26.6, 37.8] [b]	39.7 [23.7, 72.6] [b]	0.013
Glucose (mmol/L)	5.5 [5.2, 5.6] [a]	5.4 [5.2, 5.7] [a]	6.9 [6.0, 8.0] [b]	< 0.001
HbA1c (%)	5.5 [5.3, 5.7] [a]	5.5 [5.4, 5.6] [b]	6.8 [6.5, 7.5] [b]	< 0.001
2-h glucose OGTT (mmol/L)	6.0 [4.9, 6.6] [a]	7.6 [5.4, 8.0][b]	na	0.019
HOMA-IR index	0.90 [0.70, 1.32] [a]	1.27 [1.04, 1.54] [a,b]	2.15 [1.20, 3.77] [b]	0.001
Clamp-based measures				
Glucose Ra (mg/kg LBM/min)	-0.27 [-0.85, -0.10] [a]	-0.43 [-0.82, -0.05][a]	-0.12 [-0.34, 0.45][b]	0.047
Glucose Rd (mg/kg LBM/min)	10.08 [8.51, 12.20][a]	6.67 [5.44, 9.02][b]	4.13 [3.67, 5.02][c]	< 0.001
Insulin Sensitivity Index (ISI)	1.48 [1.21, 1.62] [a]	0.82 [0.67, 0.95] [b]	0.49 [0.34, 0.63][c]	< 0.001

[1] P value estimates were based on Kruskal-Wallis Test for variables expressed as median [Q1,Q3] and 1-way ANOVA for variables expressed as $\bar{x} \pm SD$ comparing distributions across 3 groups; a,b,c = T test (parametric) and Mann-Whitney U test (non-parametric) comparing distributions across 2 groups, groups without a common superscript differ at $P < 0.05$. ≠ Chi-Square test.

All values represent a fasted stated, except OGTT.

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model of assessment insulin resistance; LBM, lean body mass; ISI index = Δ Glucose Rd (mg/kg LBM/min)/clamp insulin (pmol/L) × 1000; na, not applicable; Glucose Rd, glucose rate of disposal during HEC (mg/kg LBM/min);

3.2 Dietary Data

Food group and nutrient intake assessed with a 24-h recall and 3-day dietary record are presented in **Table 6**. Food group intake did not differ between groups. The median intake for grains, and fruits and vegetables for participants was 189.1 [112.0, 243.9] g/d and 518.7 [364.6, 675.4] g/d, respectively. The median intake for protein foods was 60.5 [31.3, 123.2] g/d. The contribution from red and processed meat was 53.0 [27.0, 82.4] g/d and that from total dairy was 170.4 [87.9, 254.9] g/d. Total dairy included milk, cheese, yogurt, cream, butter, and frozen dairy products.

Participant macronutrient distribution was 50±9.4% carbohydrates, with a mean fiber intake of 21.3 [10.7, 73.5] g/d, 17.3 ± 3.8% protein and 36.0±8.5% fat. Saturated fat (10.2 [5.62, 25.3] % EI) intakes for the cohort were above the recommendations of less than 10% of total daily calories. Total energy intake differed by sex with intakes of 1801 kcal/d for females and 2446 kcal/d for males (p=0.02), No differences in macronutrient distribution and energy intakes were observed among groups.

Table 6. Self-reported nutrient intake based on a 3-d dietary record and 24-h recall

	Healthy Weight (N=20)	**Ow/Ob (N=12)**	**T2D (N=26)**	**p[1]**
FOOD GROUPS (g/d)				
Grains	203.6 [102.0, 261.8]	212.6 [146.4, 241.8]	167.8 [106.1, 228.0]	0.57
Fruits & Vegetables	533.1 [374.1, 588.3]	540.0 [345.6, 731.3]	499.2 [367.6, 631.7]	0.91
Protein Foods	78.7 [32.5, 176.7]	59.0 [36.0, 124.9]	59.8 [33.6, 126.0]	0.68
Dairy	168.3 [81.4, 237.6]	160.8 [95.5, 281.2]	178.1 [91.2, 255.0]	0.84
Red & Processed Meat	48.9 [28.8, 79.2]	39.1 [24.0, 63.8]	65.6 [28.5, 86.4]	0.49
MACRONUTRIENT & ENERGY INTAKE				
Carbohydrate (%EI)	45.7 (9.7)	50.6 (5.1)	48.9 (10.6)	0.32
Fiber (g/d)	20.5 [18.1, 30.0]	24.5 [18.2, 31.5]	20.2 [16.8, 23.7]	0.32
Sugar (%EI)	15.9 (4.5)	18.3 (7.0)	17.4 (6.8)	0.53
Protein (%EI)	17.2 (3.5)	15.3 (3.1)	18.4 (4.0)	0.06
Fat (%EI)	38.3 (8.7)	36.1 (5.1)	34.1 (9.4)	0.26
Saturated Fat (%EI)	11.3 [9.8, 13.8]	9.3 [8.3, 11.0]	9.2 [8.3, 12.5]	0.11
MUFA(%EI)	15.0 (4.2)	14.6 (3.8)	13.8 (5.0)	0.67
PUFA (%EI)	11.3 [9.8, 13.8]	9.3 [8.3, 11.0]	9.2 [8.3, 12.5]	0.11
Energy (kcal/d)	2311 [1681, 2502]	2023 [1651, 2249]	1857 [1509, 2197]	0.21

[1] P value estimates were based on Kruskal-Wallis Test for variables expressed as median [Q1, Q3] and 1-way ANOVA for variables expressed as $\bar{x}$ (SD).

Abbreviations: %EI- % of total energy intake; Ow/Ob - overweight or obese; T2D - type 2 diabetes.

3.3 Fatty Acid Distribution

A total of 57 FAs among all participants were detected, shown in **Appendix 4**, were detected with C18:1, C18:2n6 and C16:0 observed in the greatest proportions. The 11 FAs of interest are described in **Table 7**. The distribution of odd-chained FA, trans FA and BCFAs in adipose tissue are expressed as mean proportion of total FA (wt%). OCFA had the greatest proportion with C15:0 (0.91 [0.75, 1.07] wt%) followed by C17:0 (0.57 [0.52, 0.64] wt%). The proportions of the BCFAs (anteiso-C15:0, anteiso-C16:0, iso C17:0 and iso C13:0) and cis-9, trans-11 CLA were greater than the established dairy fat biomarker, tC16:1n-7, across all groups. Differences between groups were observed for the BCFAs anteiso-C15:0, anteiso-C16:0 and iso-C16:0. Anteiso-C15:0 proportions in individuals who are healthy weight were 2-fold greater than those who are overweight and obese, and 2.5-fold greater than those with T2D. Healthy weight individuals had 1.5 times greater anteiso-C16:0 proportions than those with overweight and obese. Iso-C16:0 proportions in healthy weight, and overweight and obese participants were 1.4- and 1.2-fold greater than those T2D, respectively.

