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Metabolic responses to physical activity in subjects with type 1 diabetes

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Metabolic responses to physical activity in subjects with type 1 diabetes

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To Paulo,
Mariana and André

To Celito and Anna

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ABBREVIATIONS

T1D – type 1 diabetes
T2D – type 2 diabetes
DCCT - Diabetes Control and Complications Trial
EDIC - Epidemiology of Diabetes Interventions and Complications
UKPDS – United Kingdom Prospective Diabetes Study
IDF - International Diabetes Federation
ADA – American Diabetes Association
AHA – American Heart Association
ACC – American College of Cardiology
CV – cardiovascular
CVD – cardiovascular disease
CVR – cardiovascular risk
CAD – coronary artery disease
DCCT/EDIC - Diabetes Control and Complications Trial / Epidemiology of the Diabetes Interventions and Complications
OR – odds ratio
RR – relative ratio

GLUT4 - glucose transporter type 4
GLUT1 - glucose transporter type 1
AMPK - 5' AMP-activated protein kinase or 5' adenosine monophosphate-activated protein kinase
IMCL - intramyocellular lipid
EMCL - extramyocellular lipid
^{1}H-NMR, or 1H NMR - proton nuclear magnetic resonance

VO_2max, synonym of VO_2peak - maximal oxygen uptake, in mL/kg/min
VO_2peak, synonym of VO_2max - maximal oxygen uptake, in mL/kg/min
BMI – body mass index
WHR – waist/hip ratio
MET – Metabolic Equivalent of Task (equivalent to [4.184 kJ] • kg-1 • h-1)
IPAQ - International Physical Activity Questionnaire
eGDR - estimated glucose disposal rate - in mg/kg/min
DEXA, or DXA - dual-energy X-ray absorptiometry
ECG – electrocardiogram
HOMA - homeostatic model assessment
IR – insulin resistance

GC-MS – gas chromatography-mass spectrometry
MRI – magnetic resonance imaging
MRS – magnetic resonance spectoscopy
3T – 3 Tesla
UI_IMCL – ratio olefinic/methylene protons in intramyocellular space
UI_EMCL – ratio olefinic/methylene protons in extramyocellular space
PCA – principal component analysis

VLDL – very low density lipoprotein
LDL – low density lipoprotein
IDL – intermediate density lipoprotein
HDL – high density lipoprotein
TG – triglyceride

RLP – Remnant lipoprotein cholesterol (RLPc)

Liposcience Technique
VLDLCP – Total VLDL and Chylomicron Particles (in nmol/L)
VLCP – Large VLDL and Chylomicron Particles (in nmol/L)
VMP – Medium VLDL Particles (in nmol/L)
VSP – Small VLDL Particles (in nmol/L)

LDLP – Total LDL Particles (in nmol/L)
IDLP – IDL Particles (in nmol/L)
LLP – Large LDL Particles (in nmol/L)
LSP – Total Small LDL Particles (in nmol/L)
LMSP – Medium Small LDL Particles (in nmol/L)
LVSP – Very Small LDL Particles (in nmol/L)

HDLP – Total HDL Particles (in µmol/L)
HLP – Large HDL Particles (in µmol/L)
HMP – Medium HDL Particles (in µmol/L)
HSP – Small HDL Particles (in µmol/L)

VZ – VLDL Size (in nm)
LZ – LDL Size (in nm)
HZ – HDL Size (in nm)

Partial least square (PLS) regression
LDL-C – Total LDL Cholesterol (in mg/dL)
Large LDL-C - Large LDL Cholesterol (in mg/dL)
Medium LDL-C - Medium LDL Cholesterol (in mg/dL)
Small LDL-C - Small LDL Cholesterol (in mg/dL)

HDL-C - Total HDL Cholesterol (in mg/dL)
Large HDL-C – Large HDL Cholesterol (in mg/dL)
Medium HDL-C – Medium HDL Cholesterol (in mg/dL)
Small LDL-C – Small LDL Cholesterol (in mg/dL)

VLDL-TG – Total VLDL, fraction Triglyceride (in mg/dL)
Large VLDL-TG – Large VLDL, fraction Triglyceride (in mg/dL)
Medium VLDL-TG – Medium VLDL, fraction Triglyceride (in mg/dL)

Small VLDL-TG – Small VLDL, fraction Triglyceride (in mg/dL)

LDL-TG – Total LDL, fraction Triglyceride (in mg/dL)
Large LDL-TG – Large LDL, fraction Triglyceride (in mg/dL)
Medium LDL-TG – Medium LDL, fraction Triglyceride (in mg/dL)
Small LDL-TG – Small LDL, fraction Triglyceride (in mg/dL)

HDL-TG – Total HDL, fraction Triglyceride (in mg/dL)
Large HDL-TG – Large HDL, fraction Triglyceride (in mg/dL)
Medium HDL-TG – Medium HDL, fraction Triglyceride (in mg/dL)
Small HDL-TG - Small HDL, fraction Triglyceride (in mg/dL)

I. PREFACE

Changing type 1 diabetes natural clinical history

Type 1 diabetes mellitus is an autoimmune chronic disease that has undergone drastic changes on its clinical natural history in the last decades.

Until the early XX century, the diagnosis of diabetes would mean a fatal outcome in few weeks or months. The evidence of hyperglycemia associated with abrupt polyuria, polydipsia, polyphagia and weight loss in a child or a young person determined a diagnosis of failure of insulin secretion, metabolic catabolism and death.

The introduction of treatments with exogenous insulin was the first important change in the natural clinical history of diabetes. Subjects affected by diabetes experienced then a hope of treatment and life. The main initial concern of physicians and scientists was to avoid important episodes of hypo or hyperglycemia, which could lead to hypoglycemic coma or diabetic ketoacidosis. Different classes of insulins were tested and used with success, offering better life expectancy for the affected persons.

In parallel with the provided increase of life expectancy, chronic complications related to diabetes were prone to appear. Those patients who, at that moment, benefit from the exogenous insulin, but maintained many episodes of hyperglycemia and glycemia fluctuations, developed chronic complications leading to blindness, renal failure, limbs amputations and/or cardiovascular complications as heart attack or stroke.

Aware of the consequences of hyperglycemia, researchers started to design studies promoting a more strict control of glucose levels, with the intention of minimizing chronic complications related to diabetes. Studies for type 1 diabetes (T1D) like DCCT, published in 1993 (The Diabetes Control and Complications Trial Research Group, 1993) and its follow-up EDIC (Nathan et al., 2005), and others alike for type 2 diabetes (T2D) (UK Prospective Diabetes

Study, UKPDS Group 1998), proved the reduction of complications rates and marked a new change in the natural clinical history of diabetes.

Recently, tighter glycemic control became possible with the help of new insulins, insulin infusers, glucose sensors and nutrition research. The incidence of retinopathy, nephropathy, neuropathy and their consequent serious outcomes as blindness, end renal stage disease and lower limb amputations, reduced in the past two decades, as described in the US patients with diabetes (Gregg et al., 2014). The excess risk of mortality in individuals over 20 years old with diabetes (T1D and T2D) if compared with the risk of individuals without diabetes has decreased over time in both Canada and the UK, as shown in data recently published (M Lind et al., 2013). This may be, in part, due to the earlier diagnosis, as well as to improvements in diabetes care (M Lind et al., 2013). In patients with T1D, a reduction of all-cause mortality and also of specific cardiovascular mortality could be verified, especially if associated with a good glycemic control; these rates, nevertheless, are still the double of the ones seen in subjects without diabetes (Marcus Lind et al., 2014).

Physical activity is considered as a health promoter procedure for general population and a therapeutic tool for prevention and/or treatment of several chronic diseases, like T2D, cardiovascular disease or cancer. Persons with T1D are stimulated to participate in exercise training programs and competition events. Nowadays, with the current knowledge, several elite athletes with T1D are able to compete in the same categories that the ones without diabetes, but requiring for that a strict balance among insulin adjustments, carbohydrate intake and physical activity characteristics.

Many questions may be formulated at the present time: are persons with T1D being beneficiated from physical activity as persons without diabetes? Do the subjects with T1D present the same physical conditions for exercise performance than the non-diabetic ones? Do they present different metabolic response when performing a session of exercise? Does physical activity improve lipoprotein profile generating cardiovascular benefits for the subjects with T1D? What are the characteristics of muscular composition of patients with

T1D, and are they different from subjects without diabetes? What are the factors that could be interfering?

These questions are discussed in the present thesis. Some answers were achieved and some other questions emerged. Nowadays, the availability of new technological approaches, the improvements on basic research, and the possibility to integrate the information of basic research with clinical research are improving the knowledge in biomedical science. A better understanding of physiopathology can be obtained, and with it, a better care, a better quality of life, and longer life expectancy can be offered to persons who have type 1 diabetes.

II. INTRODUCTION

1. Diabetes mellitus

1.1. Definitions and epidemiology

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2014a). Type 2 diabetes (T2D) is the more prevalent type of diabetes, and presents a worldwide increasing incidence associated with rising rates of obesity and with the worsening of lifestyle, which comprises diet habits and sedentary lifestyle (International Diabetes Federation, 2013). Type 2 diabetes is characterized by different degrees of insulin resistance with relative insulin deficiency. Many different countries are suffering the increasing incidence of this type of diabetes, in which the prevalence rates can reach, for example, 12.3% in U.S.A. population (National Center for Chronic Disease Prevention and Health Promotion, 2014) or more than 20% in some Western Pacific Islands (International Diabetes Federation, 2013). The differences on these rates depend on ethnology, weight changes and lifestyle (Figure 1).

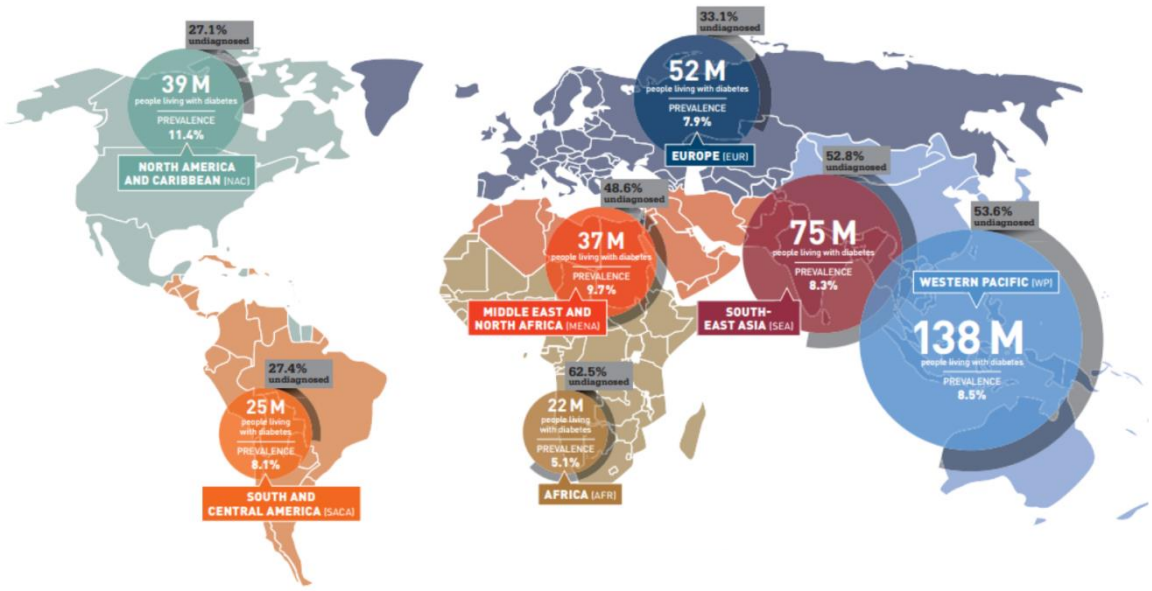


Figure 1: IDF (International Diabetes Federation) Diabetes Atlas of global diabetes prevalence. Sixth edition – 2014 update (Mena, Sea, & Lucia, 2014).

In Spain, the Di@abet.es Study (Soriguer et al., 2012) provided actualized data of prevalence of diabetes in people 18 years old and older. The Di@bet.es Study was a national, cross-sectional, population-based survey conducted during 2009–2010. The study identified that approximately 13.8% of the population had diabetes (7.8% with known and 6% with unknown diabetes) and 14.8% had prediabetes, counting for a total of 28.6% of the studied population with some carbohydrate metabolic disturbance (Figure 2). A previous review study in 2007 (Valdés, Rojo-Martínez, & Soriguer, 2007), established that the prevalence in Spain was between 10 and 15%. That data was obtained from different regional registers and counted with different diagnostic criteria. Even then, the authors called attention to the increase in diabetes prevalence that was taking place over the years.

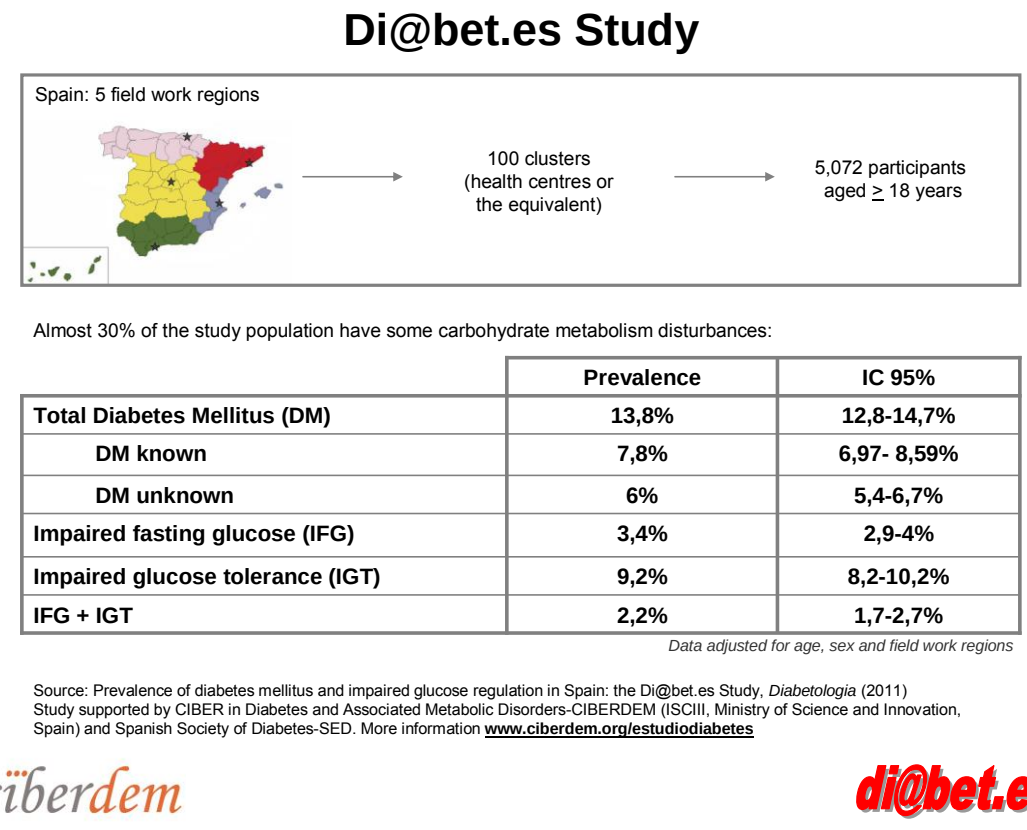


Figure 2: Official slide from diabetes prevalence in Spain from Di@bet.es Study. IFG: impaired fasting glucose. IGT: impaired glucose intolerance. IC: confidence interval (Soriguer et al., 2012)

Some other specific and less frequent types of diabetes are genetic defects of β-cell function (like maturity onset diabetes of young, or MODY); genetic defects of insulin action, like specific resistance pathologies; disease of exocrine pancreas, as chronic pancreatitis; some endocrinopathies, like Cushing Syndrome or acromegaly; drugs, like glucocorticoids; or a particular population that presents diabetes during gestation (gestational diabetes).

The second more prevalent type of diabetes is the type 1 diabetes (T1D). According to American Diabetes Association (ADA) 2015's statement (American Diabetes Association, 2015b), it accounts for 5–10% of all types of diabetes. This type of diabetes was previously encompassed by the terms insulin-dependent diabetes or juvenile-onset diabetes, due to the usual onset on early ages and early insulin requirements. It is a result of a cellular-mediated autoimmune destruction of the β-cells of the pancreas. The usual markers of

this immune destruction of the β-cell include autoantibodies to insulin (IAAs), autoantibodies to anti-glutamic acid decarboxylase (GAD), autoantibodies to the tyrosine phosphatases IA-2 and IA-2β, and the more recently described antibodies to zinc transporter 8 - ZnT8 (ZnT8As) (Lampasona et al., 2010; Vermeulen et al., 2011). Type 1 diabetes is defined by the presence of one or more specific antibodies. In addition, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and these HLA-DR/DQ alleles can be either predisposing or protective.

Type 1 diabetes has many different prevalences depending on the population studied, which could be attributed to genetics and/or to the environment (Patterson et al., 2012). According to a recent review (Canivell & Gomis, 2014), the incidence of T1D is increasing worldwide at the rate of 3-5% per year. The annual increase rate of T1D was 5.4% in children aged 0-4 years between 1989 and 2003, and the trends predict doubling the incidence in this age group by 2020. In International Diabetes Federation (IDF) 2013's reports, the estimation rate of overall annual increase of T1D is around 3% in children under 15 years old, increasing more abruptly in some Central and Eastern European countries, where the disease is less common (International Diabetes Federation, 2013).

In a recent publication, Barreiro and colleagues (Barreiro et al., 2014) could estimate the incidence of T1D in Spain, using different studies with their regional incidences. The regional incidences in children under 15 years old oscillated from 11.5 cases/100,000 inhabitants/year in Asturias to 27.6 cases/100,000 inh/year in Castilla - la Mancha. From the data obtained in that study, there was not an increment of incidence of T1D in the last 20 years in some autonomic communities (Catalonia, Extremadura, Galicia, Madrid Community, Basque Country), different from other regions (Andalucía, Aragón, Cantabria, Navarra Community and Murcia Region) that presented a raise of incidences, especially in the last 5-10 years. Just a few Spanish regions have estimative of prevalences, that varies from 0.95 cases/1,000 inh (Badajoz) to 1.53 cases/1,000 inh (Cantabria). The study estimated a mean annual incidence of T1D is 17.69 cases/100,000 inh/year in Spain, but observed the

importance of a better register for new cases and encouraged the register in the regions where these data are not available.

1.2. Physiopathology of type 1 diabetes

Type 1 diabetes is the result of β-cell destruction of pancreatic islets of Langerhans, vast majority in consequence of autoimmunity (American Diabetes Association, 2015b). Autoimmune destruction of β-cells has distinct genetic predispositions and is also related to environmental factors that are still poorly defined. Persons affected by T1D are also prone to develop associated autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac disease and others (ADA 2015).

As reviewed by Jaberi-Douraki M and colleagues (Jaberi-Douraki, Liu, Pietropaolo, & Khadra, 2014), the disease is triggered by various factors in genetically predisposed individuals. It is mediated by autoreactive β-cell specific helper CD4+ and cytotoxic CD8+ T lymphocytes that infiltrate the islets and destroy up to 90% of the total β-cell population. The destruction of β-cells ultimately leads to the reduction of insulin secretion and eventually the induction of abnormally high levels of blood glucose in these individuals. The activation and recruitment of T-cells to the islets, along with the increased release of proinflammatory cytokines, finally drive β-cell destruction and increase the workload on surviving β-cells. This, in turn, is suggested to elevate stress in the endoplasmic reticulum (ER), the compartment where various proteins including insulin are synthesized, exacerbating β-cell loss (Figure 3).