Table 7. Distribution of established and putative fatty acid biomarkers of dairy fat in subcutaneous adipose tissue[1]

FA (% total FA)	Healthy Weight	Ow/Ob	T2D	p[2]
	N = 20	N = 12	N = 26	
C15:0	0.91 [0.75, 1.07]	0.76 [0.66, 0.84]	0.76 [0.66, 0.90]	0.063
C17:0	0.57 [0.52, 0.64]	0.55 [0.49, 0.63]	0.61 [0.53, 0.69]	0.528
C16.1.n7t	0.21 [0.18, 0.26]	0.20 [0.12, 0.23]	0.24 [0.13, 0.30]	0.474
anteiso C15:0	0.47 [0.26, 0.87][a]	0.26 [0.18, 0.38][b]	0.19 [0.13, 0.42][b]	0.009
anteiso C16:0	0.35 [0.26, 0.42][a]	0.25 [0.24, 0.27][b]	0.33 [0.22, 0.40][a,b]	0.043
iso C11:0	0.35 [0.00, 0.77]	0.36 [0.12, 0.69]	0.19 [0.13, 0.38]	0.498
iso C13:0	0.25 [0.09, 0.48]	0.23 [0.12, 0.31]	0.17 [0.09, 0.35]	0.607
iso C16:0	0.17 [0.13, 0.24][a]	0.19 [0.15, 0.24][a]	0.14 [0.04, 0.18][b]	0.037
iso C17:0	0.52 [0.32, 0.61]	0.54 [0.49, 0.65]	0.50 [0.23, 0.63]	0.499
cis-9, trans-11 CLA	0.33 [0.26, 0.43]	0.30 [0.16, 0.32]	0.26 [0.18, 0.34]	0.062
trans-10, cis-12 CLA	0.04 [0.03, 0.05]	0.06 [0.03, 0.06]	0.04 [0.03, 0.06]	0.731

[1] All values are expressed as mean weight percentage of total FAs, reported as median [Q1,Q3]; L - healthy weight; Ow/Ob - overweight or obese; T2D - type 2 diabetes; CLA- conjugated linoleic acid;
[2] P-value estimates were based on Kruskal-Wallis Test comparing distributions across 3 groups; a,b = Mann-Whitney U test comparing distributions across 2 groups, groups without a common superscript differ at $P < 0.05$.

3.4 Association Between Dairy Fat Biomarkers and Clamp-Based Insulin Sensitivity

In multiple linear regression models, there was a positive correlation between 3 FA in adipose tissue and clamp-based measures of insulin sensitivity (**Table 8**). In crude models, higher proportions of C15:0, anteiso-C15:0, and cis-9 trans-11 CLA were associated with higher insulin sensitivity index (ISI) and peripheral tissue insulin sensitivity (glucose Rd). These associations were remained following adjustments for predictors of insulin sensitivity, age, sex, and BMI. In this model, C15:0 (β= 4.01 [1.37, 6.64], adj-R^2= 0.655), anteiso-C15:0 (β= 2.51 [0.707, 4.31], adj-R^2= 0.646) and cis-9 trans-11 CLA (β= 5.30 [1.04, 9.57], adj-R^2= 0.673) were positively correlated with glucose Rd. The 3 FA in these models were associated with glucose Rd, independently of age, sex, and BMI (Appendix 5). In model 2 and 3, the independent contributions of C15:0, anteiso C15:0 and cis-9 trans-11 CLA to the total variance in glucose Rd was 10-15%. BMI accounted for 25-45% of the variance, age and sex for 10-15% each and by T2D status for 7-10%. Similar results are observed for ISI, as these two variables are strongly correlated. These associations remained after further adjustment for T2D status. The variability in ISI and glucose Rd can be largely attributed to BMI, followed by age, sex and T2D status. In an additional analysis, adjustment for total dairy intake did not change these associations.

Only the fully adjusted model for anteiso-C16:0 showed a positive correlation with ISI, not glucose Rd. Greater concentrations of iso-C16:0 showed improved ISI and Rd; however, the association was no longer significant when adjusting for age, BMI, and sex. Higher proportions of adipose tissue FA were associated with lower glucose rate of appearance (Ra), an indicator for hepatic glucose production and insulin resistance. However, this inverse association was not significant. The established biomarkers of dairy fat intake, C17:0, and tC16:1n7, showed inverse associations with ISI, glucose Ra and glucose Rd but were not found to be significant.

Table 8. Multiple linear regression analysis of the relation between FA in subcutaneous adipose tissue and clamp-based measure of insulin sensitivity

FA in SAT	Model[2]	*Clamp-based measure of insulin sensitivity*					
		ISI		**Glucose Ra**		**Glucose Rd**	
		β (95% CI)	**adjusted R2**	**β (95% CI)**	**adjusted R2**	**β (95% CI)**	**adjusted R2**
C15.0	1	0.983 (0.377, 1.589)*	0.144	-0.506 (-1.361, 0.350)	0.007	6.620 (2.643, 10.600)*	0.151
	2	0.670 (0.283, 1.057)*	0.672	-0.134 (-0.939, 0.671)	0.176	4.310 (1.612, 7.007)*	0.634
	3	0.619 (0.247, 0.990)*	0.702	-0.045 (-0.833, 0.742)	0.222	4.010 (1.373, 6.643)*	0.655
C17.0	1	-0.018 (-1.114, 1.077)	-0.018	-0.291 (-1.726 , 1.144)	-0.015	-0.502 (-7.722, 6.719)	-0.018
	2	-0.066 (-0.786, 0.654)	0.598	-0.069 (-1.423, 1.284)	0.174	-0.589 (-5.537, 4.358)	0.563
	3	-0.014 (-0.699, 0.671)	0.638	-0.147 (-1.464, 1.169)	0.222	-0.284 (-5.058, 4.500)	0.593
C16.1.n7t	1	-0.054 (-0.394, 0.285)	-0.016	-0.290 (-0.729, 0.149)	0.013	-0.167 (-2.407, 2.073)	-0.018
	2	-0.037 (-0.253, 0.179)	0.598	-0.265 (-0.665, 0.135)	0.201	-0.050 (-1.536 1.435)	0.563
	3	-0.034 (-0.239, 0.171)	0.638	-0.270 (-0.657, 0.118)	0.250	-0.031 (-1.465, 1.403)	0.593
anteiso.C15.0	1	0.810 (0.442, 1.177)*	0.245	-0.431 (-0.978, 0.117)	0.025	5.230 (2.786, 7.667)*	0.234
	2	0.434 (0.171, 0.698)*	0.666	-0.102 (-0.645, 0.441)	0.176	2.710 (0.871, 4.557)*	0.624
	3	0.399 (0.146, 0.652)*	0.696	-0.042 (-0.573, 0.489)	0.222	2.510 (0.707, 4.307)*	0.646
anteiso.C16.0	1	0.885 (-0.362, 2.132)	0.018	-1.010 (-2.66, 0.629)	0.009	6.260 (-1.940, 14.460)	0.023
	2	0.680 (-0.112, 1.472)	0.619	-0.531 (-2.05, 0.991)	0.182	4.210 (-1.258, 9.683)	0.581
	3	0.832 (0.0876, 1.576)*	0.670	-0.751 (-2.24, 0.733)	0.236	5.120 (-0.135, 10.380)	0.621
iso.C11.0	1	0.313 (-0.075, 0.701)	0.028	0.066 (-0.455, 0.586)	-0.017	2.450 (-0.082, 4.988)	0.046
	2	-0.005 (-0.279, 0.270)	0.597	0.262 (-0.249, 0.773)	0.190	0.307 (-1.579, 2.193)	0.564
	3	-0.028 (-0.289, 0.233)	0.638	0.298 (-0.198, 0.794)	0.243	0.171 (-1.654, 1.996)	0.593