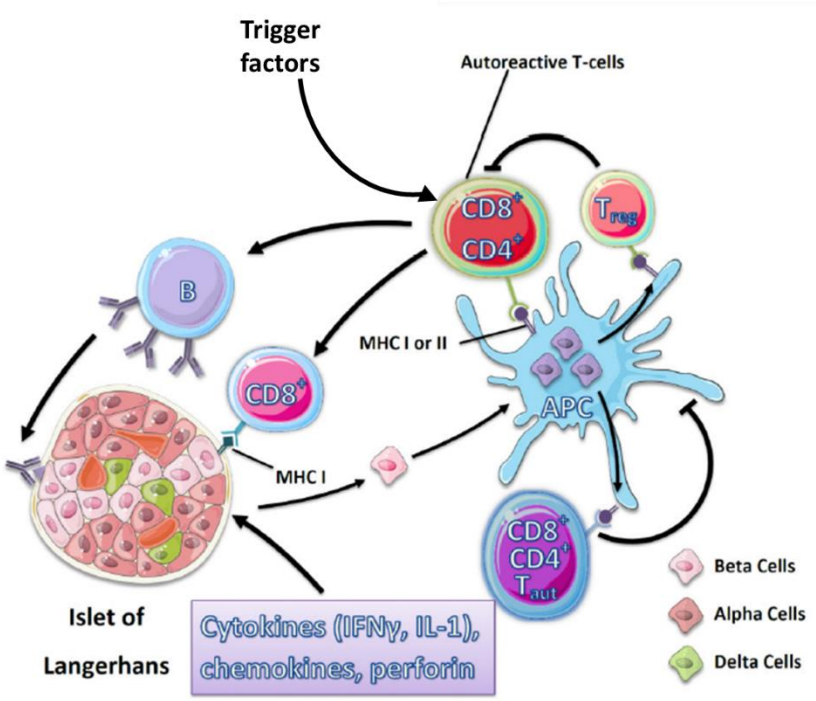


Figure 3. Scheme showing the various components of the autoimmune response in T1D. Trigger stimulus leads to the activation of various classes of islet-specific CD4+ and CD8+ T-cells (i.e., Th-lymphocytes, regulatory T-cells and autoregulatory T-cells as well as B-cells). High avidity cytotoxic T-lymphocytes destroy β-cells by either secreting harmful cytokines, or by inducing apoptosis via cell-to-cell contact. Mature B-cells release islet specific autoantibodies that may appear prior to disease onset. Figure extracted and modified from Jaberi-Douraki (Jaberi-Douraki et al., 2014)

In this type of diabetes, the rate of β-cell destruction is variable, being faster in some individuals, especially in the younger ones. Some patients, particularly children and adolescents, may present ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia, associated with classic symptoms of polyuria, polydipsia, weakness and weight loss that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years. Once the disease is stablished, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide (American Diabetes Association, 2014a).

1.3. Microvascular complications in type 1 diabetes

Chronic complications related to diabetes are primarily the micro and macrovascular diseases. The main targets of microvascular disease are eyes, kidneys and nervous system (American Diabetes Association, 2014b).

Diabetic retinopathy is a highly specific vascular complication of both T1D and T2D, with prevalence strongly related to the duration of diabetes (American Diabetes Association, 2015d). In addition to the duration of diabetes, the most important risk factors for retinopathy include chronic hyperglycemia, associated nephropathy and hypertension. Studies like DCCT could demonstrate that an intensive diabetes management with the goal of achieving near-normoglycemia could prevent and/or delay the onset and progression of diabetic retinopathy (The Diabetes Control and Complications Trial Research Group, 1995b).

Diabetic nephropathy occurs in 20–40% of patients with diabetes and is the single leading cause of end stage renal disease. Persistent albuminuria in the range of 30–299 mg/24h has been shown to be an early stage of diabetic nephropathy in T1D. Persistent albuminuria is a well-established marker of increased cardiovascular disease (CVD) risk (American Diabetes Association, 2015d). Intensive diabetes management with the goal of achieving near-normoglycemia has been shown to delay the onset and progression of increased urinary albumin excretion in patients with T1D (The Diabetes Control and Complications Trial Research Group, 1995a).

Diabetic neuropathy is a heterogeneous chronic related complication, whose symptoms vary according to the class of fibers involved (American Diabetes Association, 2015d). The most common is the distal polineuropathy with symptoms that involve small fibers, and the symptoms include pain, dysesthesias and numbness. The major clinical manifestations of diabetic autonomic neuropathy include resting tachycardia, exercise intolerance, orthostatic hypotension, constipation, gastroparesis, erectile dysfunction, sudomotor dysfunction, impaired neurovascular function, and, potentially, autonomic failure in response to hypoglycemia. Tight and stable glycemic

control, implemented as early as possible, has been shown to effectively prevent the development of distal polineuropathy and autonomic neuropathy in patients with T1D for many years (J. Albers et al., 2010; The Diabetes Control and Complications Trial Research Group, 1995c, 1998).

1.4. Cardiovascular disease in type 1 diabetes

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in individuals with diabetes. According to the last Standards of Medical Care from ADA (American Diabetes Association, 2015a), in T2D, CVD usually coexists with other cardiometabolic risk factors, like hypertension, dyslipidemia and/or obesity. In T1D, however, hypertension is often the result of underlying nephropathy. It is known that young subjects with T1D that were diagnosed in childhood have also a high risk of early subclinical and clinical CVD (Trevor J Orchard, Forrest, Kuller, & Becker, 2001; Skrivarhaug et al., 2006). The American Heart Association (AHA) categorizes children with T1D in the highest level for cardiovascular risk and recommends both lifestyle and pharmacological treatment for those with dyslipidemia, in particular, with elevated low density lipoprotein (LDL) cholesterol levels (Kavey et al., 2006).

The natural history of CVD in diabetes has been changing in the last decades with the identification of the major risk factors associated, like hypertension, hyperglycemia, dyslipidemia, obesity and smoking and with the improvement on prevention approaches and treatments. Cardiovascular disease was identified as an important cause of morbi-mortality in subjects with T1D since the ‘70 decade, and was mainly associated with nephropathy, end stage renal disease and hypertension, as referred before (Christlieb et al., 1981; Krolewski et al., 1987).

A Pittsburg register from 1984 (Dorman et al., 1984) reported a sevenfold excess in mortality risk in patients with T1D when compared with the rates expected from U.S. population of the same age. The main cause of death was

related to renal disease in younger subjects with an important increment in CVD in the ones that were older than 30 years. Krolewski and colleagues (Krolewski et al., 1987) studied the coronary artery disease (CAD) in Joslin Clinic patients. They observed that CAD mortality increased with age and was the main cause of death at ages 45-59 years. In the cumulative mortality analysis, the rates indicate that, by age 55, about 35% of the group had died from CAD and that the risk for women was similar to that for men. The study contrasted these results with the Framingham Heart Study, a population-based survey published in the same period (Lerner & Kannel, 1986), where the mortality of nondiabetic subjects at that age was 8% for men and 4% for women.

The DCCT (The Diabetes Control and Complications Trial Research Group, 1993) study represented a milestone in the treatment and evolution of chronic complications related to diabetes. The DCCT revealed a significant reduction of microvascular complications in the group that were treated more intensively (multiple insulin doses, with mean HbA1c ~ 7%) and also verified a trend toward a lower risk of CVD. The 9-year follow-up study from the DCCT, the DCCT/EDIC Study (Nathan et al., 2005), showed that participants previously randomized to the intensive arm had a significant reduction of 57% in the risk of nonfatal myocardial infarction, stoke or CVD death compared to those that were previously assigned as standard group, despite the two groups presented similar glycemic control in the follow-up trial (Figure 4).

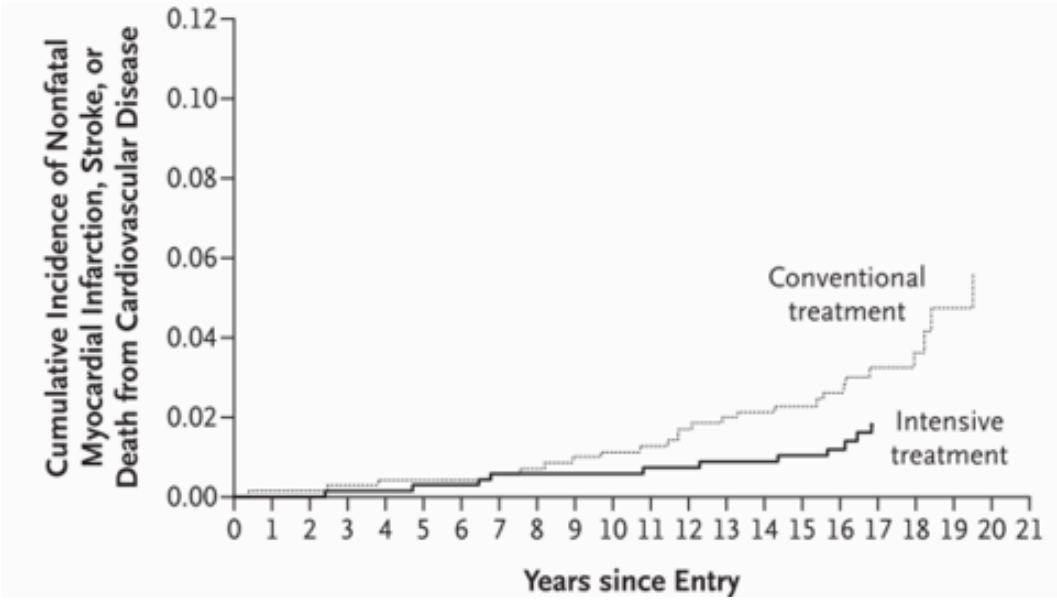


Figure 4 – First ocurrence of nonfatal myocardial infarction, stroke, or death from cardiovascular disease. Figure extracted from DCCT/EDIC Study (Nathan et al., 2005)

In 2006, Orchard and colleagues (Trevor J Orchard et al., 2006) reviewed the causes and the risk factors associated with CAD in T1D. Some complications related to diabetes, as nephropathy and autonomic cardiac neuropathy, as well as the pro-inflammatory state and alterations in lipoprotein profile, especially concerning T2D, were pointed as risk factors. The topic of discussion of the review was the weak relationship between glycemic control and CAD events in the studies that involved subjects with T1D. They indicate that DCCT/EDIC advocates a very strong benefit for early, intensive glycemic management, although it is still unknown to what degree this finding is mediated via other pathways. They finish the review by stating the importance of rigorous glycemic, lipid and blood pressure control, while better strategies are yet to be found.

Giannini and colleagues (Giannini, Mohn, Chiarelli, & H, 2011), some years later, also reviewed different aspects of macrovascular disease in T1D, focusing in the importance of recognition of the risk factors and in the importance of actions for prevention and treatment early in the childhood. The authors point out the severity degrees of atherosclerosis, intima media thickness of the

carotids and aorta, as well as endothelial dysfunction, present in the macrovascular pathophysiology of T1D. In more recent studies, it has being evidenced that fluctuations of glycemic control in patients with T1D are implicated in endothelial dysfunction, lower flow-mediated brachial dilatation and higher carotid and femoral intima-media thickness, and are imputed as aggravators for preclinical atherosclerosis (Ceriello et al., 2012; Giménez et al., 2011).

Despite advances in diabetes care, UK mortality rates in the past decade continued to be higher in patients with T1D than in those without diabetes (Soedamah-Muthu et al., 2006). A nationwide study from Scotland in 2012 (Livingstone et al., 2012) examined major CVD and deaths in T1D. The age-adjusted incidence rate ratio for first CVD event associated with T1D versus non diabetes was higher in women (RR 3.0: 95% CI 2.4-3.9 p <0.001) than men, while all-cause mortality associated with diabetes was comparable in men (RR: 2.6) and women (RR: 2.7). In Spain, in a cross-sectional study from medical records in the years 1990-2010, it was identified an overall increased rates of CVD of in subjects with T1D (OR 2.32) (Ortega et al., 2015).

In a more recent follow-up, from 1998 to 2011 in Sweden, patients with T1D presented a reduction of all-cause mortality and also of specific cardiovascular mortality, especially if associated with a good glycemic control, when compared with previous studies. Another study from Canada and UK also identified a reduction of risk mortality in individuals over 20 years old recently (Lind et al. 2013). Nowadays, the prevalence of CVD in countries like Sweden, nevertheless, is still the double of the one seen in subjects without diabetes, and the prevalence increases with the worsening of the glycemic control and with the presence of nephropathy, as pointed by Lind and colleagues in 2014 (Lind et al., 2014). These numbers indicate that relative risk for CVD is still high in the population with T1D, but it is declining with the passing of the years. This may be in part due to the earlier detection of risk factors, as well as to improvements in diabetes care.

As already referred above, there are also evidences of higher rates in coronary heart disease in women with diabetes (Peters, Huxley, & Woodward, 2014). A statement from AHA/ADA regarding CVD in T1D calls the attention to the usual lower rates of CVD in premenopausal women, compared to men, that are erased in the case of T1D (de Ferranti et al., 2014). These aspects in women with T1D again drive our attention to the necessity of better understanding the CV risk factors in order to propose better approaches.

2. Physical activity

2.1. Effects and benefits of physical activity in general population

Several studies show the importance of physical activity for both prevention and treatment of many established atherosclerotic cardiovascular risk factors, once cardiovascular disease (CVD) as the leading cause of morbidity and mortality worldwide (Eckel et al., 2014). Statements published by many organizations like the Council on Clinical Cardiology (Thompson et al., 2003), by American College of Cardiology and American Heart Association (Eckel et al., 2014) , as well as the World Health Organization (Lee et al., 2012; World Health Organization, 2014), highlighted the importance of physical activity, or structured exercise, for the management of risk factors for CVD, including hypertension, insulin resistance and glucose intolerance, dyslipidemia (elevated triglyceride and/or low high-density lipoprotein cholesterol concentrations) and obesity. Physical activity is also advised for the prevention of breast and colon cancer, for lowering the risk of depression and for the improvement of well-being in the general population.

Children and adolescents may also benefit from the promotion of healthy lifestyles. Different physical activity programs are encouraged with the objective of improving skeletal health, cardiorespiratory fitness, psychological health and blood lipid profile in different environments like school, home or institutions (Trost & Loprinzi, 2008). Health care and governmental institutions accredit the importance of physical activity for the promotion of health and prevention of diseases, and many programs of incentive are constantly being promoted (World Health Organization, 2014).

The importance of physical activity for the protection against cardiovascular mortality was recognized and published in some interesting studies in the 60’s. For example, one of the first observational studies on physical activity was performed in England (Heady, Morris, Kagan, & Raffle, 1961), and showed that drivers, that had a sedentary activity, had the double events of cardiac mortality when compared to their colleagues conductors, that had an active work.

Some important trials also verified the effect of lifestyle programs (diet and exercise) for the prevention of T2D in in-risk populations (Diabetes Prevention Program Research Group, 2009; Knowler et al., 2002; Pan et al., 1997; Ramachandran et al., 2006; Tuomilehto et al., 2001). One of the classical studies, the Diabetes Prevention Program (DPP) showed that lifestyle intervention was even superior than drugs like metformin on the prevention of the development of T2D (Knowler et al., 2002) (Figure 5).

In the case of established T2D, physical activity acts as a promoter of good glycemic control, reflected mainly in HbA1c. In addition, physical activity is able to reduce other risk factors like hypertension, obesity, insulin resistance and atherogenic lipid profile, in the way that physical activity can prevent and treat risk factors for cardiovascular disease in T2D (Boulé, Haddad, Kenny, Wells, & Sigal, 2001; Boulé, Kenny, Haddad, Wells, & Sigal, 2003; Cuff et al., 2003; Ibañez et al., 2005; Snowling & Hopkins, 2006; Thomas, Elliott, & Naughton, 2006).

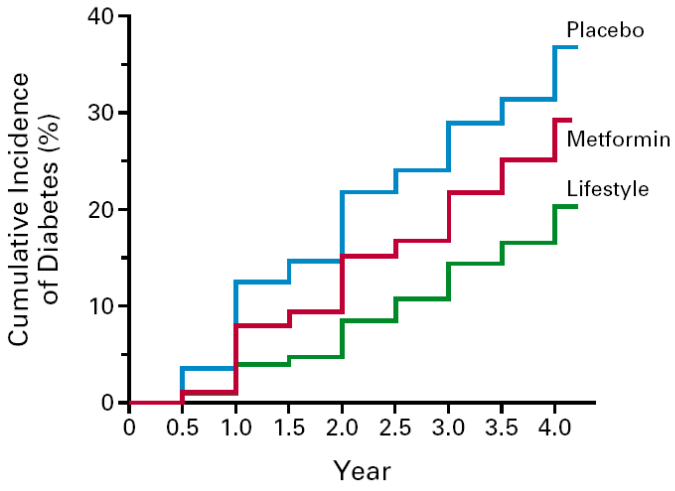


Figure 5. Cumulative incidence according to study group. Participants presented pre-diabetes and were random in three different group of intervention. The incidence of diabetes differed significantly among the three groups. Figure extracted from Knowler et al. (Knowler et al., 2002)

2.2. Effects and benefits of physical activity in type 1 diabetes

The beneficial effect of physical activity on glycemic control of patients with T1D is not so well recognized. The delicate balance among the characteristics of physical activity, the carbohydrate intake and the doses of insulin leads to a dynamic and permanent control in these patients. Studies about the benefits of glycemic control in T1D are controversial, and also complex to perform.

It is common to identify debates about the benefits of physical activity in glycemic control in this population. A multicenter study, that included 23,251 adolescents with T1D, identified a positive correlation between physical activity and lower levels of glycated hemoglobin (Herbst, Kordonouri, Scheab, Schmidt, & Holl, 2007), association recently confirmed by a study that took place in Sweden (Beraki, Magnuson, Särnblad, Aman, & Samuelsson, 2014). On the other hand, other studies did not find this correlation (Zinman, Zuniga-Guajardo, & Kelly, 1984), probably because the variables were studied following different designs, especially considering the different prescriptions of insulin and carbohydrate supplementation. Three recent meta-analysis published in 2013 (A. Kennedy et al., 2013) and in 2014 (Quirk, Blake, Tennyson, Randell, & Glazebrook, 2014; Yardley, Hay, Abou-Setta, Marks, & McGavock, 2014) concluded that nowadays there are insufficient well-designed studies to confirm the effect of exercise training on glycated hemoglobin in subjects with T1D. Nevertheless, these meta-analysis indicate that the results are promising to confirm the benefits on glycemic control, as well as they encourage physical activity for its other known benefits.

In a different systematic review, Chimen and colleagues (Chimen et al., 2012) identified several studies with different designs showing the benefits of physical activity for patients with T1D in other aspects different from the glycemic control. They recognized reduction of insulin requirements, insulin resistance, endothelial function, cardiovascular disease, mortality and improvements on physical fitness, muscle strength and wellbeing, as well as controversies about

the role of physical activity for lipids, blood pressure and microvascular complications.

Concerning the difficulties on the routinely management of the triad physical activity, carbohydrate intake and insulin doses, there is a known limitation for the practice of exercise in this group of patients, that it is the fear of hypoglycemia. It has been shown (Valerio et al., 2007) that children and adolescents with T1D get less exercise that children and adolescents without diabetes of the same age. In parallel with that, patients who have a better knowledge of the disease are those who practice more exercise (Brazeau, Rabasa-Lhoret, Strychar, & Mircescu, 2008).

There are established physical activity recommendations for T1D and T2D, published by ADA (American Diabetes Association, 2015c), in which “all children should be encouraged to engage in at least 60 min of physical activity each day; and adults should be advised to perform at least 150 min/week of moderate-intensity aerobic physical activity (50–70% of maximum heart rate), spread over at least 3 days/week with no more than 2 consecutive days without exercise”.

The demonstration that patients with T1D have an increased cardiovascular risk (CVR) when compared with non-diabetic population, as exposed above (Krolewski et al., 1987; Mason, Jenkins, Best, & Rowley, 2006), that an adequate glycemic control can reduce cardiovascular complications, such as myocardial infarction or stroke (Nathan et al., 2005), and that physical activity can reduce CVR factors (Chimen et al., 2012) make us contemplate the idea that the application of a good exercise program adapted to the dietary and insulin requirements may be useful to delay or reduce cardiovascular events in these patients.

2.3. Glucose uptake in muscle contraction

Molecular and metabolic effects of exercise are partially known, and the field of studies of physiology of exercise is growing and taking relevance in different areas of medicine.

Richter and Hargreaves (Richter & Hargreaves, 2013) wrote an interesting review about glucose uptake following muscle skeletal contraction and the relation with glucose transporter type 4 (GLUT4), based on experimental and human experiments. During exercise, the coordinated increase in skeletal muscle blood flow, capillary recruitment, GLUT4 translocation to the sarcolemma and T-tubules, and metabolism are all important for glucose uptake and oxidation. One important effect of muscle contraction is to promote the migration of the vesicles of glucose transporter GLUT4 to the membrane of the muscular cell, facilitating the glucose uptake. The activation of AMPK enzyme seems to be important in the signaling of this process by increasing the muscle glucose transport (GLUT1 and GLUT4) as well as in the induction of mitochondrial biogenesis (J. W. Kennedy et al., 1999; McGee & Hargreaves, 2006). The activation of AMPK enzyme also could be confirmed by an AMPK activator, the 5-aminoimidazole-4-carboxamide-1-D-ribofuranoside (AICAR), that provoked similar effects of glucose uptake and GLUT4 than exercise in rats (Jessen et al., 2003)

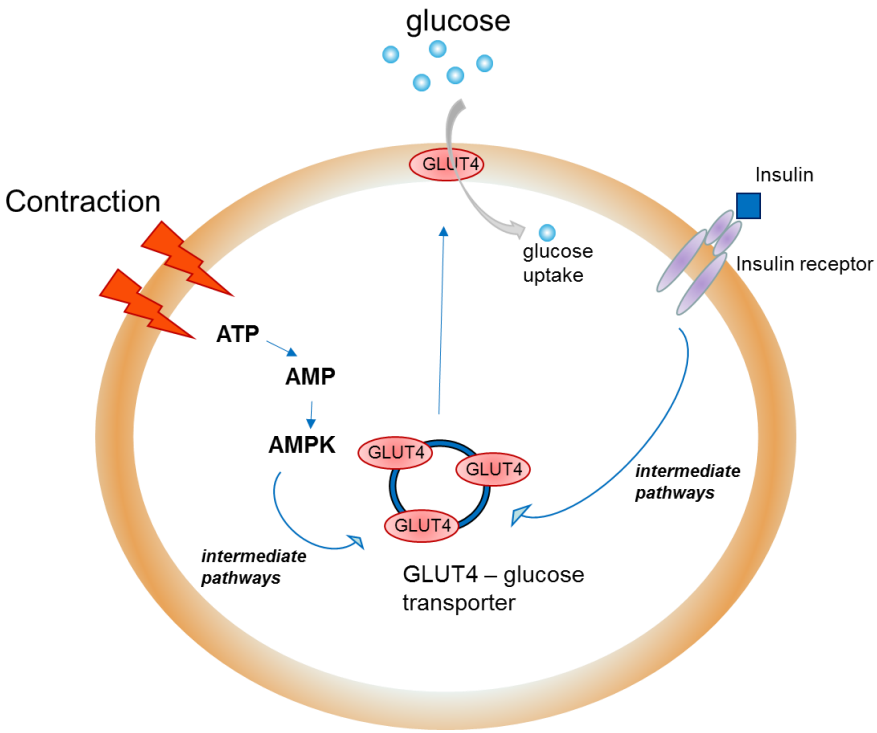


Figure 6. Schematic muscle contraction and insulin pathways for GLUT4 translocation to the surface of the cellular membrane.

The effect of muscular contraction has an additional effect with the insulin signaling to GLUT4 translocation. Insulin, by different pathways, also promotes this migration of vesicles of GLUT4 to cellular membrane (Figure 6). As a clinical consequence of physical activity, it is possible to testify an improvement on glycemic control in T2D (Boulé et al., 2001, 2003) and reductions of the requirements of insulin doses in T1D (Beraki et al., 2014). In Figure 7 it is represented an experimental study that evaluated the muscle glucose transport by the effect or insulin, muscle contraction or both.

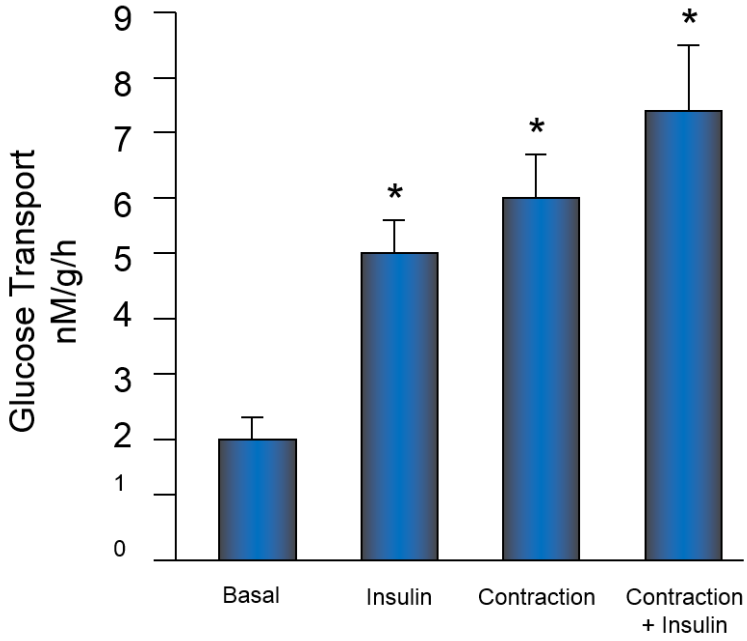


Figure 7. Rates of glucose transport in different conditions. Glucose transport were evaluated in whole gastrocnemius from lean animals were measured following basal (resting without insulin), contraction, insulin, or contraction followed by insulin (Contraction + Insulin) stimulation. Contraction increased glucose transport (*$p < 0.05$). Values are means ± SE. Adapted from Thyfault et al. (Thyfault et al., 2007).

3. Skeletal muscle composition and glucose metabolism

The skeletal muscle is one of the most important organs for the glucose metabolism and is directly related to insulin resistance and sensitivity (DeFronzo, Tobin, & Andres, 1979; Petersen et al., 2007), for this reason, it is important to review some muscle characteristics. In humans, two main fiber types are described with their particular characteristics and body distribution. Type I fibers are characterized by long period utilization, and also called slow-twitch or oxidative fibers. These fibers, in normal conditions, have a high capacity for fatty acid oxidation and insulin-stimulated glucose transport, present more mitochondria and GLUT4 content than type II fibers, and are mainly implicated in aerobic actions. By the opposite, type II fibers as type IIA (fast-twitch oxidative), and especially type IIX (fast-twitch oxidative-glycolytic), showed less intramyocellular lipid (IMCL) amounts, are more glycolytic, present lower mitochondrial and GLUT4 content, and are mainly implicated in anaerobic actions (Egan & Zierath, 2013; Schiaffino & Reggiani, 2011) (See Table 1 and Figure 8).

It is well recognized that elite athletes from resistance sports, like marathon or triathlon, have proportionally more type I fibers than control subjects, as well as sprint athletes, like 100-200 m runners, that present proportionally more type II fibers (Baguet et al., 2011; Wilson et al., 2012). There is a current discussion whether the athletes endowed with greater percentages of fast or slow twitch fibers, or the correlations between fiber types and performance be accounted for by exercise training. Some studies could not confirm the association of training with the modification of fiber type, but others point to a probable plasticity and fibers shift, especially between fibers type IIA and type IIX (Wilson et al., 2012).

Table 1 – Contractile, metabolic and morphological characteristics of human skeletal muscle fibers**

		Type II	
	Type I	**Type IIa**	**Type IIb**
General Properties			
Alternative nomenclature	SO, ST	FOG, FTa	FG, FTb
Myosin heavy chain isoform	MHC1	MHC2A	MHC2X
Contractile and metabolic characteristics	Slow twitch, high oxidative, fatigue resistant	Fast twitch, oxidative-glycolytic, fatigue resistant	Fast twitch, glycolytic, fast fatigable
Force production (power output)	Weak	Intermediate	Strong
Endurance capacity	High	Intermediate	Low
Appearance/myoglobin content	Red/high	Red/intermediate	White/low
Time to peak tension (msec)	80	30	
Recruitment threshold	All intensities	>40% VO2max	>75% VO2max
Morphological Properties			
Capillary density (capillaries per fiber)	4.2	4.0	3.2
Mitochondrial density	High	Intermediate	Low
Fiber size (cross-sectional area)	5310	6110	5600
Percent distribution in whole muscle	54 ± 12.2	32.2 ± 9.1	13 ± 7.6
Metabolic and Substrate properties			
Oxidative potential	High	Intermediate-high	Low
Glycolitic potential	Low	Intermediate-high	High
[Phosphocreatine]*	12.6	14.5	14.8
[Glycogen]*	77.8	83.1	89.2
[IMTG]*	7.1	4.2	
Exercise-type predominance	Prolonged low intensity	Moderate duration, high intensity	Short duration, maximal effort

SO, slow oxidative; ST, slow-twitch; FOG, fast oxidative-glycolytic; FT, fast-twitch; fast-glycolytic; MHC, myosin heavy chain; IMTG, intramuscular triglycerides

*mmol kg^{-1} wet weight.

**Adapted from Brendan Egan and Juleen R. Zierath (Egan & Zierath, 2013).

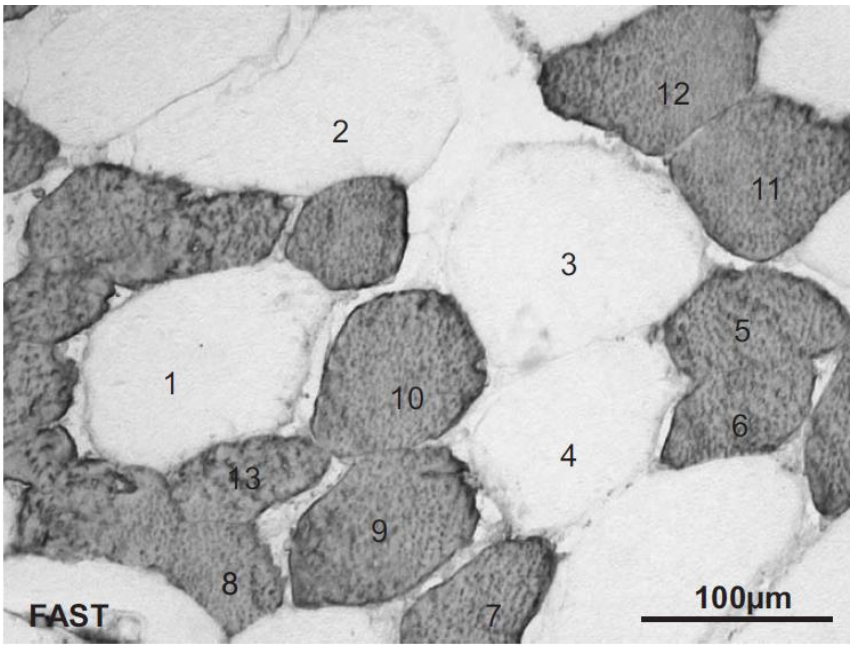


Figure 8. Skeletal muscle fiber typing in serial sections of biopsies from the vastus lateralis muscle in a patient with T1D. Immunostaining with fast myosin heavy chain (FAST). As an example, 13 fibers were identified and typed in each serial section: fibers 1 – 4 are slow fibers (type I), fibers 5-13 are fast fibers (type II). Figure extracted from Fritzsche K et al.(Fritzsche et al., 2008)

3.1. Muscular insulin resistance

Some metabolic conditions, such as T2D and obesity, have been importantly associated with muscular insulin resistance. Imbalances of muscular composition, fatty acids intramyocellular accumulations and mitochondrial dysfunction have been implicated in muscular dysfunction (Petersen, Dufour, Befroy, Garcia, & Shulman, 2004). The accumulation of IMCL has been proposed as an important predictor of insulin resistance (White, Ferguson, McCoy, Kim, & Castellano, 2006). The mitochondrial dysfunction, related to reduction of mitochondrial content, mitochondrial biogenesis and/or electron transport chain content, induces a reduction of the oxidative capacity, and is associated with lipid accumulation and reduction of insulin action (Befroy et al., 2007; Montgomery & Turner, 2015; Morino, Petersen, & Shulman, 2006; Petersen et al., 2004). In subjects with T2D and obesity, it was observed an excess of IMCL accumulation (He, Watkins, & Kelley, 2001; Kelley, Goodpaster, Wing, & Simoneau, 1999). Other studies showed IMCL accumulation even in lean insulin resistant T2D offspring (Jacob et al., 1999; Gianluca Perseghin et al., 1999) in the muscles tibialis anterior (higher content of fiber type II, less insulin sensitive) and especially in soleus (higher content of fiber type I, insulin sensitive), when analyzed by 1H NMR (proton nuclear magnetic resonance).

Some studies identified, through biopsies of vastus lateralis muscle, a less content of type I fibers in T2D and obese subjects, less GLUT4 expression, and/or less oxidative enzyme capacity especially in type I fibers when compared to control subjects (Gaster, Staehr, Beck-Nielsen, Schrøder, & Handberg, 2001; He et al., 2001; Oberbach et al., 2006; Stuart et al., 2013).

A condition called metabolic inflexibility was referred by several authors (Aucouturier, Duché, & Timmons, 2011; Battaglia, Zheng, Hickner, & Houmard, 2012; Russell, Kraemer, & Nelson, 2013; Storlien, Oakes, & Kelley, 2004). Metabolic inflexibility is characterized by impaired ability to switch from fat to carbohydrate as substrate from the fasted-to-fed transition. Patients with T2D and/or obesity are less able to shift between substrates, demonstrating different

degrees of metabolic inflexibility, fact that is associated with an excessive ectopic lipid accumulation and in lipid intermediates such as diacylglycerol and ceramide, resulting in intracellular lipotoxicity. An interesting study was performed by Kelley and collaborators (Kelley et al., 1999), in which lean and obese subjects received insulin infusion; it could be observed differences in insulin sensitivity and fat oxidation between both groups of subjects, suggesting that the triglyceride accumulation in skeletal muscle in obese subjects derives from reduced capacity for fat oxidation and, with this, a parallel insulin resistance.

It is worthy to mention that studies that analyzed high performance athletes showed a paradoxical increase in IMCL. In athletes, it is described that IMCL is associated with the increase of VO_2peak (maximal aerobic capacity); in untrained individuals, however, the higher IMCL content predicted the lower insulin sensitivity (Machann, Häring, Shick, & Stumvoll, 2004). One study showed that IMCL could increase in sedentary subjects when they participated in an exercise training program in parallel with the improvement of insulin sensitivity (Dubé et al., 2008). It has been proposed by Coen and Goodpaster (Coen & Goodpaster, 2012) that the athlete's paradox represents a convergence of two separate areas of research, one linking IMCL to fuel metabolism and the other linking IMCL to insulin resistance, as exemplified in Figure 9. This paradoxical situation indicates the need of a better understanding of the IMCL role and require of a better characterization of muscular components associated with the clinical profile.

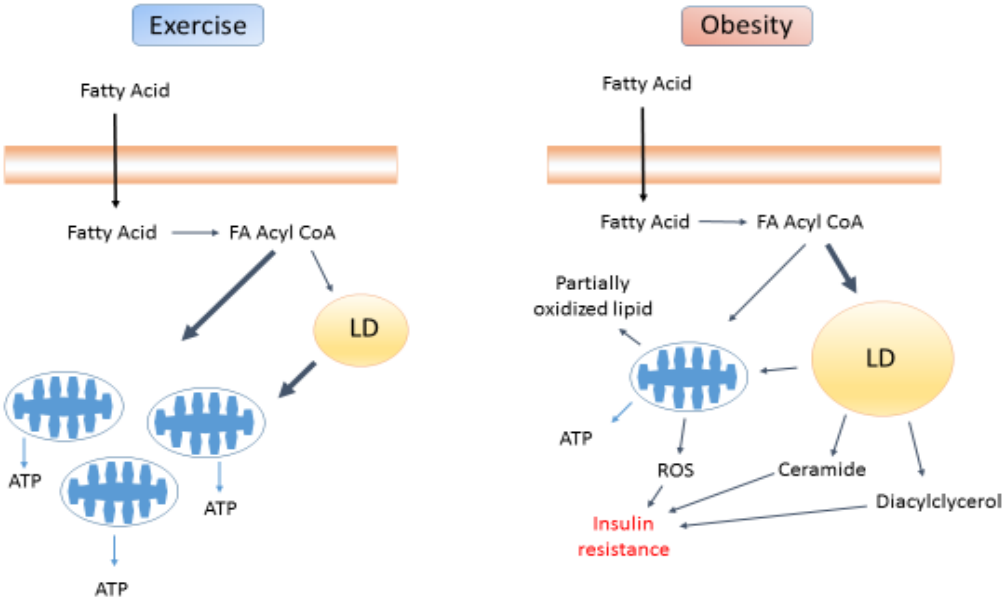


Figure 9. Role of intramyocellular lipid (IMCL) during exercise and in obesity. During exercise, fatty acid (FA) acyl CoA is oxidized by mitochondria to synthesize adenosine triphosphate (ATP). FA acyl CoA is also subdivided to lipid droplets (LDs), where it is esterified to triglyceride (TG). TG can subsequently be lyophilized to release FAs for mitochondrial oxidation. In obesity, because of lower energetic demand, most FA acyl CoA is directed to LDs. IMCL in LDs can then turn as alternate for ceramide and diacylglycerol. FA CoA oversupply to mitochondria during low energetic demand results in incomplete β oxidation and reactive oxygen species (ROS) production. Adapted from Coen and Goodpaster, 2012 (Coen & Goodpaster, 2012)

3.2. Muscular composition and type 1 diabetes

In subjects with T1D there is little information about specific muscle fiber composition. One study identified a decrease of the slow-twitch oxidative and increase of the fast-twitch fibers fractions by biopsies in vastus lateralis muscle in subjects with T1D when compared with subjects with normal glucose tolerance; and the same study also showed significantly higher glycolytic enzyme activities in all fiber types, which were correlated with HbA1c (Fritzsche et al., 2008). Another study (G Perseghin et al., 2003) verified higher accumulations of IMCL in calf muscles of adult population with T1D, mostly if there was an inadequate glycemic control, indicating also an assignment of this

organ and probable changes on its functions.

Recently it is been recognized that subjects with T1D could present different degrees of insulin resistance (Bergman et al., 2012; Liu et al., 2009). Insulin resistance was tested through hyperinsulinemic-euglycemic clamps in teenagers with T1D and in non-diabetic controls. It was evidenced that teenagers with T1D presented higher insulin resistance and lower cardiovascular fitness (determined by VO_2peak) than their counterparts, and it was also identified that insulin resistance was inversely correlated with VO_2peak. The same study also analyzed the intra and extramyocellular lipid contents (IMCL and EMCL) in soleus and tibialis anterior muscles from both groups of subjects, however no differences were identified between them (Nadeau et al., 2010).

4. Lipoproteins

4.1. Lipoproteins in general population

A correct equilibrium of lipoprotein profile is directly related to cardiovascular health. Dyslipidemia is one of the most important risk factors involved in cardiovascular disease (CVD) (American Hearth Association, 2002) in addition to cigarette smoking, hypertension, family history of premature coronary heart disease, age and diabetes. It is recognized that low-density lipoprotein (LDL) plays a major role in the promotion, development and progression of atherosclerosis through mechanisms that involve endothelial dysfunction, activation of inflammatory pathways and lipid oxidation and accumulation (Morris, Ballantyne, Birtcher, Dunn, & Urbina, 2014). Besides the LDL, triglycerides and triglycerides-rich lipoprotein also play an important role as risk for cardiovascular disease (Miller, Ginsberg, & Schaefer, 2008; Morris et al., 2014; Nordestgaard & Varbo, 2014).

Another lipoprotein, the high-density lipoprotein (HDL), is considered as an effective biomarker for predicting cardiovascular risk protection, and its role was reviewed by Raver and Hovingh (Rader & Hovingh, 2014). Many prospective studies have confirmed that HDL-cholesterol (HDL-C) is a strong, consistent, and independent predictor of incident cardiovascular events (myocardial infarction, ischemic stroke). Strong data also consider HDL-C as a predictor of incident cardiovascular events in the setting of secondary prevention in individuals who have already been diagnosed with cardiovascular disease.

Lipoproteins are complex particles composed of different proteins which transport fat molecules. The fats carried include cholesterol, triglycerides and phospholipids; the amounts of each can vary considerably. Lipoproteins classically are classified by molecular size and density and are ordered from largest to smallest as: chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (See Figure 10). The most abundant apolipoproteins

of HDL particles are apo A-I and apo A-II; the LDL particles, by contrary, present the apolipoprotein B-100.

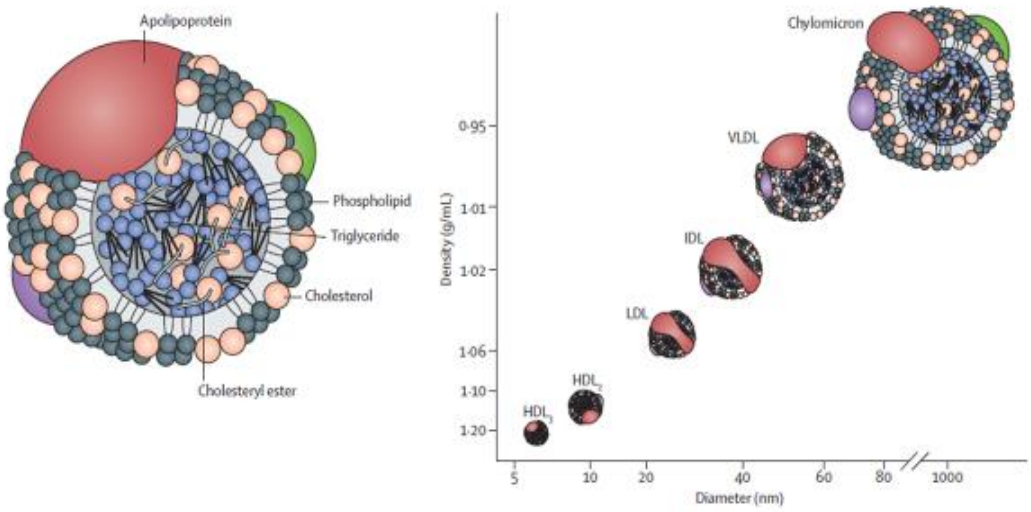


Figure 10. Structure, diameters and density of lipoproteins. In the left, lipoprotein structure; and in the right, the different lipoproteins displaced by diameters and densities. Adapted from Ridker P., 2014 (Ridker, 2014).