iso.C13.0	1	0.142 (-0.328, 0.612)	-0.011	-0.099 (-0.717, 0.519)	-0.016	0.687 (-2.416, 3.791)	-0.014
	2	-0.024 (-0.323, 0.276)	0.598	0.019 (-0.544, 0.582)	0.174	-0.450 (-2.504, 1.604)	0.564
	3	0.029 (-0.258, 0.316)	0.638	-0.061 (-0.612, 0.491)	0.222	-0.144 (-2.149, 1.861)	0.593
iso.C16.0	1	1.200 (0.298, 2.106)*	0.097	-1.060 (-2.288, 0.166)	0.034	8.540 (2.637, 14.440)*	0.115
	2	0.483 (-0.143, 1.109)	0.615	-0.365 (-1.563, 0.834)	0.180	3.410 (-0.888, 7.709)	0.583
	3	0.391 (-0.212, 0.993)	0.649	-0.220 (-1.396, 0.956)	0.223	2.870 (-1.333, 7.073)	0.607
iso.C17.0	1	-0.249 (-0.880, 0.381)	-0.007	0.403 (-0.421, 1.228)	-0.001	-1.110 (-5.283, 3.055)	-0.013
	2	-0.075 (-0.492, 0.342)	0.598	0.286 (-0.495, 1.067)	0.182	-0.327 (-3.196, 2.542)	0.563
	3	-0.123 (-0.520, 0.273)	0.640	0.360 (-0.399, 1.119)	0.235	-0.614 (-3.390, 2.162)	0.595
C18.2. cis.9.trans.11	1	1.250 (0.273, 2.219)*	0.0891	-0.570 (-1.910, 0.771)	-0.005	9.490 (3.203, 15.770)*	0.125
	2	0.723 (0.072, 1.373)*	0.632	0.037 (-1.241, 1.315)	0.174	5.300 (0.860, 9.743)*	0.605
	3	0.723 (0.109, 1.337)*	0.673	0.037 (-1.205, 1.278)	0.221	5.300 (1.040, 9.567)*	0.637
C18.2. trans10.cis12	1	0.265 (-4.640, 5.169)	-0.018	2.850 (-3.539, 9.237)	-0.004	3.740 (-28.6, 36.071)	-0.017
	2	0.447 (-2.640, 3.535)	0.598	2.570 (-3.196, 8.330)	0.186	5.070 (-16.1, 26.260)	0.565
	3	0.457 (-2.473, 3.387)	0.638	2.550 (-3.042, 8.147)	0.234	5.120 (-15.3, 25.560)	0.595

[1] Values expressed as β coefficients (95% CIs);
[2] Model 1, unadjusted; model 2, adjusted for age, sex, and BMI; model 3, adjusted as for model 2 + T2D status;
*Statistically significant association based on t-tests, $P < 0.05$.

Discussion

The present study explored the association between 11 established and potential biomarkers of dairy fat in adipose tissue and clamp-based measures of IS. In this cross-sectional analysis, we observed a positive association between C15:0, anteiso-C15:0, and CLA cis-9, trans-11 in adipose tissue, and peripheral insulin sensitivity assessed by using an HEC. These correlations remained after adjusting for age, sex, BMI, and T2D status. An inverse association was observed between adipose tissue FA and glucose Ra, a proxy of hepatic insulin resistance, but was not significant. These findings offer insight into the role of dairy consumption in preventing T2D and encourage further investigation into new biomarkers of dairy fat intake.

Only one other study has assessed this association with established biomarkers of dairy fat in adipose tissue. In contrast to our findings, C17:0 ($\beta = 3.5$ [0.3, 6.6]), but not C15:0, was positively correlated to clamp-based insulin sensitivity when adjusted for BMI, smoking, alcohol intake, and physical activity [119]. This discrepancy may reflect the differences in the population, overweight elderly Swedish men (n=719), and the adjustment for lifestyle covariates. The results observed in plasma and serum are scarce and inconsistent. Total serum C15:0, not t16:1n-7, has been shown to positively correlate to insulin sensitivity as assessed by intravenous GTT in 659 adults [118]. Similar associations were observed for plasma phospholipid C17:0, but not C15:0 or t16:1n-7, in 86 adults with metabolic syndrome [29]. Only one cross-sectional study assessed this relationship with HEC-derived data, and observed a direct association between plasma phospholipid t16:1n-7 and hepatic and systemic insulin sensitivity in 32 adults [30]. However, each of these associations was attenuated and no longer significant after adjustments for covariates. The associations observed in our study may be attributed to measuring FA in adipose tissue, which serves as a longer-term FA depot and shows stronger correlations with total dairy intake compared to plasma and serum [82]. Other factors may include sample size, adjusted covariates, and methods used to assess insulin sensitivity and FA.