One important aspect, more recently recognized, is the size of the lipoprotein particles and subfractions, which can be determined through different methods, and especially by nuclear magnetic resonance (NMR) lipoprotein analysis (Mallol, Rodriguez, Brezmes, Masana, & Correig, 2013). The size of LDL particles (LDL-P) varies such that particles with more triglycerides and fewer cholesteryl esters result in smaller and denser LDL (Ridker, 2014). Different studies evidence that elevated plasma concentrations of small dense LDL are correlated with high plasma triglycerides and low HDL levels, and also that reduced size and increased density of LDL have been associated with increased coronary heart disease (CHD) risk, (Diffenderfer & Schaefer, 2014; Morris et al., 2014).

The classical determination of LDL-cholesterol (LDL-C) is a measure of the cholesterol content of LDL-P. The determination of LDL-C can vary significantly between individuals and in response to drug or lifestyle intervention; it has been shown, however, that LDL-P could be better than LDL-C in predicting incident events (Toth et al., 2014). As well as for LDL-C, the conventional determination of HDL-cholesterol (HDL-C) is a measure of the cholesterol content of HDL-particle (HDL-P). In a similar way to LDL-P, HDL-P have been studied, and it could be evidenced that the amount and sizes of HDL-P are also helping on elucidating its role. An independent association with other cardiovascular risk for coronary artery disease was identified and it could be a good marker for cardiovascular risk protection (Harchaoui et al., 2009).

Recent findings are changing some concepts about the HDL functionality, concerning its components and sizes (Hovingh, Rader, & Hegele, 2015; Soran, Hama, Yadav, & Durrington, 2012). In HDLs, it has been identified distinct molecule species of lipids, as well as several proteins compounds, characteristics that confer antioxidative, anti-inflammatory, cytoprotective, vasodilatory, antithrombotic and anti-infection actions (Rached, Chapman, & Kontush, 2015; Soran et al., 2012). The exact role of HDLs, however, is not yet completely known since the direct clinical effect of cardioprotection has been recently questioned. One of the points of reflection is that drugs that increased HDL concentrations failed to prove direct CV protection (Hovingh et al., 2015), despite the known association of HDL levels and cardioprotection.

4.2. Lipoproteins and physical activity

Physical activity is considered as a protective factor against CVD and also a promotor of beneficial effects on lipid and lipoprotein profiles in general population. For instance, HDL-C and LDL-C, as well as changes in LDL and HDL subfractions and particle sizes, have been identified in several studies as modifiable in result of physical activity interventions (Halverstadt, Phares, Wilund, Goldberg, & Hagberg, 2007; Spate-Douglas & Keyser, 1999; Trejo-

Gutierrez & Fletcher, 2007), along with other observational prospective studies (Mora, Cook, Buring, Ridker, & Lee, 2007).

The study of different particle compositions and sizes of lipoproteins are helping in the knowledge about the influence of physical activity. Sedentary behavior was associated with small VLDL-particles (VLDL-P), large LDL-P and TG while high and moderate activities were positively associated with large HDL-P, average HDL-size, Apo A1 and HDL-C, in a recent study that evaluated healthy subjects that registered their physical activity (Aadland, Andersen, Anderssen, & Kvalheim, 2013). The same study identified that, for women, the association between physical activity with large HDL-P and average HDL size was quite strong, whereas a weaker association was detected for conventional HDL-C. An example of an interesting study of endurance exercise was the one published by Halverstadt and colleagues (Halverstadt et al., 2007) that proposed a training for 24 week in subjects between 50 and 75 years-old and analysed lipoprotein and lipid profile using both conventional and NMR measures. That study showed a significant decrease in total cholesterol, triglycerides and LDL-C, and also an increase in HDL-C subfractions (HDL3-C and HDL2-C - smaller and bigger, respectively). It was also identified a decreased of large and small VLDL-P, total, medium, and very small LDL-P and small HDL-P. Mean VLDL-P size also decreased significantly and mean HDL-P size increased significantly with exercise training. These changes were independent of baseline body fat and body fat changes with training.

The American Heart Association and the American College of Cardiology (AHA/ACC) stablished guidelines on lifestyle management to reduce CVR (Eckel et al., 2014). These guidelines are based on evidences from important trials and meta-analysis that studied the effect of diet and physical activity in order to modify lipids and to promote blood pressure reduction. Associated with diet guidance, there are recommendations for adults to engage in aerobic physical activity to reduce LDL-C and non–HDL-C that would be: 3–4 sessions per week, lasting on average 40 min per session, and involving moderate- to vigorous-intensity physical activity. The same statement indicates that aerobic

physical activity alone or resistance exercise training, compared to control intervention, have no consistent effect on HDL-C.

As already pointed out in the section above, HDL particles are highly heterogeneous. Independent variables can exert effects on the lipoprotein profiles, like genetics, diet and several environmental factors. These different influences could explain some discrepancies on the results of different interventional physical activity studies and individual responses to exercise. Some recent studies are evidencing effects of genetics on HDL changes (Blazek, Rutsky, Osei, Maiseyeu, & Rajagopalan, 2013). This genetic variability and polymorphisms have been studied in different steps of the pathway of HDL particles, like in Apo-I, ATP-binding cassette transporter A1 (ABCA1), lipoprotein lipase (LPL), hepatic lipase, cholesterol ester transfer lipoprotein (CETP) and others (Blazek et al., 2013; Hovingh et al., 2015), and may condition to distinct responses to physical activity interventions.

4.3. Lipoproteins in type 1 diabetes

As already reviewed above, patients with T1D have a higher prevalence of CVD and cardiovascular mortality than subjects without diabetes in the same age. In the last years, the rates of CVD in T1D are decreasing, but are still far from non-diabetic population. The identification of risk factors for CVD is crucial for the prevention and the treatment of this condition.

American Diabetes Association (ADA) (American Diabetes Association, 2015a) states that patients with T2D have an increased prevalence of lipid abnormalities, contributing to their high risk of CVD, and that less evidence of this association exists for T1D. ADA stablishes that, although the data are not definitive, similar lipid-lowering goals for both groups of patients with T1 or T2D should be considered, particularly if they have other CVR factors. This recommendation is also corroborated by the joint of ADA and American Heart

Association (AHA) in the specific case of T1D, published in 2014 (de Ferranti et al., 2014).

This publication from AHA/ADA also calls the attention to the fact that the lower rates of CVD in premenopausal women, when compared to men, are erased in the case of T1D (de Ferranti et al., 2014), as already mentioned in item 1.4 (Cardiovascular disease in T1D), and the causes are yet to be clarified. There are some evidences that indicate that lipoprotein profile in premenopausal women with diabetes is less cardioprotective than in women without diabetes. According to Masding and colleagues (Masding, Stears, Burdge, Wootton, & Sandeman, 2003), after testing a meal containing a stable isotope in women with and without T2D, the premenopausal advantage in clearance of dietary lipid is not seen in premenopausal diabetic women. They suggest that this is likely to promote an atherogenic lipoprotein profile and may contribute to the loss of cardiovascular disease protection seen in diabetic women. Another study, by Maahs and colleagues, analyzed the lipoprotein profile in men and women with and without T1D submitted to hyperinsulinemic-euglycemic clamp. Among men, those with T1D had less VLDL and more HDL cholesterol than control subjects. In women, however, those with T1D had more LDL cholesterol with an apparent shift of cholesterol distribution within LDL to smaller (atherogenic) LDL fractions when compared with those without diabetes. Moreover, they identified that insulin resistance was associated with a more atherogenic lipoprotein cholesterol distribution in all men and in women with T1D (Maahs et al., 2010).

The early observations of lipids and lipoprotein profile in patients with T1D revealed pro-atherogenic features, such as hypercholesterolemia and hypertriglyceridemia, and were particularly associated with poor glycemic control (Patti et al., 1995; Vergès, 2009) and nephropathy (Jenkins et al., 2003; T J Orchard, Stevens, Forrest, & Fuller, 1998). In the nineties, studies from Europe and U.S. identified similar rates of CVD in subjects with T1D in that regions, but with different pattern of dyslipidemia: low HDL-C in EURODIAB and hypertriglyceridemia in the U.S. group (T J Orchard et al., 1998).

More recent studies identified that an adequate metabolic control could improve these characteristics. Epidemiological evidences show that lipids and lipoproteins are normal in subjects with T1D if good glycemic control is maintained and the patient does not have microalbuminuria or clinical nephropathy (Jenkins et al., 2003; Lehmann, Kaplan, Bingisser, Bloch, & Spinas, 1997). Some studies question the role of glycemic control, as in the work of Guy and colleagues (Guy et al., 2009), when it was identified that young patients with T1D presented higher levels of small LDL particles than subjects without diabetes, independent of their glycemic control. Different findings were described by Alberts and colleagues, in which poor glycemic control was related to more dense LDL particles (J. J. Albers et al., 2008). On the other hand, Caixàs and colleagues observed that, after intensification of insulin treatment for the optimization of glycemic control in patients with T1D, VLDL particles, triglycerides, total LDL-C and HDL-C reached the levels of the control group, while the pattern of small LDL-C was also similar to that of the non-diabetic group, with no changes after optimization (Caixàs et al., 1997).

Insulin has an important role in lipoprotein metabolism. Vergès and Howard (Howard, 1987; Vergès, 2009) review this topic indicating different functions of insulin, for example, in the inhibition of hormone-sensitive lipase. The effect is an antilipolytic action of insulin, that promotes the storage of triglycerides in the adipocytes, while reduces the release of free fatty acids from adipose tissue into circulation. Another described effect of insulin is the inhibition of VLDL production by the liver. Insulin is also a potent activator of lipoprotein lipase (LPL), which promotes the catabolism of triglyceride-rich lipoprotein levels, also reducing the plasma triglyceride levels (Figure 11). It is common to identify, in the clinical practice, that patients with T1D under inadequate control due to insulin deficiency treatment are more prone to present hypertriglyceridemia. Low levels of serum triglycerides are evidenced as well when the patient presents an adequate glycemic control.

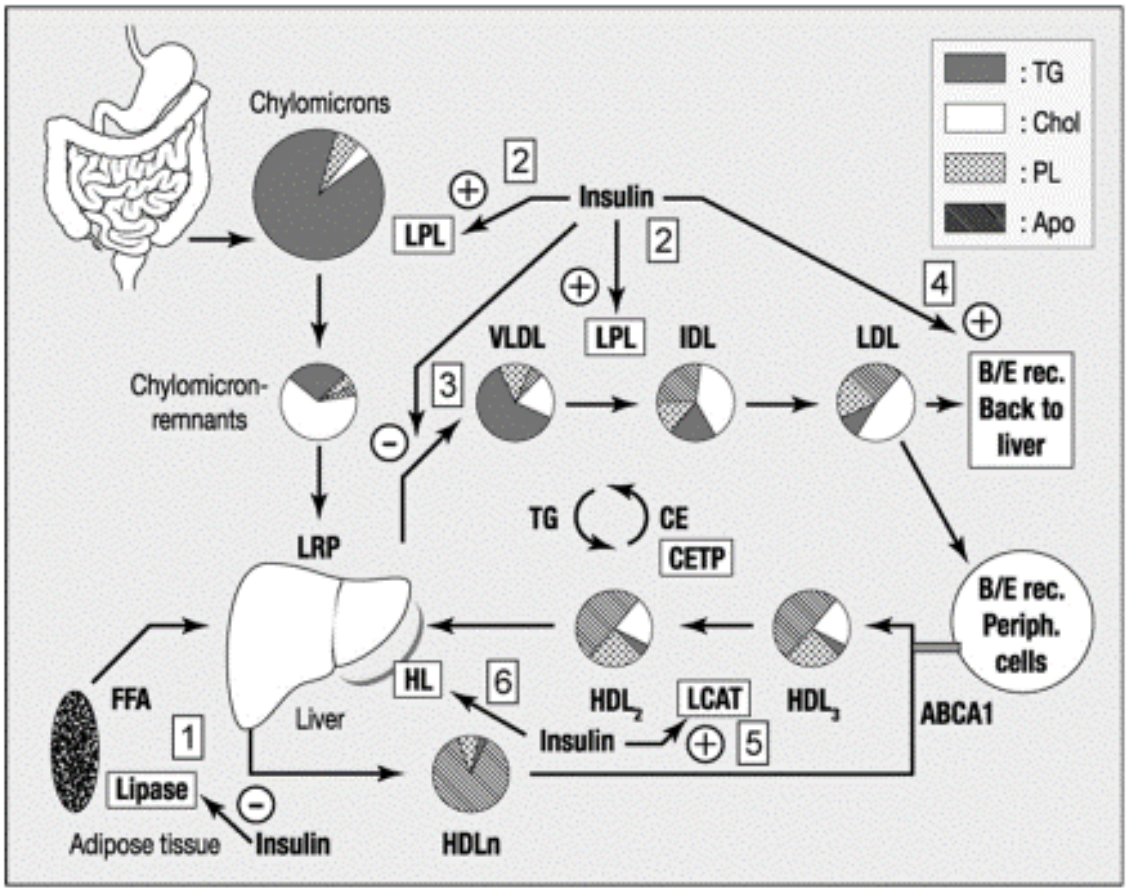


Figure 11. Main effects of insulin on lipoprotein metabolism. HDL_2 – smaller HDL particles; HDL_3 – bigger HDL particles. LPL: lipoprotein lipase; HL: hepatic lipase; CETP: Cholesteryl Ester Transfer Protein; LCAT: lecithin-cholesterol acyl transferase; FFA: free fatty acids; B/E rec.: receptor B/E (LDL receptor); CE: cholesterol esters. 1) Insulin inhibits hormone-sensitive lipase. 2) Insulin activates LPL. 3) Insulin inhibits hepatic VLDL production. 4) Insulin increases LDL B/E receptor expression. 5) Insulin activates LCAT. 6) Insulin activates HL. Figure extracted from Vergès, 2009 (Vergès, 2009).

Few information of the effect of physical activity in subjects with T1D is available, concerning changes in lipoprotein profile. From the EURODIAB IDDM Complications Study, where 3159 patients with T1D were evaluated, it was demonstrated a positive relationship with degree of physical activity and HDL-C and HDL_3-C, and negative for Total-C/HDL-C ratio and triglycerides in men. In women, however, both Total-C and LDL-C had a significantly positive relationship with the degree of physical activity (Idzior-Walus et al., 2001). That study, however, indicated the smoking habit and the central obesity as important factors that influenced the lipoprotein profile. In interventional studies

of 3 months of aerobic exercise in patients with T1D, it was described an increment of HDL-C in one protocol (Rigla et al., 2000) and an increase in the HDL3-C associated with a reduction of abdominal fat and blood pressure (Lehmann et al., 1997) in another one. An additional study with a protocol of endurance training improved the lipid profile in already physically active men with T1D, in the ratio HDL-C/LDL-C, independently of effects on body composition or glycemic control (Laaksonen et al., 2000).

As already mentioned above, the exact role of HDLs are not fully understood (Hovingh et al., 2015). In the particular case of patients with T1D, the expected protection effect of HDLs profile on coronary artery calcification is not so clear as in control subjects, as shown in one study that used 1H NMR methodology (Colhoun et al., 2002). Nevertheless, a prospective study associated coronary artery disease with lower rates of large HDL and higher rates of medium HDL in T1D patients, also measured by 1H NMR (Soedamah-Muthu, Chang, Otvos, Evans, & Orchard, 2003). These features are bringing new concepts and opening new fields of research and could better explain the association of lipoprotein profile and cardiovascular disease in patients with T1D.

III. HYPOTHESIS AND AIMS

Physical activity produces a beneficial effect on general population associated with the delay of development of cardiovascular diseases and improvement of life quality. It is recognized that structured physical activity improves glycemic control in patients with type 2 diabetes and ameliorates cardioprotective lipoprotein profile in general population.

Despite the improvements in diabetes care, patients with type 1 diabetes (T1D) still have a high incidence of cardiovascular disease and cardiovascular mortality, with evidences of affectation even in the youth. In these patients, there are contradictory data about the benefits produced by physical activity on the metabolic control.

Nowadays, subjects with T1D can achieve a good metabolic control and avoid or delay chronic complications, but a possible hyperinsulinemia induced by exogenous insulin administration in addition to some degree of insulin resistance could result in different metabolic responses to exercise. In this sense, the role of physical activity in this condition is poorly known.

Based on these considerations, we hypothesize that physical activity can induce beneficial metabolic responses, promoting changes in intramyocellular lipid content and inducing modifications in lipoprotein profile in subjects with type 1 diabetes.

The aims of this study are:

1. To describe the clinical characteristics and fitness capacity of a cohort with type 1 diabetes compared to non-diabetic counterparts.

2. To analyze the metabolomic profile induced by acute exercise in subjects with type 1 diabetes compared to non-diabetic counterparts.

3. To investigate the muscular lipids content in subjects with type 1 diabetes in relation to their level of physical activity.

4. To compare the lipid and lipoprotein profile in serum of subjects with type 1 diabetes considering their level of physical activity.

IV. STUDY POPULATION, METHODS AND RESULTS

STUDY POPULATION, METHODS AND RESULTS

The studies were based on the aims proposed and will be presented as 4 studies, as follows.

AIM 1 – STUDY 1

Clinical charactestics and aerobic exercise capacity in subjects with and without type 1 diabetes

Rationale

Persons with type 1 diabetes (T1D) are encouraged to carry an active life for the promotion of health care and wellbeing, as all the population. Regular physical activity prevents or delays type 2 diabetes (T2D) in persons with prediabetes and, in persons with established T2D, promotes better glycemic control (Knowler et al., 2002; J Lindström et al., 2013; Jaana Lindström et al., 2003; Wing et al., 2013).

In T1D, the effect of physical activity in the improvement of glycemic control is controversial (Kennedy et al., 2013; Landt, Campaigne, James, & Sperling, 1985; Yardley, Hay, Abou-Setta, Marks, & McGavock, 2014; Zinman, Zuniga-Guajardo, & Kelly, 1984), and available studies are limited by the difficulties on the assessments of physical activity or fitness, the small number of patients, or control of the variables (Herbst, Kordonouri, Scheab, Schmidt, & Holl, 2007). In addition, the management of T1D is particularly complex and dynamic, due to the needs of accurate administration of insulin coordinated with the carbohydrate intake and physical activity volume, in order to maintain optimal glycemic control. Therefore, taking into account the particularities faced in the

management of T1D, studies that analyze the fitness characteristics of this population are limited.

The enhancement in insulin sensitivity related to physical activity is also on debate in T1D. Studies that conducted programs of exercise (Landt et al., 1985), demonstrated improvement of insulin sensitivity by clamp techniques, but could not demonstrate a parallel improvement on blood glucose control. Other studies, comparing T1D athletes with sedentary ones, showed decrease in required insulin doses, a worse glycemic control and an increased lipid utilization in the trained group, without any improvement on insulin sensitivity (Ebeling, Tuominen, Bourey, Koranyi, & Koivisto, 1995).

Individuals with T1D may present some impaired fitness-related components and alterations in their cardiorespiratory responses to exercise. To assess differences in fitness, Nguyen and colleagues (Nguyen et al., 2014) studied three groups of children: eight with good glycemic control (stable HbA1c for 9 months: ≤ 7.5%), eight with poor glycemic control (HbA1c ≥ 9%) and eight healthy controls. Children with poor glycemic control presented lower VO_2peak (maximal oxygen uptake) compared to the control ones (poor-control: 33.2 ± 5.6 mL/kg/min; control: 43.5 ± 6.3, $p < 0.01$). Children with T1D and good glycemic control presented similar results compared to the other two groups. This study also identified a negative correlation between VO_2peak and HbA1c ($R = - 0.54$, $p<0.001$), concluding that a poor glycemic control could compromise aerobic muscle capacity. Stettler and colleagues (Stettler et al., 2006) studied the influence of eu- or hyperglycemia on exercise capacity. Different from Nguyen (Nguyen et al., 2014), they looked for differences in the current glycemic state, through eu- or hyperglycemic clamps, and concluded that these different conditions do not affect the peak power output in a bicycle ergometer test.