Established biomarkers of dairy fat have consistently shown an inverse association with the incidence of T2D [23-27]. Contrary to expected results, the present study observed an inverse association between C17:0 and t16:1n7, and IS. Similar trends were shown with t16:1n7 in a multiethnic cohort [118]. This may be due to the contribution of t16:1n7 from partially hydrogenated oils, as samples from this cohort were collected during the phase-out period (2018-

2020) of trans fats imposed by Health Canada [134]. It is suggested that the association between trans fats, and coronary heart disease and all-cause mortality is driven by industrial sourced trans fats, rather than ruminant ones [3]. Further, C17:0 shows weaker correlations with total dairy intake as compared to C15:0 [82, 135], indicating that it may serve as a less specific marker. Increased dairy intake is proposed to reduce liver fat content, eliciting their positive effects on insulin action [30, 136]. However, a 20-week supplementation of C17:0 in a high-fat diet did not improve insulin resistance or hepatic metabolism in mice [137]. This study showed that only C15:0 improved insulin-mediated AKT phosphorylation, which may drive the insulin sensitivity benefits. This offers a potential mechanistic explanation for our observed results that increased adipose tissue C15:0 concentrations improved ISI and glucose Rd. Other mechanisms by which C15:0 operate include enhancing insulin-sensitizing and glucose uptake through increased myotube GLUT4 translocation [125] and improving mitochondrial function by serving as anaplerotic intermediates of the citric acid cycle [126, 127]. C15:0 and C17:0 have shown anti-inflammatory and glucose regulatory action in humans attributed to the improved serum adipokine and chemokine profiles [124, 128-130, 137]. Among the established biomarkers, C15:0 shows the most robust correlation with total dairy intake [82] and could reflect the influence of the bioactive components of dairy, such as calcium, probiotics, and protein [69]. The exact mechanism by which dairy-derived FA influence insulin sensitivity is unclear and warrants further investigation.

The relationship between individual BCFAs and CLAs in adipose tissue and insulin sensitivity has not been reported. To our knowledge, this is the first study to show that anteiso-C15:0 is positively associated to peripheral insulin sensitivity. It has been reported that the sum of adipose BCFAs (including iso-C15:0, anteiso-C15:0, and iso-C17:0) was directly associated (ρ=0.59) with skeletal muscle insulin sensitivity in an unadjusted model [122]. Similarly, total serum BCFAs were inversely associated with HOMA insulin resistance in individuals with morbid obesity [121]. Adipose tissue is a site of BCFA *de novo* synthesis via branched-chain amino acid (BCAA) catabolism [138]. BCFAs in serum and adipose were reduced in participants with obesity than in healthy weight participants and increased following weight loss [121, 122, 139]. Likewise, our study found higher *anteiso* proportions in healthy weight participants compared to those with obesity and T2D. This trend coincides with increased FA synthase gene expression and clearance of circulating BCAA [121]. Considering the association of increased

BCAA with both skeletal insulin resistance and metabolic dysfunction [140], this inverse relationship with BCFAs may clarify their beneficial action on IS. This may be reflected in our study as insulin sensitivity was influenced by BMI, potentially implying that the metabolism of these FA is altered with adiposity. Other mechanisms could be at play, as BCFAs have been associated with reduced inflammation and serum triacylglyceride concentrations [20, 121]. Regarding CLA cis-9 trans-11, our findings support the observed reduced risk of T2D with increased adipose CLA cis-9 trans 11 concentrations [141]. However, clinical studies are inconclusive on the effects of its supplementation on insulin sensitivity [142]. The evidence of CLA bioactivity on glucose metabolism is conflicting and the mechanisms of action are largely unknown.

Fatty acids were assessed in adipose tissue owing to its stronger correlations with total dairy intake and individual FA biomarkers compared to serum and plasma [82]. Adipose tissue FA concentrations were expressed as a percent of total FA as in previous studies. Compared to our findings, C15:0 and C17:0 were reported at lower concentrations in overweight older men (0.20 and 0.31, respectively), in women with obesity (0.18 and 0.21, respectively) and in a large cohort (0.19 and 0.21, respectively) [91, 119, 143]. Adipose t16:1n-7 was also found at lower concentrations (0.08) [91]. Only one study assessed BCFAs in adipose tissue and found lower concentrations for anteiso-C15:0 (0.016) and iso-C17:0 (0.087) [122]. Inconsistencies may be due to differences in FA analytic methods, the number of FA detected, and population characteristics. We observed greater concentrations of individual BCFAs than the commonly assessed dairy fat biomarker, t16:1n7. In addition, their primary dietary source is from dairy foods, and minimal endogenous synthesis in humans makes these FA ideal candidates as biomarkers for assessing dairy fat intake [20].

Analysis of Fatty Acids

The method used for FA isolation and quantification was not without challenges, posing some issues and uncertainty in FA identification. The Folch method for lipid extraction was optimized for adipose tissue by use of an ultrasonic processor for homogenization and by adding extra washing and extraction steps. Roughly 20 mg of SAT was processed in duplicate due to the limited amount of sample. However, due to intrinsic variability in the extraction procedure, triplicate with less tissue per replicate could have helped reduce analytical variability. The

chloroform-methanol solution included BHT and internal standards C15:0-d3, C17:0-d3, and C19:0. BHT interfered with the elution of some FA (i.e.; C13:0); therefore, a different antioxidant should be selected moving forward. C19:0 was present in adipose tissue samples and contributed to total C19:0 in a variable manner among participant, which prevent us from using this FA as an internal standard. C21:0 should be used as a "true" internal control. C15:0-d3 and C17:0-d3 were included as stable isotope internal standard, but we were unable to unambiguously distinguish these and the endogenous FA. Stable isotopes are seldom used in current literature assessing OCFA, which commonly use FID detection, and it may be best not to include these as standards or use heavier isotopes when commercially available. For these FA of low concentration, these are essential tools to validate their identity as well as to minimize systematic errors [84].

Derivatization of lipids from adipose tissue was completed using BF_3-MeOH (60°C for 60 min) after testing and comparing various incubation times, reagents, and temperatures. To quantify FAMEs, the processing (optimizing integration parameters) and acquisition (optimizing selected ion monitoring and scan modes) methods in the OpenLab software were developed to detect and report the FAMEs. After much troubleshooting, the final method was able to detect and identify peaks with sufficient resolution. Baseline increases in the chromatographs were observed after running several samples, potentially because of the tissue matrix. This was resolved by a regular column burning; 15 min following daily runs and 10-h overnight bake following 3 days of runs (equivalent to 50 samples). Subsequently, a n-hexane blank was run alone to verify the return of the baseline. To validate the GC-MS method, parameters including selectivity, reproducibility, and recovery were performed [144]. The identification of FA was performed by comparing the RT to the purchased standards (GLC reference standard (Nu-Chek Prep, 674), BCFA FAME (Larodan, 90-1053), CLA FAME (Sigma, O5632) and 19:0 FAME (Cayman Chemicals, 20607)) and to mass spectral libraries. This was critical for identifying isomers, as MS is unable to locate double bond positions. This is a strength as much research within this field not only use FID detection, incapable of providing structural information for double checking FA identity, but also only use a GLC reference standard with fewer than 40 FA [84]. Given the low concentrations of the biomarkers of interest and the fact that varying detection parameters (i.e.; column length and split ratio) lead to different elution times, this step is critical to ensure correct FA identification. However, the use of spectral library searches was

unreliable for FA excluded from reference standards as there were multiple possible identities with low probability (<40%) of compound certainty. Therefore, there were some unidentifiable FA and presence of contaminants. The most abundant FA in the sample tissue, i.e.; C18:2n-6, were broadened and obscured the detection of the surrounding FA. This was potentially due to the saturation of the detector owing to the tissue matrix. This may be resolved by injecting the sample at a 1:5 or 1:50 split or using FID detection which provides a greater range.