The association of a reduced lung function with diabetes has been described. The lung may be another organ adversely affected by diabetes. Komatsu and colleagues (William Ricardo Komatsu et al., 2005) described a reduction of VO_2peak in adolescents with T1D compared to matched controls ones. Some years later, the same group of researchers (William R Komatsu, Barros Neto,

Chacra, & Dib, 2010) analyzed pulmonary function and exercise capacity in persons with T1D and controls. They described a reduction ($p < 0.001$) in forced expiratory volume in the first second (FEV1) in the group of athletes with T1D compared to non-athletes and controls (athletes or not); however, the aerobic capacity was similar between athletes with T1D and control athletes. Other studies did not identify differences in respiratory capacity when compared with control population, but showed a reduction in VO_2peak in subjects with T1D when compared with controls (Benbassat et al., 2001). Niranjan and colleagues (Niranjan, McBrayer, Ramirez, Raskin, & Hsia, 1997), in a cross-sectional study, analysed 14 control subjects, 9 subjects with T1D and chronically elevated levels of HbA1c; and other 9 subjects with T1D with chronically near-normal levels of HbA1c. The authors evidenced that chronically hyperglycemic patients presented an impaired maximal work load and oxygen uptake that were associated with restrictions of lung volume, lung diffusing capacity, and stroke index during exercise. The patients in normoglycemic control showed less impairment than the hyperglycemic patients, but some parameters were different from the control group. In the same study, patients with T1D (both groups) presented lower VO_2peak than control subjects, but a not commented and important aspect was the older age of the patients when compared with the control participants.

Insulin resistance is a condition described especially in T2D. Insulin resistance is associated with a clinical condition called Metabolic Syndrome, that is characterized by the clinical features of hypertension, abdominal obesity, hyperglycemia, high triglycerides and low HDL-cholesterol, and recognized as a risk factor for cardiovascular disease (American Hearth Association, 2002; International Diabetes Federation, 2006). Type 1 diabetes is a different entity, but shares many clinical characteristics with T2D like hyperglycemia and the association with cardiovascular disease, and micro- and macrovascular complications. Different degrees of insulin resistance are also being accepted as an additional condition in patients with T1D (Chillarón et al., 2011; Llauradó et al., 2012). Searching for a clinical approach to identify insulin sensitivity on T1D, Williams and colleagues (Williams, Erbey, Becker, Arslanian, & Orchard, 2000) described the eGDR (estimated glucose disposal rate) from euglycemic-

hyperinsulinemic claps performed in T1D patients. The equation was defined in 24 patients with T1D (male-to-female ratio 12/12) from the Pittsburgh Epidemiology of Diabetes Complications Study, with mean age 35.5 years, BMI 27 kg/m2, HbA1 9.9%, and unspecified ethnicity. This equation uses clinical characteristics for the approximation of insulin sensitivity that involve waist/hip ratio, hypertension and glycated hemoglobin. Higher levels of eGDR correspond to higher insulin sensitivity. Several studies have been using this index to approach insulin resistance or sensitivity in patients with T1D (Chillarón et al., 2009; Girgis, Scalley, & Park, 2012; Llauradó et al., 2012; Olson et al., 2002).

The objective of this analysis was to evaluate patients with T1D, on their habitual physical activities, and study possible impairment of fitness and clinical characteristics.

Subjects and procedures

Subjects

To study clinical characteristics and fitness of type 1 diabetes (T1D), we compared a group of 129 subjects affected by T1D with 136 subjects without diabetes (controls).

The subjects with T1D were outpatients derived from Hospital Clinic de Barcelona. The individuals that were studied as controls came from the staff of Hospital Clínic de Barcelona and Laboratory of Diabetes and Obesity (IDIBAPS). All the people that joined this evaluation received written and verbal explanations about the procedures and signed an informed consent for participation in the study. This study was approved by Ethics and Research Committees of Hospital Clinic de Barcelona (CEIC nº Register: 2009/4933).

The study was performed at Diabetes Research Clinical Unit, a research division of IDIBAPS and Hospital Clínic de Barcelona. One of the research

interests of this Unit is the study of exercise and physical activity related to diabetes; and, for that, we had the collaboration of several people that accepted the invitation and volunteered to participate in the study. These people presented different levels of fitness and different physical activity habits, both in T1D and control groups.

Men and women, age between 18 and 55 years old, without cardiorespiratory pathologies or osteomuscular limitations were included in the study. Subjects with T1D with advanced chronic complications related to diabetes and/or cardiovascular disease were excluded. Patients with incipient retinopathy, albuminuria lower than 300 mg/24h and/or initial peripheral neuropathy, defined by slight reduction of vibratory sensitivity (values lower than 25 V in biothesiometer measurement, but higher than 15 V) (Duke et al., 2007), were allowed to participate.

It was defined as type 1 diabetes all the patients with diagnostic in childhood or adolescence, classical history of weight loss, polydipsia, polyuria, and that needed treatment with insulin immediately after the diagnosis or some few weeks later. Some patients, moreover, had registers of positive autoantibodies (anti-GAD and/or anti-IA2). Hypertension was defined as systolic blood pressure ≥ 135 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or in use of anti-hypertensive drugs. The anti-hypertensive drugs were from the classes of angiotensin converting enzyme inhibitors or antagonist receptors angiotensin II and/or diuretics. Dyslipidemia was defined as triglycerides ≥ 150 mg/dl and/or LDL-C ≥ 130 mg/dl and/or HDL-C < 50 mg/dl for women or < 40 mg/dl for men. The chronical use of statins was also a criterion for dyslipidemia (American Hearth Association, 2002; International Diabetes Federation, 2006).

Procedures

a. Anamnesis, physical evaluation and anthropometry

Initially, all the participants answered questions about medical history, familial history, habitual physical activity, practice of exercise or habitual sport, and

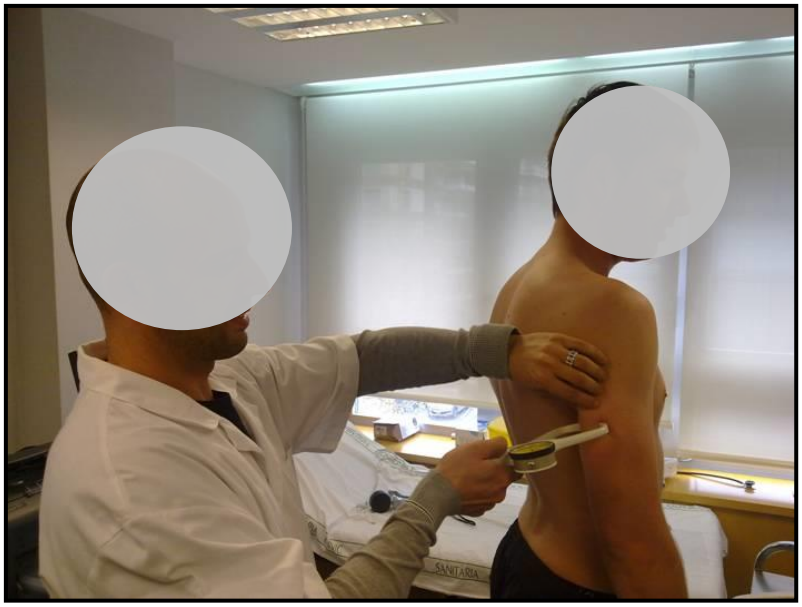
answered the International Physical Activity Questionnaire, short form (IPAQ) (“International Physical Activity Questionnaire,” n.d.).

IPAQ is an easy and reproducible questionnaire, used in many countries, which facilitates comparison among different populations. Individuals were asked to report the number of days and the duration of the vigorous, moderate, and walking activities they had undertaken during the previous week (Sjöström, Oja, Hagströmer, Smith, & Bauman, 2006). These data were quantified, and an estimated MET (Metabolic Equivalent of Task) was attributed to each activity. One MET was defined as the energy spent sitting quietly (equivalent to [4.184 kJ] • kg-1 • h-1). Estimated MET-min per week in this questionnaire, taking the different intensities of the activity components into account, were calculated by multiplying reported weekly minutes spent in different activities by 8 METs for vigorous, by 4 METs for moderate, and by 3.3 METs for walking activities, respectively. Energy expenditure per individual was obtained by adding the MET-minutes of the three activity components. According to IPAQ score, each individual was assigned to one of three categories: high, moderate, and low physical activity (“International Physical Activity Questionnaire,” n.d.). Those individuals who did not reach a minimum of minutes and/or days per week of vigorous, moderate or walking activities, following IPAQ classifications, met the criteria for ‘low’ physical activity levels and were thus considered as sedentary individuals (Guthold, Ono, Strong, Chatterji, & Morabia, 2008; “International Physical Activity Questionnaire,” n.d.; Sjöström et al., 2006) (Annex 1).

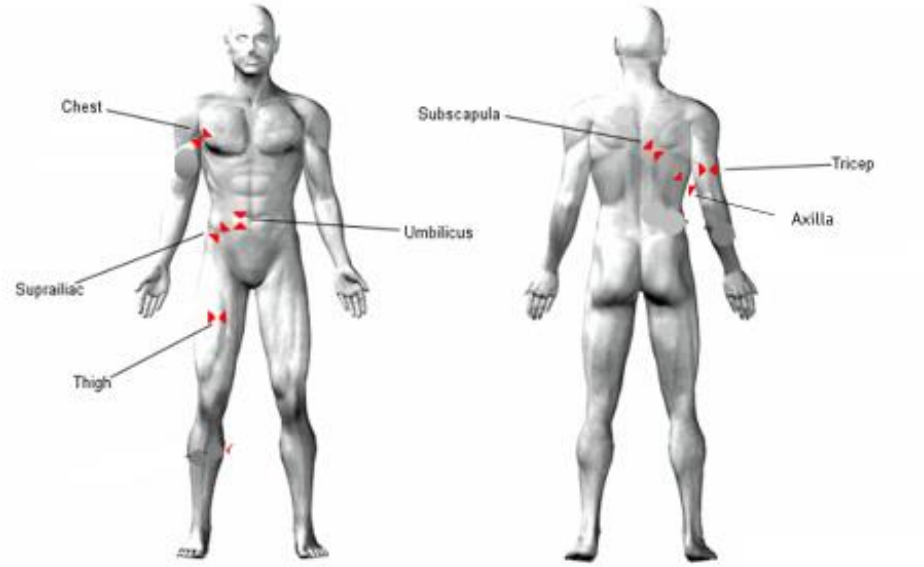
Baseline clinical characteristics such as height, weight, BMI, waist and hip circumferences, and total and fat body composition were obtained. Body composition was evaluated by different methods. The first one was the skinfold thickness measurements (Jackson and Pollock equations) (Image 1); the second, bioimpedance, that was obtained though multifrequency bioimpedance (MediSystem, Sano Care Human Systems) and abdominal impedance (Tanita ViScan AB-140) (Image 2); and the third, total body and abdominal composition, measured by densitometry using DXA (Lunar iDXA body composition, GE Healthcare) (Image 3) (Alvero Cruz, Diego Acosta, Fernández Pastor, & Romero, 2005a, 2005b, 2005c).

Image 1 - The skinfold thickness measurements

1.a. Skinfold measurement



1.b. Skinfold anatomic references



1.c. Skinfold caliper (Holtain, U.K.)

Image 2 - Bioimpedance assessment

2.a. Multifrequency bioimpedance (MediSystem, SanoCare Human Systems)

2.b. Abdominal impedance (Tanita ViScan AB-140)

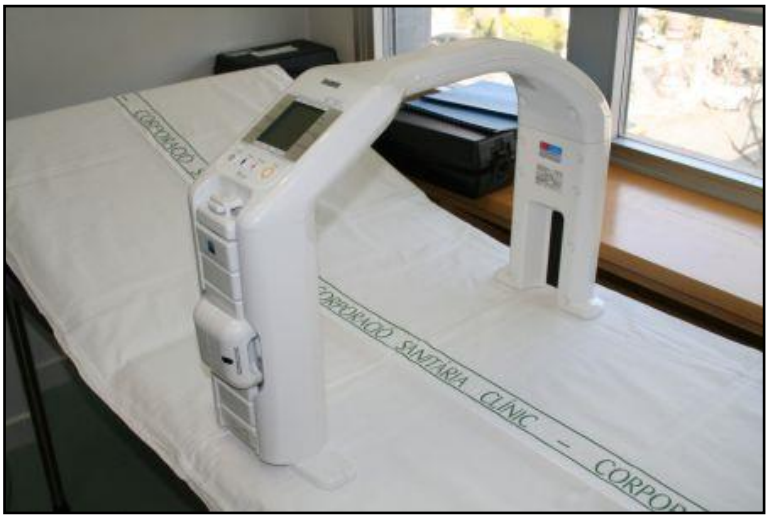
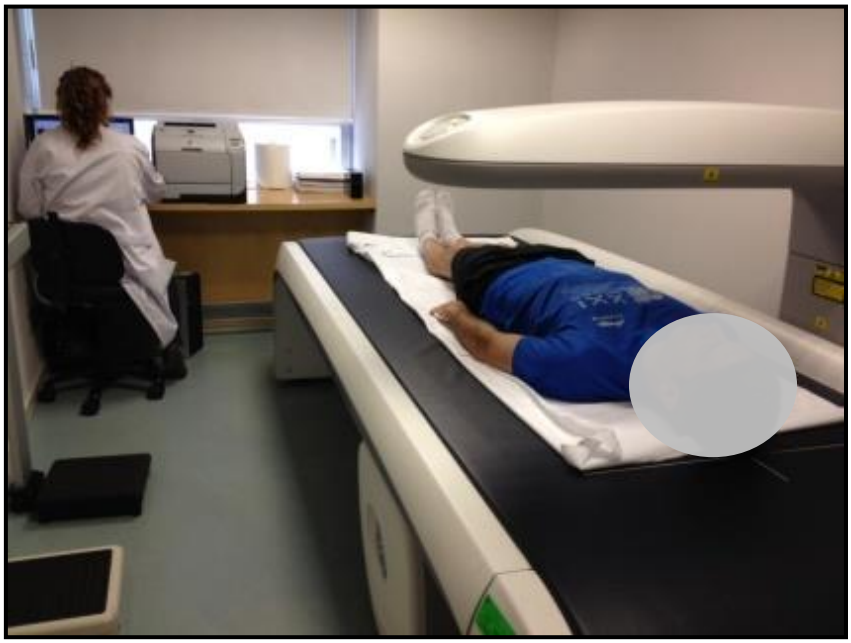
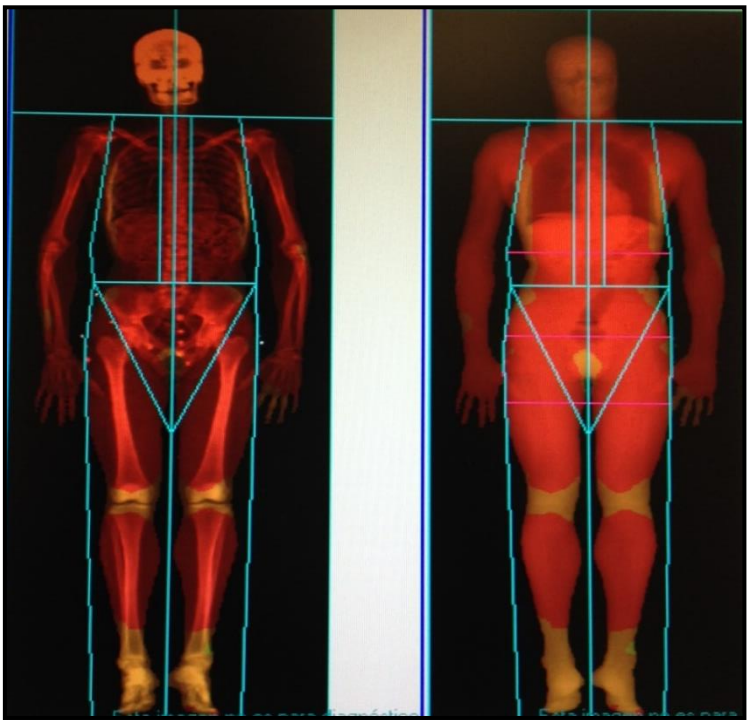


Image 3 - Total body composition measured by densitometry using DXA

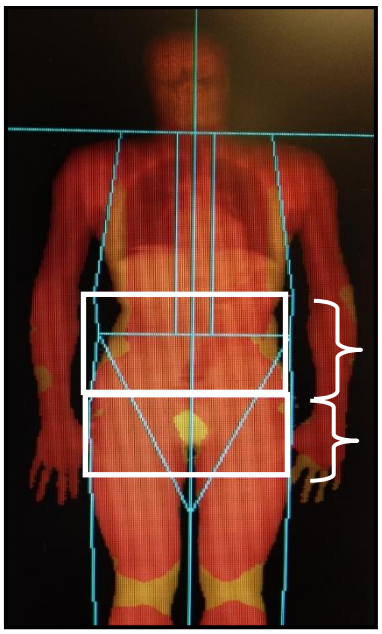
3.a. DXA densitometer (GE Healthcare, Lunar iDXA)



3.b. Example of general image acquisition



3.c. Example of abdominal and gynecoid segments acquisition



Arterial blood pressure was measured using a blood pressure monitor (Hem-703 C, Omron, Barcelona, Spain) after several minutes of rest. After some minutes in supine position, blood pressure was verified; immediately after the measurement, the subject was asked to assume the seated position and blood pressure was verified again 1 minute later. The difference of 20 mmHg or more between both systolic blood pressure measurements was defined as orthostatic hypotension (Vinik, Maser, Mitchell, & Freeman, 2003). Patients had cardiologic evaluation by clinical examination, rest electrocardiogram (ECG) and ECG by cycle-ergometer test.

All subjects were also evaluated for peripheral neuropathy by symptoms, physical examination and biothesiometry exploration on first toes and metatarsal heads localizations (Bio-thesiometer, Bio-Medical Instrument Company, Newbury, OH, U.S.). Subjects with T1D were included in the study if presented no symptoms of peripheral polineuropathy and no more than slight alteration on biothesiometry exploration (vibratory perception lower than 25 volts) (J. B. Dyck & Dyck, 1999; Schmid, Neumann, & Brugnara, 2003; Tesfaye et al., 2010).

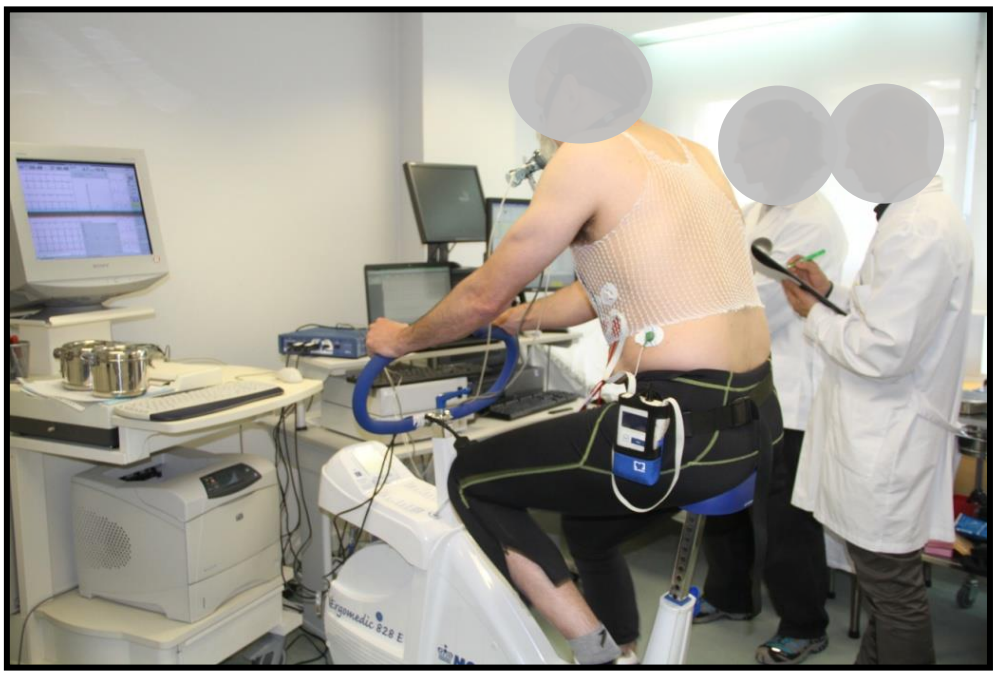
From medical registers, we had access to albuminuria results and ophthalmological examination from patients with T1D. All patients included in the study presented normal albuminuria or persistent albuminuria values below 300 mg/24h, and normal retinal exam or incipient retinopathy. Patients with persistent albuminuria above 300 mg/24h, or severe nonproliferative or proliferative retinopathy were excluded (American Diabetes Association, 2015).

b. Physical Fitness Evaluation

Maximal oxygen uptake or peak (VO_2peak) was determined by using a maximal progressive incremental exercise test on a friction-braked cycle-ergometer (Monark 828E, Monark Sweden). After a 3 min warm-up period at a power output of 25-W, workload was increased by 25-W every minute until exhaustion. Oxygen uptake was monitored during exercise using a computerized, open circuit gas-collection system (Vmax Spectra, version v12.0, Sensor Medics

Corp, VIASYS Healthcare Inc, Yorba Linda, CA, U.S.), and VO_2peak was determined at the point of highest oxygen consumption over a 15-s period. VO_2peak was confirmed using established physiological criteria, including a respiratory exchange ratio above 1.15, oxygen uptake reaching a plateau despite an increased work rate, a heart rate near 95% of the age-predicted maximum value, or the incapacity to maintain the marked load (Image 4).