Even with the advancements in our method, improvements are required. The GC-MS correction factors used to calculate FA concentration (wt%) for FA not included in the reference standards were assumed to be 1. The values of the OCFA presented in this study are an overestimation as they reflect the co-elution of both the endogenous and internal standards added. The order of FA elution occasionally differed between participant replicates and required manual correction. Moving forward adaptations should include optimizing acquisition methods to be more selective for FA of interest, adjusting sample injection through dilution and increasing split ratio, and completing all necessary validation tests, such as calibration curve. Therefore, the identity of these FA cannot be fully ascertained, and the interpretation of the presented results should be made with caution.

As for future research in this field, greater transparency is required in methods used for FA analysis. This study used a 100-m column which allows for greater resolution of FAMEs and is particularly important for isomers separation (i.e., CLAs and trans-FA). However, most studies use shorter columns (30 m), FID detection, lack internal standards and less specific reference standards, described in **Appendix 1.** This makes it not only more difficult to reproduce, but to trust that the FA reported are those detected. This field requires standards for analytical methods and reporting requirements, including a full description of chromatography programming. Future studies are needed to validate the current findings troubleshooting and implementing the challenges in the FA protocol.

Strengths, Limitations and Future Directions

To our knowledge, this is the first study to assess odd-chained FA, trans-FA, and individual BCFAs in adipose tissue and to explore their association with insulin sensitivity assessed by HEC. The quantification of FA using GC-MS provides greater sensitivity, a lower detection limit and a well-established database for FA identification when compared to the commonly used FID

[84]. In contrast to common protocols for FA quantification in this field, our method involved GC method validation, the use of internal standards, and reliance on multiple reference standards. Although a positive correlation was found, a causal relationship cannot be inferred because of the cross-sectional nature of the study. The HEC was an effective method for studying whole body and tissue insulin sensitivity. However, it was not an optimal model for examining the correlation between variables of interest and glucose Ra, since the HEC was precisely designed to completely suppress glucose Ra. To address this limitation, a clamp involving lower insulin infusion rates is necessary to allow for a greater range of Ra values and to examine of the association between FA and hepatic insulin sensitivity. Sex differences could not be computed due to an uneven ratio among groups. Although the primary determinants of insulin sensitivity were adjusted for, additional covariates, such as lifestyle and dietary factors, may contribute to this relationship.

The association between biomarkers of dairy fat intake in serum lipid fractions, cholesterol esters and phospholipids, and insulin sensitivity should be explored. Current literature on this relationship is inconclusive which may be because most studies have relied on surrogate indices of insulin sensitivity, which lack sensitivity and specificity of HEC. Moreover, the studies have measured the FA biomarkers in different blood lipid fractions, some of which may not reflect dairy fat intake. It would be valuable to investigate how adipose tissue FA profiles are reflected in plasma phospholipid fractions, or even erythrocytes, which are more accessible samples to collect. To understand the relationship between biomarkers of dairy fat and IS, it would be valuable to explore the correlation of FA concentrations with total dairy intake, dairy product (cheese, milk, yogurt), fat content (reduced, regular), and processing method (fermented, non-fermented). In addition, the potential of BCFA concentrations in serum and adipose tissue as biomarkers of dairy fat intake could be examined based on these preliminary results. These studies would provide a more comprehensive understanding on the role of dairy intake on insulin sensitivity in humans.

We conclude that increased proportions of FA (C15:0, anteiso-C15:0, and CLA cis-9, trans-11) in adipose tissue are associated with higher insulin sensitivity. The results offer a possible explanation for the previously observed association between biomarkers of dairy fat intake and T2D. As a preliminary study, these findings require confirmation with a validation study.

Additional studies are needed to assess the relationship between BCFAs and dairy intake to explore their potential as biomarkers.

Conclusion

In the present study, we evaluated the association between established biomarkers of dairy fat intake (C15:0, C17:0, t16:1n-7) in adipose tissue and clamp-based measures of insulin sensitivity. In a multivariable analysis adjusted for age, sex, BMI and T2D status, FA C15:0, anteiso-C15:0, and CLA cis-9, trans-11 in subcutaneous adipose tissue were positively associated with peripheral insulin sensitivity. Additional studies are required to confirm the observed results. The results imply that the improvement of insulin sensitivity elucidates the association between the established biomarkers of dairy fat intake and T2D. To further understand the relationship between biomarkers of dairy fat and insulin sensitivity, its essential to investigate the correlation between these FA and dairy consumption. In addition, there is need to explore the potential of BCFAs as biomarkers of dairy fat intake. This field requires greater transparency and implementation of robust methods, particularly concerning the analytical techniques used for FA identification.

Appendix

Appendix 1. Analytical methods used for dairy fatty acid analysis

Study	Tissue	Fraction	FA Measure	Internal Standard	Lipid extraction	Fractionation	Derivatization	Column	Detection method	FA detected
Berriozabalgoitia et al 2021	Plasma Erythrocytes	n/a		undecanoic (11:0), tridecanoic (13:0), and nonadecanoic (19:0) acids	SPE (modified method of Bondia-Pons)	n/a	2.5 mL of 0.5 M sodium methoxide in methanol followed by 2.5 mL of 14% boron trifluoride in methanol	100 m CpSil88 capillary column	FID	43
Baylin et al 2002	Adipose	n/a			hexane and isopropanol (3:2 by vol) mixture	n/a	methanol and acetyl chloride	fused silica, capillary *cis/trans* column (100 m × 250 mm internal diameter, with a 0.20-µm film; SP2560; Supelco, Bellefonte, PA)	FID	35
Nestel et al 2014	Plasma	PL		C13:0				SGE BPX70		
Santaren et al 2014	Serum	Total	C15:0 t16:1n-7		Folch	n/a	sulfuric acid/methanol	30-m HP-88 capillary column	FID	35
Kratz et al 2015	Fasting plasma	PL, FFA			Folch	1-dimensional TLC	methanol-benzene 4:l with acetyl chloride (Lepage method)	Supelco fused-silica 100-m capillary column SP-2560	FID	46
Zong et al (2014)	Erythrocyte	Total	*trans*-18:1 isomers		hexane and isopropanol	n/a	methanol and sulfuric acid	Agilent 6890 GC; SP-2560 capillary column: 100 m × 0.25 mm internal diameter × 0.2 µm film;	FID	

Study	Tissue	Fraction	FA Measure	Internal Standard	Lipid extraction	Fractionation	Derivatization	Column	Detection method	FA detected
Johnston et al 2016	Fasting serum	PL, CE		2-diheptade-canoyl-sn-glycero-3-phosphocholine and cholesteryl heptade-canoate (17:0)	Folch	TLC			FID	22
Forouhi et al., 201)	Plasma	PL			Folch	SPE	14% boron trifluoride (BF3)/methanol solution	HP-88, 30 m length, 0·25 mm internal diameter, film thickness of 0.2 µm	FID	37
Ma et al., 2015	Plasma	PL	palmitic stearic , oleic , myristic , 7-hexadecenoic , and vaccenic acid			one-dimensional TLC	methanol-benzene 4:l with acetyl chloride (Lepage method)	Supelco fused silica capillary column SP-2560 (100 m × 0.25 mm, 0.2 μm)	FID	
Mozaffarian et al., 2013	Fasting blood	PL ,CE, TAG	*trans* 16:1n–7		Using a mixture of chloroform:methanol (2:1, v/v) under nitrogen	TLC	14% boron trifluoride in methanol, incubated at 80°C for 90 min, and then extracted with petroleum ether	Varian CP7420 capillary 100m column	FID	27
Mozaffarian et al., 2013	Plasma	PL	*trans* 16:1n–7		Folch	One dimenaional TLC	direct transesterification using methods of Lepage	SP-2560 fused-silica 100m capillary column	FID	41
Yakoob et al ., 2016	Plasma, Erythrocytes	Total	15:0, 17:0, t16:1n7		N/a	N/a	Methanol and acetyl chloride	fused silica, capillary *cis/trans* column (100 m × 250 mm internal diameter, with a 0.20-µm film; SP2560; Supelco, Bellefonte, PA)	GLC-FID	40

Study	Tissue	Fraction	FA Measure	Internal Standard	Lipid extraction	Fractionation	Derivatization	Column	Detection method	FA detected
Krachler et al., 2008	Erythrocyte (EMFA)	Total	14:0, 15:0, 16:0,17:0, 18:0,18:2n-6,18:3n-3, 20:3n-6, 20:4n-6,20:5n-3,22:4n-6,22:5n-3,22:6n-3		N/a	N/a	2 ml 5% H2SO4 in methanol, transmethylated at 60oC overnight	25-m glass capillary column coated with SLP OV-351	GLC	
Santaren et al., 2019	Serum	PL, TAG, CE, FFA		17:0 in FFA; cholesteryl heptadecanoate; 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine; 1,2,3-triheptadecanoylglycerol	Folch method	TLC; heptane/diethyl ether/glacial acetic acid (60:40:2)	14% boron trifluoride in methanol at 100 °C for 1 h	100-m CP-Sil 88	FID	52
Sawh et al., 2021	Plasma	Total	C15:0, C17:0, iso-C17:0, and anteiso-C17:0	d31 C16:0, d3 C14:0, d3 C15:0, d3 C17:0, d3 C18:0, d2 C18:1n9, d3 C22:0, and d4 C24:0	Folch	N/A	500 µL 2% H2SO4 in MeOH and incubation at 50 °C for 2 hours	Select FAME column (100 m × 0.25 mm i.d.)	MS	
Prangere t al., 2019	Plasma	CE,PL, TAG	Trans-C18:1(n-7) and CLA	CE (100 µl of a solution of 50.1 mg C17:0/100 ml chloroform-methanol, 2:1 v/v) and TG (100 µl of a solution of 19.9 mg of C19:0/100 ml chloroform-methanol, 2:1 v/v	Folch	aminopropyl SPE columns	methanolic-HCL	200 m × 0.25 mm polar column (CP Select for FAME)	FID	
Brevik et al., 2005	Serum, Adipose		15:0, 17:0		Serum: Folch (dichloromethane/methanol (2/1; vol/vol) Adipose: hexane/isopropanol (3/2; vol/vol)			100-m-long SP 2560 column (diameter 0.2 mm, phase layer 0.20 mm, Supelco, CA, USA)	GLC	

Appendix 2. Fatty acid proportions in dairy products

FAs	Milk					Cheese	Butter
	Mean in retail milk (g/100 of FA)[1]	US milks (wt%)[2]	Retail milk (%wt/w of total FA, and of total BCFAs)[3]	Retail milk (%wt)[4]	Retail milk (%wt)[5]	Cow cheeses	Canadian butter wt% [2]
Even SFA							
C4:0	4.15	4.5				1.1	5.31
C6:0	2.13	2.3				2.4	2.81
C8:0	1.19	1.3				1.8	1.56
C10:0	2.59	2.7				3.4	3.14
C12:0	2.87	3				2.4	3.39
C14:0	9.53	10.6				12.5	10.78
C16:0	28.08	28.2				27.9	28.13
C18:0	11.68	12.6				12.8	10.62
Sum SFA	62.22	65.2					65.74
OCFA							
C11:0	-	0.3					0.3
C13:0	-	0.2					0.2
C15:0	0.89	1			1.104		1
C17:0	0.52	0.6			0.557		0.57
Sum OCFA	1.41	2.1			1.661		2.07
TFA							
t16:1n7	0.29						0.27
t18:1n7	1.48	1.7					
BCFA							
isoC13:0				0.02-0.03	0.04		0.13
isoC14:0		0.1	0.13(6.4)	0.08-0.22	0.089		0.15
iso C15:0		0.7	0.13(6.6)	0.13-0.44	0.224		0.3
iso C16:0			0.31 (14.9)	0.17-0.45	0.209		0.29
iso C17:0		0.7	0.26 (12.7)	0.26-0.56	0.272		0.52
Iso C18:0			0.04 (1.9)	0.01-0.09			0.09
anteiso C13:0		-		0.07-0.09	0.083		
anteiso c15:0			0.56 (27.5)	0.37-0.93	0.462		0.49
anteiso c17:0			0.61 (29.9)	0.11-0.76	0.501		0.5
Sum BCFA			2.04	1.66-3.44	2.213		2.47
MUFA							
c14:1	0.82					0.9	0.9
C16:1	1.48	1.8				1.2	1.38
C18:1	23.58	21.4				20.8	20.84
PUFA							
c18:2	3.19	2.9				1.2	2.25
c18:3	0.83					0.8	0.56
CLA							
C18:2 cis9,t11	0.55					0.8	
others						0.005	