Image 4 - Maximal oxygen uptake in cycle-ergometer
(Monark 828E, Monark Sweden / Vmax Spectra, version v12.0, Sensor Medics Corp, VIASYS Healthcare Inc, Yorba Linda, CA, U.S.)



VO_2peak determination

c. Standard biochemical determinations and eGDR

Subjects with type 1 diabetes had usual laboratory determinations: glycemia (glucose-oxidase method, Advia 2400 Siemens Diagnostics, Deerfield, IL, U.S.); glycated hemoglobin (HbA1c) (high-performance liquid chromatography [HPLC]); total-cholesterol (TC), HDL-cholesterol (HDL-C) and

triglycerides (TG) (molecular absorption spectrometry) and LDL-cholesterol (LDL-C) (Friedewald equation); and albuminuria (immunoturbidimetric assay).

Estimated glucose disposal rate (eGDR) was calculated by the equation originally developed by Williams and colleagues (Williams et al., 2000) for use in patients with T1D. This equation result of eGDR is expressed in milligrams per kilogram per minute and it has a good correlation with values measured with a euglycemic-hyperinsulinemic clamp. The equation was modified to be use with HbA1c instead of HbA1 (Wáden et al., 2005):
eGDR = 24.4 – 12.97*(waist-to-hip ratio) – 3.39*(hypertension) – 0.60*(HbA1c), where no hypertension is assigned as 0 or hypertension as 1, based on blood pressure ≥ 140/90 and/or antihypertensive medication.

d. Statistical Analysis:
Mean and standard deviation were used to express the clinical and biochemical results obtained. ANOVA or Student test were used to compare continuous variables; Mann-Whitney U test for non-parametrical variables; chi-square was used for dichotomous variables; and Spearman tests for variable correlations. It was assumed as significant difference a p value < 0.05.

Results

An initial overview of subjects that participated is presented in Table 1.1. One-hundred twenty-nine patients with type 1 diabetes (T1D) and 136 subjects without diabetes (controls – CT) were evaluated. The subjects in both groups participated in different training modalities - like running, swimming, cycling, tennis, soccer –, or performed habitual walking or recreational bicycling, or even no activity at all. All participants presented normal cardiac evaluation and no one presented orthostatic hypotension or peripheral vasculopathy.

Subjects with T1D presented similar age (36.7 ± 11.6 years in T1D vs. 35.8 ± 8.8 in CT), similar clinical history of hypertension, dyslipidemia or active smoking than control subjects. The groups were also not different when compared by body composition, considering body mass index (BMI) or total fat percentage by skinfolds, or total and abdominal fat percentage, by bioimpedances, or DXA. The proportion of low, moderate and high active subjects were not different between groups, as well as estimated energy consumption evaluated by METs in IPAQ-SF questionnaire. The groups were different on the proportion of men and women, fact that could justify the differences in weight, height and VO_2peak (Table 1.1).

Subjects with T1D were compared separately by gender (Table 1.2). Men and women presented the main differences in the field of body composition, analyzed by different methods: BMI, skinfolds, bioimpedance and DXA. Moreover, women were less active than men (IPAQ classification p= 0.003) and presented lower VO_2peak (32.3 mL/kg/min ± 10.3 vs. 23.3 ± 7.2, $p < 0.001$). In biochemical analysis, women presented higher levels of HDL cholesterol and higher eGDR index.

Aware of these differences in weight, height, body composition and VO_2peak (Tables 1.1 and 1.2), men and women were analyzed separately. Table 1.3 presented data from women, and in Table 1.4, data from men. Women with T1D presented similar age, body composition, physical activity levels and cardiorespiratory capacity than control women.

Men with T1D presented similar body composition and physical activity than control men (Table 1.4). In the T1D group, men were older (37.6 ± 11.4 vs. 34.5 ± 6.7 years, p = 0.028) and presented a lower cardiorespiratory capacity (32.3 ± 10.3 vs. 38.5 ± 11.6 mL/kg/min, p = 0.001) when compared with control ones. Age was studied as a covariate, and with this correction, the VO_2peak difference between the diabetes and control groups remained different (p = 0.003).

Table 1.1 – Overview of clinical characteristics of all subjects

Total *(265)*	***Type 1 diabetes*** *(129)*	***Controls*** *(136)*	***p***
Sex (Men / Women)	98 / 31	75 / 61	**< 0.001**
Age (years)	36.7 ± 11.6	35.8 ± 8.8	ns
Hypertension (n)	31	10	**< 0.001**
Dyslipidemia (n)	28	8	**< 0.001**
Active smoking (n)	32	34	ns
BODY COMPOSITION			
Weight (kg)	74.8 ± 12.3	70.8 ± 12.6	**0.01**
Height (m)	1.74 ± 0.07	1.71 ± 0.09	**0.008**
BMI (kg/m^2)	24.6 ± 3.2	24 ± 2.9	ns
Waist (cm)	84.4 ± 11	81.8 ± 10.1	ns
WHR (Waist/hip ratio)	0.84 ± 0.07	0.82 ± 0.07	ns
Total fat % (skinfolds)	18.9 ± 7.6	18.6 ± 7.4	ns
Total fat % (bioimpedance)	21.2 ± 6.9	21.1 ± 7.4	ns
Abdominal fat % (bioimpedance)	10.3 ± 6	9.9 ± 11.4	ns
Total fat % (DXA)	26.1 ± 8.7	27.7 ± 8.8	ns
Abdominal fat % (DXA)	30.1 ± 12.3	31.5 ± 11.6	ns
PHYSICAL FITNESS			
Physical Activity (low / moderate / high)	29 / 47 / 53	37 / 47 / 52	ns
IPAQ – SF (METs/week)	2411 ± 1934	2492 ± 2192	ns
VO_2peak (mL/kg/min)	30.3 ± 10.6	33.4 ± 11.8	**0.031**

Table 1.2 – Clinical and biochemical characteristics of men and women with type 1 diabetes

T1D (129)	***men (98)***	***women (31)***	***p***
Age (years)	37,6 ± 11,4	33,2 ± 11,5	ns
Evolution of diabetes (years)	16.4 ± 10.2	13.4 ± 9.6	ns
Hypertension (n)	26	5	ns
Dyslipidemia (n)	19	9	ns
Active smoke (n)	20	12	ns
CHRONIC COMPLICATIONS			
Retinopathy (n)	17	3	ns
Nephropathy (n)	4	1	ns
Neuropathy (n)	9	1	ns
Macrovascular disease (n)	0	0	-
Doses of insulin (Total Units/day)	47.8 ± 18.1	32.8 ± 14.7	**<0.001**
Units of insulin / body weight (U/kg)	0.61 ± 0.22	0.51 ± 0.21	**0.026**
BODY COMPOSITION			
BMI (kg/m^2)	24.9 ± 2.8	23.1 ± 3.2	**<0.001**
Waist (cm)	86.5 ±10.6	77.5 ±9.3	**<0.001**
WHR (Waist/hip ratio)	0.86 ±.06	0.77 ±.05	**<0.001**
Total fat % (skinfolds)	16.6 ± 6.6	26.1 ± 5.9	**<0.001**
Total fat % (bioimpedance)	19.4 ± 5.9	27.4 ± 6.6	**<0.001**
Abdominal fat % (bioimpedance)	11.1 ± 6.4	7.4 ± 2.9	**<0.001**
Total fat % (DXA)	23 ± 7.3	34.5 ± 6.6	**<0.001**
Abdominal fat % (DXA)	28 ± 12.4	36.2 ± 10.1	**<0.001**
PHYSICAL FITNESS			
Physical Activity (low / moderate / high)	21 / 29 / 48	8 / 18 / 5	**0.003**
SF-IPAQ (METs/week)	2459 ± 1759	2120 ± 1285	ns
VO_2peak (mL/kg/min)	32.3 ± 10.3	23.3 ± 7.2	**<0.001**
BIOCHEMICAL ANALYSIS			
Glycemia (mg/dl)	159.1 ± 69.4	166.5 ± 49.2	ns
Total cholesterol (mg/dl)	177.5 ± 33.7	194.1 ± 34.8	ns
LDL cholesterol (mg/dl)	110 ± 24.1	118.8 ± 26.7	ns
HDL cholesterol (mg/dl)	53 ± 11.8	62.6 ± 12.2	**0.004**
Triglycerides (mg/dl)	72.5 ± 33.8	63.4 ± 32.1	ns
Glycated hemoglobin (%)	7.2 ± 1	7.5 ± 1.2	ns
Creatinine (mg/dl)	0.98 ± 0.26	0.79 ± 0.09	**0.008**
eGDR (mg/kg/min)	8 ± 1.9	9.2 ± 2	**0.019**

Table 1.3 - Clinical characteristics in women with type 1 diabetes and controls

WOMEN *(92)*	***Type 1 diabetes*** *(31)*	***Controls*** *(61)*	***p***
Age (years)	33.2 ± 11.5	37.6 ± 11	ns
BODY COMPOSITION			
Weight (kg)	64.1 ± 10.8	61.5 ± 8.9	ns
Height (m)	1.65 ± 0.06	1.63 ± 0.06	ns
BMI (kg/m^2)	23.5 ± 3.8	22.9 ± 2.8	ns
waist (cm)	77.5 ± 9.5	77.1 ± 9.7	ns
WHR (waist/hip ratio)	0.77 ± 0.05	0.78 ± 0.07	ns
Total fat % (skinfolds)	26.1 ± 5.9	23.3 ± 7	ns
Total fat % (bioimpedance)	27.4 ± 6.6	24.3 ± 8.9	ns
Abdominal fat % (bioimpedance)	7.3 ± 2.8	3.7 ± 5.3	ns
Total fat % (DXA)	34.5 ± 6.6	33.5 ± 7.9	ns
Abdominal fat % (DXA)	36.2 ± 10.1	34.9 ± 12.5	ns
FISICAL FITNESS			
Physical Activity (low / moderate / high)	8 / 18 / 5	20 / 28 / 13	ns
SF-IPAQ (METs/week)	2120 ± 2285	1841 ± 1634	ns
VO_2peak (mL/kg/min)	23.3 ± 7.2	26.8 ± 8.8	ns

Table 1.4 – Clinical characteristics in men with type 1 diabetes and controls

MEN *(173)*	***Type 1 diabetes*** *(98)*	***Controls*** *(75)*	***p***
Age (years)	37.6 ± 11.4	34.5 ± 6.7	**0.028**
BODY COMPOSITION			
Weight (kg)	78.1 ± 10.8	78.3 ± 9.9	ns
Height (m)	1.76 ± 0.06	1.77 ± 0.06	ns
BMI (kg/m^2)	25 ± 3	24.9 ± 2.7	ns
Waist (cm)	86.5 ± 10.6	85.9 ± 8.4	ns
WHR (waist/hip ratio)	0.86 ± 0.06	0.86 ± 0.05	ns
Total fat % (skinfolds)	16.6 ± 6.6	15 ± 5.4	ns
Total fat % (bioimpedance)	19.3 ± 5.9	18.7 ± 4.8	ns
Abdominal fat % (bioimpedance)	11.2 ± 6.4	11.9 ± 14.2	ns
Total fat % (DXA)	23 ± 7.3	23 ± 6.3	ns
Abdominal fat % (DXA)	28 ± 12.4	28.6 ± 10	ns
PHYSICAL FITNESS			
Physical Activity (low / moderate / high)	21 / 29 / 48	17 / 19 / 39	ns
SF-IPAQ (METs/week)	2459 ± 1759	3044 ± 2477	ns
VO_2peak (mL/kg/min)	32.3 ± 10.3	38.5 ± 11.6	**0.001**

A remarkable number of highly active men was included in this study, fact that permitted a specific analysis of this subgroup of subjects, with the intention of understanding better this difference in cardiorespiratory capacity in T1D men. Table 1.5 shows the data of 48 patients with T1D and 39 controls. Both groups presented similar age, body composition and estimated energy consumption evaluated by METs in IPAQ-SF questionnaire, but, despite these similitudes, a lower cardiorespiratory capacity in T1D group was verified when compared with the control one (36.4 ± 10.2 vs. 42.8 ± 12.5 mL/kg/min, p = 0.016).

We also studied a possible effect of an incipient chronic complication related to diabetes in the group of men with T1D. Chronic complications allowed in this study were incipient retinopathy, presence of persistent albuminuria (≥ 30 mg/24h and ≤ 300 mg/24h) and/or initial peripheral neuropathy (values lower than 25 V in biothesiometer measurement, but higher than 15 V). Clinical and laboratorial characteristics of patients with T1D were also explored in this analysis (Table 1.6). In this specific analysis, 8 subjects presented incipient retinopathy, 2 presented persistent albuminuria, and 4 presented initial peripheral neuropathy (12 subjects with any combination). The group with any chronic complications was older (38.4 ± 8.9 vs.30.6 ± 9.9 years old, p = 0.02) and presented more years of evolution of the diabetes (22.1 ± 12.3 vs. 11.2 ± 7, p=0.01). Despite of these differences, in our group of patients, early chronic complications did not influence the cardiorespiratory capacity (Table 1.6).

Table 1.5 – Clinical characteristics of high active men with type 1 diabetes and controls

MEN *(87)*	***Type 1 diabetes*** *(48)*	***Controls*** *(39)*	***p***
Age (years)	32.6 ± 10.2	35.5 ± 6.5	ns
Hypertension (n)	8	5	ns
Dyslipidemia (n)	6	4	ns
Active smoking (n)	10	8	ns
BODY COMPOSITION			
BMI (kg/m^2)	24.8 ± 2.7	25 ± 2.5	ns
Waist (cm)	84.8 ± 9.7	85.3 ± 8	ns
WHR (waist/hip ratio)	0.85 ± 0.05	0.85 ± 0.05	ns
Total fat % (skinfolds)	14.7 ± 6.4	13.2 ± 4.8	ns
Total fat % (bioimpedance)	18.8 ± 5.8	18.3 ± 4.6	ns
Abdominal fat % (bioimpedance)	9.3 ± 5.4	9.3 ± 4	ns
Total fat % (DXA)	20.4 ± 6.8	20.9 ± 5.2	ns
Abdominal fat % (DXA)	23.5 ± 12	25.4 ± 9.2	ns
PHYSICAL FITNESS			
SF-IPAQ (METs/week)	3793 ± 1425	4473 ± 2551	ns
VO_2peak (mL/kg/min)	36.4 ± 10.2	42.8 ± 12.5	**0.016**

Table 1.6 – Clinical characteristics of high active men with type 1 diabetes concerning incipient chronic complications related to diabetes

High active men with T1D *(48)*	***Any chronic incipient complication*** *(12)*	***Without chronic complications*** *(36)*	***p***
Age (years)	38.4 ± 8.9	30.6 ± 9.9	**0.02**
Evolution of diabetes (years)	22.1 ± 12.3	11.2 ± 7	**0.01**
Hypertension (n)	3	5	ns
Dyslipidemia (n)	0	5	ns
Active smoking (n)	1	8	ns
Units of insulin (U/day)	42.5 ± 9.6	48.1 ± 19.8	ns
BODY COMPOSITION			
BMI (kg/m^2)	25.1 ± 2.4	24.7 ± 2.9	ns
Waist (cm)	85.5 ± 7.9	84.6 ± 10.4	ns
WHR (waist/hip ratio)	0.83 ± 0.04	0.85 ± 0.05	ns
Total fat % (skinfolds)	17.5 ± 4.6	13.7 ± 6.7	ns
Total fat % (bioimpedance)	20.5 ± 2.4	18.3 ± 6.5	ns
Abdominal fat % (bioimpedance)	9.1 ± 3	9.3 ± 6.1	ns
Total fat % (DXA)	21.4 ± 5.3	20.1 ± 7.3	ns
Abdominal fat % (DXA)	23.6 ± 8.7	23.5 ± 13	ns
PHYSICAL FITNESS			
SF-IPAQ (METs/week)	3615 ± 1577	3852 ± 1390	ns
VO_2peak (mL/kg/min)	38.5 ± 10.5	35.5 ± 10.1	ns
eGDR (mg/kg/min)	8.4 ± 1.3	8.3 ± 1.8	ns

We also compared highly and less active men with T1D (Table 1.7). We could identify that highly active men were younger than less active (35.5 ± 10.2 vs. 43.9 ± 9.6 years old, $p < 0.001$), presented less years of evolution of diabetes, had less hypertension and less dyslipidemia. In almost all parameters of body composition, less active men presented higher percentage of total and abdominal fat. In the biochemical analysis, the only parameter that presented a difference between groups was fasting glycemia (high 144 ± 55 mg/dl vs. low 192 ± 83, $p = 0.014$). EGDR was higher in the highly active group (8.3 ± 1.6 mg/kg/min vs. 7 ± 2.1, $p = 0.024$).

EGDR correlations with continuous physical activity variables in T1D are presented in Table 1.8 (all T1D subjects), Table 1.9 (men with T1D) and Table 1.10 (women with T1D). EGDR did not correlate with the physical activity variables, named VO_2peak or METs by IPAQ-SF. A negative correlation of eGRD with total fat percentage (by DXA) in men was found. Considering that the data of waist/hip ratio takes part of the formula for the estimation of insulin sensitivity (eGDR), it is a coherent result. Other correlations that could be verified were the percentage of total fat inversely with physical fitness (VO_2peak) or with physical activity amount (METs).

We looked for a possible correlation between fitness (VO_2peak) and glycemic control (HbA1c), but it was not significant.

Finally, we compared the different methods for the determination of body fat composition. The total fat percentage estimated by DXA was positively correlated with total fat percentage by bioimpedance ($p > 0.001$, $R = 0.741$) and with total fat percentage by skinfolds ($p < 0.001$, $R = 0.892$). For abdominal fat percentage, the determination by DXA was positively correlated with abdominal by bioimpedance ($p < 0.001$, $R = 0.651$).

Table 1.7 - Clinical characteristics of men with type 1 diabetes, comparing high physical activity with low physical activity

Men with T1D *(69)*	***High physical activity*** *(48)*	***Low physical activity*** *(21)*	***p***
Age (years)	32.5 ± 10.2	43.9 ± 9.6	**< 0.001**
Evolution of diabetes (years)	14 ± 9.8	19.4 ± 9.2	**0.03**
Hypertension (n)	8	10	**0.014**
Dyslipidemia (n)	5	8	**0.011**
Active smoking (n)	9	3	ns
Units of insulin (U/day)	46.8 ± 17.9	54.4 ± 17.3	ns
BODY COMPOSITION			
BMI (kg/m^2)	24.8 ± 2.7	26.2 ± 3.7	ns
Waist (cm)	84.8 ± 9.7	92.7 ± 12.7	**0.013**
WHR (waist/hip ratio)	0.85 ± 0.05	0.9 ± 0.07	**0.01**
Total fat % (skinfolds)	14.7 ± 6.4	19.8 ± 6.4	**0.005**
Total fat % (bioimpedance)	18.8 ± 5.8	20.8 ± 7.9	ns
Abdominal fat % (bioimpedance)	9.3 ± 5.4	16.5 ± 7.4	**< 0.001**
Total fat % (DXA)	20.4 ± 6.8	28.3 ± 6.1	**< 0.001**
Abdominal fat % (DXA)	23.5 ± 12	36.7 ± 9.1	**< 0.001**
PHYSICAL FITNESS			
SF-IPAQ (METs/week)	3793 ± 1425	488 ± 387	**< 0.001**
VO_2peak (mL/kg/min)	36.4 ± 10.2	25.1 ± 7.3	**< 0.001**
BIOCHEMICAL ANALYSIS			
Fasting glycemia (mg/dl)	144 ± 55	192 ± 83	**0.014**
HbA1c (%)	7.2 ± 1.1	7.2 ± 1.2	ns
Total cholesterol (mg/dl)	172 ± 37	184 ± 34	ns
HDL-cholesterol (mg/dl)	52.9 ± 13.7	53.2 ± 8.6	ns
LDL-cholesterol (mg/dl)	104.9 ± 30	116.6 ± 24	ns
Triglycerides (mg/dl)	69.2 ± 31.5	70.7 ± 48.3	ns
eGDR (mg/kg/min)	8.3 ± 1.6	7 ± 2.1	**0.024**

Table 1.8 – Correlations eGDR with physical fitness variables and total fat percentage in type 1 diabetes (men and women)

	METs [a]	***VO_2peak***	***eGDR***
Total Fat % (DXA)	**R = - 0.46** **p < 0.001**	**R = - 0.614** **p < 0.001**	R = - 0.146 p = 0.19
METs [a]		**R = 0.517** **p < 0.001**	R = 0.186 p = 0.078
VO_2peak (mL/kg/min)			R = 0.076 p = 0.48

[a] IPAQ-SF in METs/week

Table 1.9 - Correlations eGDR with physical fitness variables and total fat percentage in men with type 1 diabetes

	METs [a]	***VO_2peak***	***eGDR***
Total fat % (DXA)	**R = - 0.389** **p < 0.001**	**R = - 0.419** **p < 0.001**	**R = - 0.393** **p = 0.002**
METs [a]	-	**R = 0.472** **p < 0.001**	R = 0.23 p = 0.055
VO_2peak (mL/kg/min)		-	R = 0.124 p = 0.323

[a] IPAQ-SF in METs/week

Table 1.10 - Correlations eGDR with physical fitness variables and total fat percentage in women with type 1 diabetes

	METs [a]	***VO_2peak***	***eGDR***
Total fat % (DXA)	**R = - 0.471** **P < 0.001**	**R = - 0.581** **p < 0.001**	R = - 0.291 p = 0.201
METs [a] (per week – SF-IPAQ)	-	**R = 0.451** **p < 0.001**	R = 0.33 p = 0.88
VO_2peak (mL/kg/min)		-	R = 0.153 p = 0.066

[a] IPAQ-SF in METs/week

Discussion

The present analysis is based on an observational study of patients with T1D and subjects without diabetes that volunteered to participate in this evaluation. The patients that were included in the final analysis did not present advanced chronic complications related to diabetes. In the group with diabetes there was a higher proportional presence of men than in the control group. Some clinical characteristics were found to be different between the groups with or without diabetes: a higher prevalence of hypertension and dyslipidemia and a lower VO_2peak, a marker of physical fitness level.

VO_2peak may be conditioned by some factors like age, gender, genetics and physical training (Powers & Howley, 2015). Thus, we separated the subjects by gender (Tables 1.3 and 1.4) to review the variables in more detail. The analysis of women showed no differences between the ones with T1D and the control

ones. On the other hand, men with T1D presented lower VO_2peak than the ones without diabetes, even after the adjustment for age. We are aware about the small number of subjects, especially women, and a possible different physical training between the subjects. Due to that, we specifically compared highly active men with and without T1D (Table 1.5), and VO_2peak presented sustained lower rates in T1D. A lower VO_2peak in subjects with T1D was already observed by some studies (William Ricardo Komatsu et al., 2005; Nguyen et al., 2014), but not confirmed by others (Benbassat et al., 2001; William R Komatsu et al., 2010), as stated in the Rationale. The glycemic control is referred as a factor that could influence VO_2peak, but no association was found in our study, probably due to the adequate glycemic control presented by the subjects that participated in this analysis reflected by glycated hemoglobin (HbA1c) of 7.2 ± 1 % in men and 7.5 ± 1.2 in women.

EGDR in an easy clinical index to estimate insulin sensitivity in subjects with T1D. In the present analysis, the difference of eGDR index between men and women must be interpreted carefully because one of the elements of the equation of eGDR is waist/hip ratio (WHR), and women constitutionally present lower values of WHR (Table 1.2).

It is described that eGDR is associated with microvascular complications (Chillarón et al., 2009; Girgis et al., 2012), subclinical atherosclerosis (Olson et al., 2002) and low grade inflammation (Llauradó et al., 2012) and it also predicts coronary artery disease (Orchard et al., 2003; Orchard, Costacou, Kretowski, & Nesto, 2006). In the present study, we analyzed just a few patients with any chronic complication (n = 12), but they did not present difference in the eGDR index compared to the ones without complications (Table 1.6). It is demonstrated that sedentary patients had lower eGDR (Wáden et al., 2005) than active ones, and we could obtain similar results when we compared highly active with less active men (Table 1.7), identifying that low active subjects presented higher prevalence of hypertension and higher waist/hip ratio (criteria for eGDR) and a probable higher insulin resistance.

Finally, we did not obtain correlations of physical fitness (VO_2peak) and physical activity levels (METs) with eGDR (Tables 1.8, 1.9 and 1.10), but VO_2peak with total body fat percentage.

The results suggest a lower physical fitness of men with T1D when compared with control men. Despite the careful characterization of the participants in relation to clinical data, body composition, metabolic control and chronic complications, we believe that studies with a higher number of subjects must be performed to confirm these results.

AIM 2 – STUDY 2

Metabolomics approach for analyzing the effects of exercise in subjects with type 1 diabetes mellitus

In the second study, we proposed to analyze the metabolic response to an acute exercise in subjects with T1D, in order to understand the main metabolic aspects of this response and to verify if there were differences compared to subjects without diabetes.

For this, we worked with the Metabolomics Platform from Universitat Rovira i Virgili, which performed a non-target metabolomic analysis from the serum of the subjects. This metabolomics analysis was explored by two approaches: ^{1}H-NMR and GC-MS.

To avoid gender interference in the results, we invited just men to participate. The volunteers were invited from the ones that had already participated in the baseline evaluation in de Diabetes Research Clinical Unit, from the series explained in Study 1. Ten men with T1D and 11 men without diabetes participated in the Study 2.

The exercise proposed was 30 minutes of cycle-ergometer at 80% maximal aerobic capacity, calculated from of each individual VO_2peak, already determined in the first evaluation. The tests were performed at first hour in the morning, in fasting state. Participants with T1D were oriented to do not inject fast-action insulin in the morning. Capillary blood determinations were performed before, during and after the 30 min of the exercise test in subjects with T1D. Blood samples were taken before and just after the 30 minutes of exercise from all subjects.

The results are described in the following publication:

Brugnara L, Vinaixa M, Murillo S, Samino S, Rodriguez MA, Beltran A, et al. (2012) Metabolomics Approach for Analyzing the Effects of Exercise in Subjects with Type 1 Diabetes Mellitus. PLoS ONE 7(7): e40600. doi:10.1371/journal.pone.0040600

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Metabolomics Approach for Analyzing the Effects of Exercise in Subjects with Type 1 Diabetes Mellitus

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Abstract

The beneficial effects of exercise in patients with type 1 diabetes (T1D) are not fully proven, given that it may occasionally induce acute metabolic disturbances. Indeed, the metabolic disturbances associated with sustained exercise may lead to worsening control unless great care is taken to adjust carbohydrate intake and insulin dosage. In this work, pre- and post-exercise metabolites were analyzed using a ^{1}H-NMR and GC-MS untargeted metabolomics approach assayed in serum. We studied ten men with T1D and eleven controls matched for age, body mass index, body fat composition, and cardiorespiratory capacity, participated in the study. The participants performed 30 minutes of exercise on a cycle-ergometer at 80% VO_2max. In response to exercise, both groups had increased concentrations of gluconeogenic precursors (alanine and lactate) and tricarboxylic acid cycle intermediates (citrate, malate, fumarate and succinate). The T1D group, however, showed attenuation in the response of these metabolites to exercise. Conversely to T1D, the control group also presented increases in α-ketoglutarate, alpha-ketoisocaproic acid, and lipolysis products (glycerol and oleic and linoleic acids), as well as a reduction in branched chain amino acids (valine and leucine) determinations. The T1D patients presented a blunted metabolic response to acute exercise as compared to controls. This attenuated response may interfere in the healthy performance or fitness of T1D patients, something that further studies should elucidate.

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Introduction

Type 1 diabetes mellitus (T1D) is a lifelong metabolic disorder of usual acute onset in children, adolescents and young adult people. Over time, micro and macro vascular co-morbidities develop in patients with T1D which are closely related to metabolic control [1]. In addition to these complications, the management of T1D is particularly complex and challenging. It is well known that, in order to maintain optimal glycemic control, T1D patients need accurate administration of insulin coordinated with a balanced diet and an adequate level of physical activity.

Exercise plays a crucial role in the prevention and treatment of several chronic diseases, including glucose intolerance states, type 2 diabetes [2–4] and diseases of the cardiovascular system [5,6]. Moreover, it has been demonstrated that exercise improves the quality of life in the general population [7]. However, the beneficial effects of exercise in patients with T1D are not fully proven, given that exercise may occasionally induce acute metabolic disturbances, mainly related to insulin treatment. Nevertheless, children and adolescents with T1D are encouraged to exercise regularly as a means of improving social integration and cardiovascular health [8]. Thus, a better understanding of the effects of exercise on the metabolic response in T1D patients will allow clinicians to prescribe exercise to their patients with greater clarity.

Metabolomics enables the systematic assessment of the abundant changes of low molecular weight compounds present in biological samples, using high-throughput sample analysis techniques (GC-MS, NMR or HPLC-MS) and computer-assisted multivariate pattern-recognition techniques [9]. Metabolomics is enriching our current understanding of both the physiologic and physiopathologic processes underlying diabetes mellitus [10–12]. Moreover, recent metabolomic-based studies have described the first metabolic signatures of exercise in human plasma [13–15]. For example, Lewis et al [13] described the metabolic changes in tricarboxylic acid cycle, fatty acid oxidation and lypolisis in the plasma of healthy subjects exposed to different intensities and durations of exercise.

Most of the *in vivo* studies investigating the metabolic pathways of T1D have been performed under strictly controlled conditions using hyperinsulinemic euglycemic clamp techniques [16,17] or in situations of insulin withdrawal [12]. Although these studies have provided invaluable new insights into the metabolic disturbances present in T1D, the experimental conditions used are dissimilar to everyday life. To the best of our knowledge, no study to date has

applied a metabolomics approach prior to and following exercise in subjects with T1D. We hypothesize that (a) an acute bout of exercise will result in changes in the systemic metabolic profile and that (b) these parameters will be different in patients with T1D in comparison to healthy controls. The aim of this study is to analyze the metabolic changes induced by a short-term session of acute exercise performed by T1D patients and their corresponding non-diabetic counterparts. A comprehensive ^{1}H-NMR and GC-MS untargeted metabolomics approach was applied to serum samples taken from all participants.

Methods

Participants

Ten recreationally active male patients with T1D, recruited by the Department of Endocrinology (Hospital Clinic, Barcelona), and eleven non-diabetic controls matched for sex, age, body mass index (BMI) and similar physical activity, recruited from a research institute (IDIBAPS, Barcelona), were enrolled in the study.

The T1D patients participating in the study had diabetes for a total of 14±8.4 years, undetectable C-peptide levels and good glycemic control, as determined by glycated hemoglobin A1c. Total body composition was measured by densitometry using DXA (Lunar iDXA body composition, GE Healthcare). Patients with chronic complications related to diabetes were excluded. All patients presented microalbuminuria values below 30 mg/L, normal retinal exam by direct and indirect retinoscopy, normal peripheral neurologic evaluation by clinical exploration and biothesiometry (Bio-thesiometer, Bio-Medical Instrument Company, Newbury, OH, U.S.), and normal. resting 12-lead electrocardiogram (ECG) and normal exercise testing by upright cycle-ergometer (25 W/3 min) [18]. At the time of testing, none of the T1D participants were taking any form of prescription medication, except for long-acting basal insulin analogue glargine (Sanofi-Aventis, U.S.) and fast-acting insulin analogue aspart (Novo Nordisk, Denmark).

Experimental Procedures

All subjects were required to visit the Diabetes and Exercise Research Unit of the Hospital Clínic on two separate occasions. On the first visit, all subjects were fully briefed and familiarized with the experimental procedures. Baseline clinical characteristics such as height, weight, BMI and total and fat body composition where also obtained, and each subject was required to complete an evaluation of current physical activity using the International Physical Activity Questionnaire [19]. Maximal oxygen uptake (VO_2max) was determined by using a maximal progressive incremental exercise test on a friction-braked cycle-ergometer (Monark 828E, Monark Sweden). After a 3 min warm-up period at a power output of 25-W, workload was increased by 25-W every minute until exhaustion. Oxygen uptake was monitored during exercise using a computerized, open circuit gas-collection system (Vmax Spectra, version v12.0, Sensor Medics Corp, VIASYS Healthcare Inc, Yorba Linda, CA, U.S.), and VO_2max was determined at the point of highest oxygen consumption over a 15-s period. VO_2max was confirmed using established physiological criteria, including a respiratory exchange ratio above 1.15, oxygen uptake reaching a plateau despite an increased work rate, and a heart rate near 95% of the age-predicted maximum value.

On the second visit, conducted at the same time (8:30am) a week later, subjects performed an acute bout of 30 minutes of intense exercise at 80% VO_2max (individually calculated during the preliminary session) on a cycle-ergometer. Subjects performed 3 to 6 minutes of warm-up until achieving a fixed cardiac frequency. Prior to exercise, all participants fasted overnight for a 12 hour period, following a balanced meal consisting of approximately 55% carbohydrates, 30% fat, and 15% proteins. Furthermore, T1D patients had received their last short-acting insulin injection before dinner at 8 p.m. and their long-acting insulin injection at 10 p.m. All subjects were asked to avoid strenuous exercise and alcohol consumption the day prior to the acute exercise protocol.

Blood determinations. Fasting blood samples were obtained before and after the short-term intensive exercise intervention. Glycemia (glucose-oxidase method, Advia 2400 Siemens Diagnostics, Deerfield, IL, U.S.) and insulinemia (quimioluminiscent method, Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.) were determined in serum samples. For the metabolomic measurements, serum was obtained once blood had been allowed to clot at room temperature for 30 min and after centrifugation at 4°C at 5000 rpm for 10 min. Samples were kept at - 80°C until further metabolomic analysis.

Serum ^{1}H-NMR metabolomics. Serum samples were thawed, vortexed and allowed to stand for 10 min prior to NMR analysis. For NMR measurements 430 µl of serum were transferred into 5 mm NMR tubes. A double tube system was used: an internal tube (o.d. 2 mm, supported by a Teflon adapter) containing the reference substance (sodium 3-trimethylsilyl [2, 2, 3, 3-d4] propionate (TSP) 9.9 mmol/l, $MnSO_4$ 0.47 mmol/l in 99.9% D_2O) was placed coaxially into the NMR sample tube (o.d. 5 mm). This double tube system was kept at 4°C in the sample changer until analysis was performed. Spectra were acquired at a ^{1}H observation frequency of 600.20 MHz at a temperature of 300 K using an Avance III-600 Bruker spectrometer equipped with an inverse TCI 5 mm cryoprobe®. The Carr-Purcell-Meiboom-Gill (cpmg, spin-spin T_2 relaxation filter) pulse sequence with a fixed spin-spin relaxation delay of 200 ms was applied to acquire ^{1}H-NMR spectra for all serum samples, in order to minimize the broad signals arising from lipoprotein and albumin in the NMR spectra. For each sample, 128 transients were collected into 32 K data points using a spectral width of 12 kHz with a relaxation delay of 2 s and an acquisition time of 1.36 s. A line-broadening function of 0.3 Hz was applied to all spectra prior to Fourier transform.

Serum GC-MS metabolomics. A second aliquot of serum (100µL) was used for GC-MS analysis according to Agilent's specifications [20]. Each aliquot was spiked with 20 µl internal standard solution (1 µg.µL-1 succinic-d4 acid; Sigma-Aldrich). After protein precipitation using 900 µl of cold methanol/water (8:1 v/v), samples were centrifuged for 10 minutes at 4°C. 200 µL of the supernatant were transferred to a GC autosampler vial and spiked with 20 µl of myristic acid-d27 (Sigma Aldrich), used as the internal standard for retention time lock (RTL system provided in Agilent's ChemStation Software), and lyophilized overnight (Lyotrap freeze dryer). Samples were methoximated by incubating lyophilized serum residues in 50 µl of methoxyamine in pyridine (0.3 µg/µL) for 16 hours at room temperature. Silylation was subsequently done using 30 µL of N-methyl-N-trimethylsilyltrifluoroacetamide with 1%trimethylchlorosilane (MSTFA +1% TMCS, Sigma) for 1 hour at room temperature. Samples were automatically injected into a GC–MS system (HP 6890 Series gas chromatograph coupled to a mass selective detector model 5973) equipped with a J&W Scientific DB 5-MS+DG stationary phase column (30 m × 0.25 mm i.d., 0.1 µm film) (Agilent Technologies). The injector temperature was set at 250°C, and the helium carrier flow rate was kept constant at 1.1 mL/min. The column temperature was held at 60°C for 1 min, then increased to 325°C

at a rate of 10°C/min and held at 325°C for 10 min. The detector operated in the electron impact ionization mode (70 eV) and mass spectra were recorded after a solvent delay of 4 min with 2.46 scans per second (mass scanning range of m/z 50–600; threshold abundance value of 50 counts). The source temperature and quadrupole temperature were 230 and 150°C, respectively.

Ethics

Written informed consent was obtained from all subjects prior to participation. The experimental protocol was approved by the Research and Ethics committees of the Hospital Clínic de Barcelona, in accordance with the Declaration of Helsinki.

Data Analysis and Statistical Methods

The acquired CPMG ^{1}H-NMR spectra were phased, baseline-corrected and referenced to the chemical shift of the α-glucose anomeric proton doublet at 5.23 ppm. Pure standards compound reference in BBioref AMIX (Bruker) was used; HMDB and Chenomx databases were used for metabolite identification. After baseline correction, intensities of each ^{1}H-NMR region identified in the CPMG 1D-NMR spectra were integrated using the AMIX 3.8 software package (Bruker, GmBH). Each region was normalized to the ERETIC (Electronic REference To access *In vivo* Concentrations) signal [21].

Raw GC/MS files were exported into the platform-independent netCDF (*.cdf) and loaded into XCMS software (version 1.6.1) based on R-program version 2.4.0 (R-Foundation for statistical computing, www.Rproject.org), where peak peaking, integration and alignment in the time domain were performed. Integrated intensities of each m/z-retention time pair (MZRT) were obtained for each one of the samples used in the study. These intensities were normalized to internal standard succinic acid-d4. The AMDIS program (Automated Mass Spectral Deconvolution and Identification System, National Institute of Standards and Technology, Gathersburg, MD, U.S.A.) was run for peak annotation, and both the Fiehn GC/MS Metabolomics RTL Library and NIST mass spectral databases were used for identification. Table S1 shows detailed identification parameters for both GC-MS and ^{1}H-NMR determined metabolites.

Baseline metabolic differences between T1D and control groups were evaluated using the Mann-Witney U run test. Effects of exercise intervention on the independent control and T1D groups were assessed using the Wilcoxon exact rank sum tests. Repeated measures ANOVA were used to determine diabetes×exercise interactions. A statistically significant interaction indicates that control and T1D responded differently to the acute exercise protocol for a given metabolite. To account for multiple testing, q-values were computed for all systematic univariate tests outlined above by applying the FDR (False Discovery Rate) procedure described by Storey et al [22]. In all cases statistical significance was set at $q \leq 0.1$.Data (pre-) processing, data analysis, and statistical calculations were performed with Matlab (Matlab version 6.5.1, Release 13, The Mathworks, 2003).

Results

Clinical and Biochemical Baseline Characteristics

Anthropometric and fitness data are summarized in Table 1. In ten patients with T1D and eleven controls matched for age, height, weight, and body mass index (BMI), no differences were found in relation to the percentage of body fat composition and cardio respiratory capacity evaluated by VO_2max.

At baseline (Table 2), subjects with T1D presented higher glucose and insulin levels than controls. Moreover, untargeted ^{1}H-NMR and GC-MS metabolomic profiling results showed elevated levels of the tricarboxylic acid cycle intermediates (TCAIs) malate and citrate in T1D. Glycerol was also increased in the T1D group, whereas lysine levels were significantly lower as compared to the control group.

Table 1. Clinical characteristics of T1D patients and control population.

	Control	T1D	p-values
Subjects	11	10	ns
Age (years)	32.5±8.8	35.1±8.4	ns
Evolution of diabetes (years)	–	14±8.4	–
Height (m)	1.76±0.06	1.76±0.05	ns
Weight (kg)	75.9±8.6	75.6±5.8	ns
BMI (kg/m^2)	24.7±2.6	24.3±1.7	ns
Fat percentage (%, by DXA)	23.9±5.7	21.7±6.5	ns
IPAQ (METs min/week)	2550±995	2630±241	ns
VO_2max (mL/kg/min)	34±9.1	35±6.5	ns
Units of insulin glargine	–	31±7.9	–

Values are reported as mean values ± SD.
ns: not significant.
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Metabolic Changes Induced by Short-term Intensive Exercise

Figure 1A shows a comparison of the mean net percent variation following exercise in the individual T1D and control groups for glucose and insulin. After 30 minutes of intensive exercise, glucose levels varied differently according to diabetic condition. The T1D group, hyperglycaemic at baseline, showed significant glucose depletion with acute exercise unlike their control counterparts. Insulin levels also varied differently with the exercise according to diabetic condition. While insulin levels rose

Table 2. Baseline analytical differences of T1D patients and control population.