Abbreviations: Fatty acid (FA), Saturated FAs (SFA), odd chain FAs (OCFA), branched chain FAs (BCFA), polyunsaturated FAs (PUFA), monounsaturated FAs (MUFA), trans FAs (TFA), conjugated linoleic acid (CLA), percentage of total FAs (%wt); O'Donnell-Megaro et al., 2011[1]; Jenson et al., 2002 [2]; Ran-Ressler et al.,2014 [3];Taormina et al., 2020 [4];Vlaeminck et al., 2006[5]

Appendix 3. Participant Characteristics stratified by sex and group

	Healthy Weight		Ow/Ob		T2D		p[1]
	F (N=11)	M (N=9)	F (N=10)	M (N=2)	F (N=12)	M (N=14)	
Age (years)	28 [24, 52]	33 [25, 54]	50 [36, 57]	35 [32, 39]	55 [49, 62]	55 [49, 58]	0.002
BMI (kg/m^2)	22.9 [21.7, 23.7]	23.7 [21.6, 26.5]	28.4 [26.4, 34.5]	28.7 [28.2, 29.3]	30.9 [27.5, 37.7]	29.3 [28.4, 33.2]	< 0.001
Waist (cm)	74.4 [71.6, 78.4]	85.60 [79.9, 86.0]	94.3 [83.0, 103.9]	94.4 [91.9, 96.8]	97.7 [86.2, 111.3]	104.0 [98.3, 115.0]	< 0.001
LBM (kg)	40.0 (6.68)	57.1 (12.2)	45.2 (8.0)	63.3 (0.64)	43.8 (7.0)	59.9 (9.6)	0.34
Triglycerides (mmol/L)	0.88 [0.71, 1.15]	0.82 [0.65, 0.96]	1.07 [0.89, 1.79]	0.91 [0.78, 1.03]	1.47 [0.80, 1.94]	1.40 [0.98, 1.92]	0.008
Total cholesterol (mmol/L)	4.40 (0.95)	4.09 (0.58)	4.90 (1.11)	4.85 (0.93)	4.74 (0.92)	4.19 (1.22)	0.28
LDL cholesterol (mmol/L)	2.66 [1.54, 2.87]	2.30 [2.02, 2.70]	2.69 [2.28, 3.46]	3.19 [2.90, 3.48]	2.41 [2.27, 2.73]	2.04 [1.49, 3.62]	0.20
HDL cholesterol (mmol/L)	1.61 [1.33, 1.73]	1.28 [1.14, 1.44]	1.31 [1.12, 1.56]	1.24 [1.22, 1.26]	1.32 [1.07, 1.54]	1.02 [0.90, 1.22]	0.016
Non-HDL cholesterol (mmol/L)	3.00 [1.95, 3.40]	2.70 [2.30, 3.10]	3.15 [2.80, 4.23]	3.60 [3.25, 3.95]	3.20 [2.83, 3.78]	2.60 [2.50, 4.05]	0.17
Systolic pressure (mm Hg)	113.4 (14.4)	128.4 (13.1)	125.7 (13.6)	128.5 (19.1)	134.4 (21.5)	135.54 (10.5)	0.012
Diastolic pressure (mm Hg)	63.1 (9.6)	70.4 (10.6)	69.0 (8.9)	75.0 (18.4)	68.7 (9.9)	75.23 (8.1)	0.20
Insulin (pmol/L)	17.7 [14.01 26.0]	28.6 [20.7, 33.6]	32.9 [26.3, 38.5]	28.6 [27.8, 29.3]	47.6 [24.6, 78.3]	34.9[24.3, 67.8]	0.013
Glucose (mmol/L)	5.2 [5.1, 5.6]	5.6 [5.4, 5.6]	5.4 [5.1, 5.6]	5.7 [5.5, 5.8]	6.5 [5.9, 7.8]	7.3 [6.5, 8.5]	< 0.001
HbA1c (%)	5.5 [5.2, 5.7]	5.5 [5.3, 5.6]	5.5 [5.4, 5.7]	5.4 [5.4, 5.5]	6.7 [6.3, 7.2]	7.05 [6.7, 7.7]	< 0.001
2-h glucose OGTT (mmol/L)	6.1 [4.8, 6.5]	6.0 [4.9, 6.5]	7.6 [5.8, 8.1]	6.0 [5.1, 7.0]	na	na	0.019
HOMA-IR index	0.71 [0.61, 1.07]	1.19 [0.93, 1.33]	1.29 [1.00, 1.59]	1.20 [1.14, 1.27]	2.23 [1.30, 4.03]	1.96 [1.24, 3.39]	0.001
Clamp-based measures							
Glucose Rd (mg/kg LBM/min)	11.82 [9.19, 13.28]	9.83 [8.21, 10.51]	7.36 [5.74, 9.07]	5.81 [5.49, 6.12]	4.81 [3.84, 7.90]	3.82 [3.29, 4.38]	< 0.001
Glucose Ra (mg/kg LBM/min)	-0.26 [-1.34, 0.38]	-0.29 [-1.05, -0.070]	-0.49 [-1.13, 0.39]	-0.21 [-0.39, -0.020]	-0.26 [-1.29, 1.50]	0.040 [-0.50, 1.86]	0.047
Insulin Sensitivity Index (ISI)	1.47 [1.21, 1.84]	1.48 [1.24, 1.56]	0.82 [0.66, 1.04]	0.79 [0.74, 0.84]	0.54 [0.43, 0.81]	0.46 [0.27, 0.62]	< 0.001

[1] P value estimates were based on Kruskal-Wallis Test for variables expressed as median [Q1, Q3] and 1-way ANOVA for variables expressed as x(SD).
All values represent a fasted stated, except OGTT.
Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model of assessment insulin resistance; LBM, lean body mass; ISI index = Δ Glucose Rd (mg/kg LBM/min)/clamp insulin (pmol/L) × 1000; Glucose Rd, glucose rate of disposal during HEC (mg/kg LBM/min);

Appendix 4. Distribution of total fatty acid detected in adipose tissue using GC-MS

Fatty Acid	Mean wt % of total FA[1]	SD	Detected in n participants
iso.C11.0	0.35	0.36	44
C12.0	0.45	0.59	53
iso.C13.0	0.29	0.30	54
C14.0	5.28	1.82	58
C14.1.n5t	0.18	0.18	56
C14.1.n5	0.93	0.51	58
anteiso.C15.0	0.40	0.33	57
C15.0	0.83	0.21	58
C15.1.n5t	0.019	0.04	17
iso.C16.0	0.18	0.15	57
C15.1.n5	0.25	1.10	39
C16.0	16.61	2.47	58
C16.1.n7t	0.29	0.42	55
C16.1.n9cis..LC.	1.71	0.69	58
C16.1.n7	6.70	2.12	58
C16.1n5..LC.	0.35	0.50	52
UFA	0.028	0.055	17
anteiso.C16.0	0.32	0.11	58
C17.0	0.59	0.13	58
C17.1n7	0.18	0.21	43
iso.C17.0	0.46	0.23	57
C18.0	4.06	1.02	58
C18.1	30.79	4.06	58
C18.2n.6tt.9.12	0.50	2.07	58
UFA	0.00	0.02	2
C18.2n3.6..LC.	0.06	0.12	14
C19.0	3.30	4.99	58
C18.2n.6cc	18.07	2.35	58

C19.1n.12t	0.20	0.68	53
C19.1n.9t	0.08	0.04	57
C18.3n.6	0.18	0.14	58
C20.3n.3.6.9..LC.	0.16	0.24	56
C18.3n.3	1.72	0.71	58
C20.0	0.37	0.80	46
C18.2.cis.9.trans.11	0.28	0.15	58
C18.2.trans10..cis12	0.05	0.03	56
C20.1n.11..LC.	0.12	0.24	39
C20.1n9	0.88	0.32	58
C18.2.n9.12..LC.	0.12	0.21	56
C22.2n.9.16..LC.	0.05	0.04	49
C20.2n.9	0.06	0.052	51
UFA	0.03	0.084	22
C21.0	0.01	0.054	1
C20.2n.6	0.35	0.10	58
C20.3n.9.12.15..LC.	0.053	0.06	55
C20.3n.6.9.15	0.075	0.18	38
C20.3n.6	0.36	0.17	57
C20.4n.6	0.93	0.35	58
C22.0	0.001	0.01	2
C22.1n.9t	0.008	0.02	12
C22.1n.9	0.20	0.09	57
C20.5n.3	0.23	0.11	58
C23.0	0.01	0.04	7
C22.2n.6	0.01	0.05	5
C22.4	0.28	0.16	58
C22.5n.6	0.05	0.025	56
C24.1.n.9	0.0040	0.022	3

[1] All values are expressed as mean weight percentage of total FAs

Abbreviations: FA- fatty acid; LC- FA identity with low confidence; UFA – unidentified FA;

Appendix 5. Individual associations of covariates from multiple linear regression analysis

Fatty acid in SAT	Model	Independent variable	*Clamp-based measure of insulin sensitivity*					
			ISI			**Rd_HEC**		
			β (95% CI)	**p value**	**R2**	**β (95% CI)**	**p value**	**R2**
C15.0	1	C15:0	0.983 (0.377,1.589)	0.002	0.159	6.620 (2.64, 10.6)	0.002	0.166
	2	C15:0	0.67 (0.283, 1.057)	0.001	0.185	4.31 (1.612, 7.007)	0.002	0.162
		Age	-0.014 (-0.02, -0.008)	<0.001	0.289	-0.073 (-0.116, -0.031)	0.001	0.183
		Sex (M)	-0.103 (-0.267, 0.062)	0.216	0.029	-1.788 (-2.935, -0.641)	0.003	0.156
		BMI	-0.05 (-0.064, -0.035)	<0.001	0.471	-0.315 (-0.416, -0.214)	<0.001	0.424
	3	C15:0	0.619 (0.247, 0.990)	0.002	0.177	4.008 (1.373, 6.643)	0.004	0.152
		Age	-0.01 (-0.017, -0.004)	0.002	0.163	-0.051 (-0.098, -0.005)	0.031	0.086
		Sex (M)	-0.063 (-0.223, 0.098)	0.436	0.012	-1.553 (-2.689, -0.416)	0.008	0.127
		BMI	-0.042 (-0.057, -0.027)	<0.001	0.377	-0.271 (-0.378, -0.164)	<0.001	0.332
		T2D	-0.253 (-0.456, -0.05)	0.015	0.108	-1.483 (-2.92, -0.045)	0.043	0.076
anteiso C15:0	1	anteiso C15:0	0.810 (0.442 , 1.18)	<0.001	0.258	5.230 (2.790 , 7.670)	<0.001	0.247
	2	anteiso C15:0	0.434 (0.171, 0.698)	0.002	0.171	2.714 (0.871, 4.557)	0.005	0.141
		Age	-0.013 (-0.019, -0.007)	<0.001	0.260	-0.068 (-0.111, -0.024)	0.003	0.157
		Sex (M)	-0.106 (-0.272, 0.06)	0.204	0.030	-1.817 (-2.977, -0.656)	0.003	0.157
		BMI	-0.047 (-0.062, -0.031)	<0.001	0.416	-0.296 (-0.402, -0.190)	<0.001	0.371
	3	anteiso C15:0	0.399 (0.146, 0.652)	0.003	0.161	2.507 (0.707, 4.307)	0.007	0.13
		Age	-0.01 (-0.016, -0.003)	0.005	0.14	-0.046 (-0.093, 0.001)	0.055	0.069
		Sex (M)	-0.066 (-0.228, 0.096)	0.418	0.013	-1.578 (-2.728, -0.428)	0.008	0.127
		BMI	-0.039 (-0.055, -0.024)	<0.001	0.328	-0.253 (-0.364, -0.142)	<0.001	0.286
		T2D	-0.254 (-0.459, -0.05)	0.016	0.107	-1.499 (-2.955, -0.043)	0.044	0.076

C18:2 c9, t11	1	C18:2 c9, t11	1.250 (0.273, 2.220)	0.013	0.105	9.490 (3.200, 15.800)	0.004	0.14
	2	C18:2 c9, t11	0.723 (0.072, 1.373)	0.030	0.086	5.302 (0.860, 9.743)	0.020	0.097
		Age	-0.013 (-0.020, -0.007)	<0.001	0.237	-0.066 (-0.111, -0.022)	0.004	0.145
		Sex (M)	-0.087 (-0.267, 0.092)	0.335	0.017	-1.638 (-2.863, -0.412)	0.010	0.119
		BMI	-0.053 (-0.068, -0.038)	<0.001	0.482	-0.334 (-0.437, -0.231)	<0.001	0.442
	3	C18:2 c9, t11	0.723 (0.109, 1.337)	0.022	0.097	5.304 (1.040, 9.567)	0.016	0.107
		Age	-0.009 (-0.016, -0.002)	0.012	0.112	-0.041 (-0.089, 0.006)	0.089	0.055
		Sex (M)	-0.038 (-0.211, 0.135)	0.665	0.004	-1.344 (-2.546, -0.141)	0.029	0.088
		BMI	-0.044 (-0.06, -0.028)	<0.001	0.377	-0.28 (-0.389, -0.171)	<0.001	0.338
		T2D	-0.291 (-0.502, -0.079)	0.008	0.128	-1.726 (-3.193, -0.26)	0.022	0.097

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