	Control (n = 11)	T1D (n = 10)	q-values
Biochemical determinations			
Glucose (mg/dl)	90.27±2.25	202.7±24.36	5.52×10^{-4}
Insulin (UI/L)	6.96±1.31	18.62±4.64	0.022
C-peptide (ng/ml)	–	undetectable	–
Glycated hemoglobin (%)	–	6.9±1	–
Metabolomics analysis (arbitrary units)			
Lysine	0.039±0.0036	0.025±0.0029	0.023
Glycerol	0.039±0.0022	0.045±0.0039	0.064
Citrate	0.006±0.0004	0.008±0.0005	0.052
Malate	0.001±0.0001	0.002±0.0002	0.083

Values are reported as mean values ±SEM. Selected quantitative ions relative to internal standard areas are used in the case of GC-MS measurements. Selective ^{1}H-NMR regions relative to ERETIC digital signals are used in the case of NMR measurements. Two-sided p-values are calculated using Mann-Witney test. Statistical significance was set as $q<0.1$.
doi:10.1371/journal.pone.0040600.t002

significantly with exercise in the T1D group, control exercisers showed a net decrease of insulin, which did not account for statistical significance. Although after short-term acute exercise both T1D and control groups showed a significant net increase in circulating levels of gluconeogenic precursors (alanine and lactate), this increase was less pronounced in the T1D group (Figure 1B). In addition to alanine and lactate, pyruvate increased significantly with exercise in the control group but not in the T1D group.

A significant enrichment in TCA cycle intermediates citrate, malate, fumarate, and succinate was observed after acute exercise in the peripheral blood of both control and T1D groups (Figure 2). However, α-ketoglutarate levels significantly increased only in the case of control exercisers. In general terms, there was less accumulation of TCA cycle intermediates in serum in the T1D group. No changes in other TCAIs were identified (isocitrate, succinil-coA and oxalacetate), nor was any interaction observed between the groups of T1D and controls in the metabolites that increased following exercise.

Exercise significantly increased glycerol and oleic and linoleic acid concentrations in the control group exclusively. This effect was attenuated in the T1D group (Figure 3A). There was no interactive effect observed between the T1D and control groups in these products of lipolysis. Branched chain amino acids (valine and leucine) were lower following short-term acute exercise in the control group (Figure 3B). These changes were paralleled by significantly increased levels of alpha-ketoisocaproic acid (2-KIC) in the same group. This increase was shown to be a diabetes-dependent trend (p-interaction diabetes×exercise = 0.05) but not statistically significant after FDR correction at the established conventional significance level ($q \leq 0.1$). Worth mentioning is lysine, which also showed a diabetes-dependent effect with the exercise.

Metabolite identification parameters are presented in supporting table (Table S1).

Discussion

The principal aim of this study was to analyze the metabolomic profile at rest and after a short period of intense exercise in patients affected by T1D to provide a comprehensive insight into the physiological effects of exercise on this particular population. Based on serum sample analysis, we have compared the metabolic response to short term acute exercise in T1D and healthy subjects using an untargeted metabolomics approach (GC-MS and ^{1}H-NMR). Our findings revealed similar metabolic events in T1D patients and their control matched exercisers, although the T1D patients showed an attenuation of overall metabolic response after intense short-term exercise.

As it is commonly known, in order to increase energy supply during intense short-term exercise, glycogen breakdown is induced

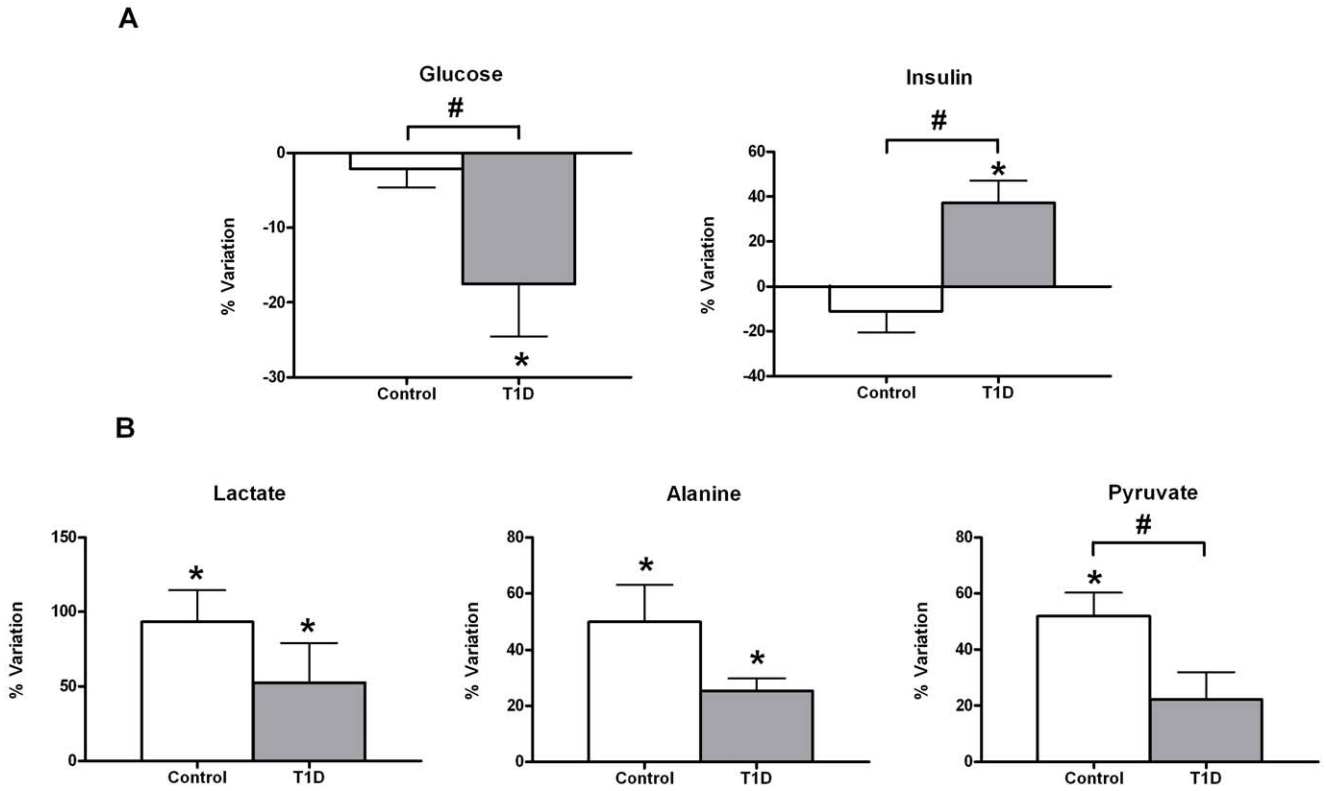

Figure 1. Relative changes in insulin and glucose and in gluconeogenic precursors in response to acute exercise. Relative changes in insulin and glucose (A) and in gluconeogenic precursors (B) in response to 30 minutes of acute exercise (80% VO_2max). Percentage of variation was calculated for each individual as the levels of a certain metabolite after exercise minus the levels of the same metabolite prior to exercise relative to the former. Data are shown as mean±sem of net percent variation for T1D and control groups. A positive value of percentage of variation indicates that metabolic levels have increased in mean with exercise, whereas a negative mean denotes the opposite. *Indicates a significant variation in metabolic levels with exercise (Wilcoxon rank-summed test for the comparison of a particular metabolite level prior to and after exercise in the independent T1D and control exercisers, $q<0.1$). #Indicates a significant diabetes×exercise interaction for a particular metabolite (Repeated-measures ANOVA, $q<0.1$). Insulin and glucose data correspond to biochemical measurements, whereas lactate, alanine, and pyruvate were evaluated in ^{1}H-NMR spectra according to Table S1.
doi:10.1371/journal.pone.0040600.g001

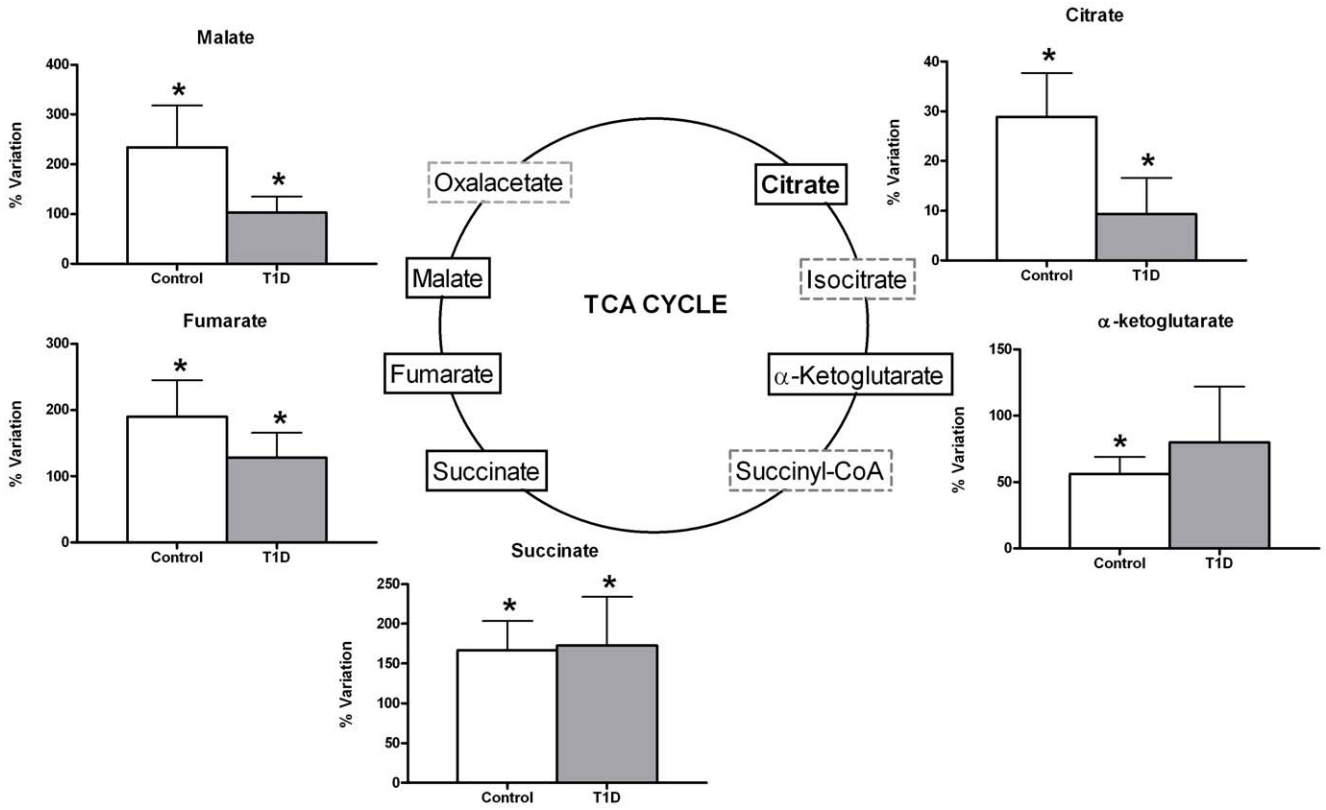

Figure 2. General significant enrichment in TCA cycle intermediates (TCAIs) in peripheral blood after acute exercise. Monitored using GC-MS (malate, fumarate, α-ketoglutarate) and NMR (citrate and succinate). Data are mean±sem of net percent variation with exercise. *Indicates a significant variation in metabolic levels with exercise (Wilcoxon rank-summed test for the comparison of a particular metabolite level prior to and after exercise in the independent T1D and control exercisers, $q<0.1$).
doi:10.1371/journal.pone.0040600.g002

to provide the substrate for activating anaerobic glycolysis, resulting in the accumulation of pyruvate and lactate in plasma. Recently, Lewis et al [13] confirmed these results by using LC-MS-based metabolomics in a non-diabetic population. Our data, using a different metabolomic approach based on ^{1}H-NMR and GC-MS techniques, showed that the non-diabetic group presented an increase in serum lactate and pyruvate concentration after exercise, indicating that glycolysis was activated, as previously reported by other groups [13,23]. However, the T1D patients elicited only an increase in lactate levels having blunted the pyruvate response, suggesting that exercise induces a reduced activation of glycogenolysis and glycolysis in the T1D group as compared to the control group. Given the inhibitory effect of insulin on glycogen breakdown, the higher insulin levels observed in the T1D group could explain in part the attenuated glycogenolytic response.

Our analysis demonstrated a significant increase in TCAIs (malate, citrate, succinate, fumarate and α-ketoglutarate) in the control subjects in response to exercise. These results are in accordance with a previous metabolomics-based study investigating the plasma signature of exercise [13], in which accumulated levels of malate, fumarate and succinate were reported following 60 minutes of exercise in a healthy cohort. Other studies have demonstrated that an increase in the total concentration of TCAIs is necessary to enhance and maintain TCA cycle flux during strenuous exercise [24]. In addition, a positive correlation has been demonstrated between the total concentration of TCAIs and the estimated TCA cycle flux in human skeletal muscle taken from muscle biopsies during exercise [25]. In the case of patients with T1D, our results also pointed to a less significant accumulation of TCAIs after exercise, which might also compromise the TCA flux rates. Given that many of the reactions that lead to a net influx of TCAIs are directly or indirectly dependent on the level of pyruvate and that elevated concentrations of pyruvate appear to be necessary for the anaplerosis pathway [25], we suggest that the insufficient increase in pyruvate concentrations in T1D subjects might have an impact on TCA cycle replenishment. In this sense, our findings of an attenuated enrichment of TCAIs in the serum of T1D patients in response to a short period of intense exercise indicate that the activation of the TCA cycle flux rates might be affected. This suggests that the T1D group has a compromised oxidative aerobic system as compared to the control group, as the T1D group did not show a significant turnover of TCA metabolites.

Concerning lipolysis, our data demonstrated that the healthy controls showed an increase in free fatty acids and glycerol in response to exercise, however, this response was attenuated in the T1D group. The increase in lipolysis after acute exercise had been previously reported in healthy individuals [13,26]. Under conditions of hyperinsulinemia induced by clamp techniques, a suppression of the intramuscular and subcutaneous adipose tissue lipolysis was demonstrated in healthy volunteers [27,28]. In another study performed in obese men, a delay in the lipolytic activation was observed together with an increase in plasma insulin levels following 30 minutes of acute resistance exercise, a finding that the authors attributed to an increase in insulin levels

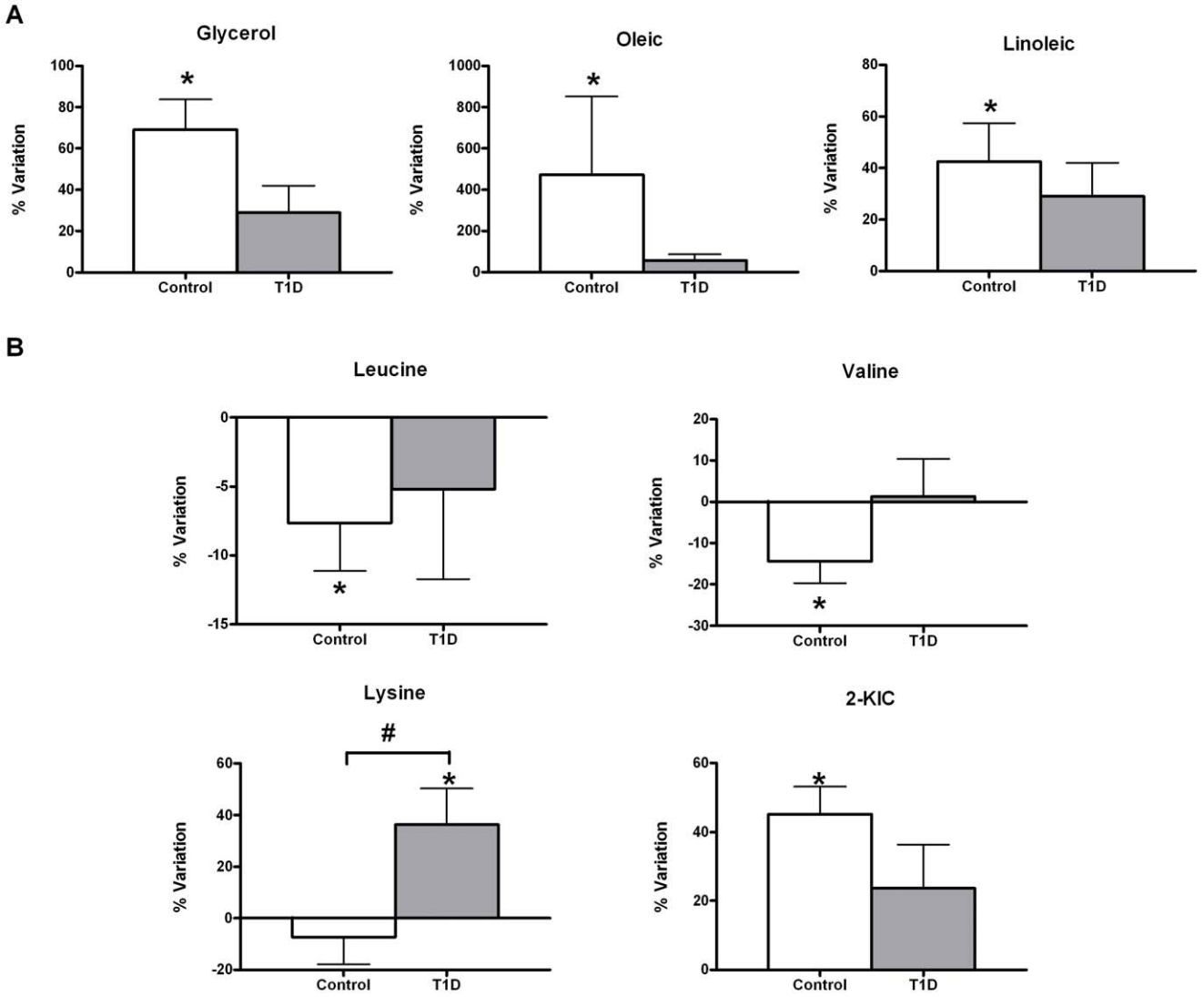

Figure 3. Relative changes in lypolysis (A) and BCAA metabolism (B) with acute exercise. Data are mean±sem of net percent variation. *Indicates a significant variation in metabolic levels with exercise (Wilcoxon rank-summed test for the comparison of a particular metabolite level prior to and after exercise in the independent T1D and control exercisers, $q<0.1$). #Indicates a significant diabetes×exercise interaction for a particular metabolite (Repeated-measures ANOVA, $q<0.1$).
doi:10.1371/journal.pone.0040600.g003

[26]. The increased insulin levels found in the T1D group in our study could have a role in the attenuated lipolytic action observed following exercise.

Our results demonstrated that control exercisers presented a significant reduction in leucine levels. It is well established that exercise increases energy expenditure, resulting in the promotion of amino acid catabolism in general and, in particular, in the oxidation of branched chain amino acids, mainly leucine [13,29]. In parallel to the reduction of leucine, we observed an increase in circulating levels of 2-KIC, which is the first step in leucine degradation. This increase in 2-KIC is in concordance with a previous reported study on the metabolomic profiling performed on urine samples of men following acute exercise [15]. Our results suggest that the effect induced by exercise on protein catabolism was attenuated in the diabetic patients, in agreement with other authors who have previously demonstrated a decrease in protein catabolism in T1D patients [17]. Moreover, there are evidences confirming that elevated insulin levels induced by infusion in healthy men promote muscle protein anabolism by inhibiting protein breakdown [30] and elicit the ability to stimulate glucose uptake and alanine transport, thus suppressing protein degradation in skeletal muscle [31]. Taking all these data into consideration, we propose that the high insulinemia levels induced by exogenous insulin administration in our T1D group could be the cause of reduced protein breakdown, as demonstrated by the minor alterations observed in the levels of leucine and 2-KIC following exercise.

Of special note, we detected an increase in insulin serum levels in all the T1D patients after 30-minute of acute exercise. This increase may explain in part the attenuation in the metabolomic response of all energetic substrates. Pharmacokinetic studies have shown that the peak action of insulin glargine is usually within the first 3 or 4 hours after injection [32], and in our protocol, samples were taken 10 hours later. One possible explanation for the increased insulin concentration detected in the serum of our T1D group could be that intense exercise induces the lipolysis of

subcutaneous adipose tissue where insulin is stored and rapidly released into the circulation. In line with this hypothesis, Davison et al [33] previously reported an exercise-induced lipolysis effect by monitoring the release of liposoluble vitamins from the subcutaneous tissue into the bloodstream, an effect which may be responsible for the increased insulin levels observed in our T1D patients.

Although we speculate that high insulin levels induced by exogenous treatment may be responsible for the attenuated response of metabolites to acute exercise, alternative explanations must also be considered. For example, the possible presence of insulin resistance (IR), an important condition described in T1D patients, cannot be ignored. Some authors have considered that supra-physiologic levels of exogenous insulin [34] and hyperglycemia per se [35] could be responsible for IR in T1D patients. Insulin, not the rate of glucose disposal per se, regulates glycogen synthesis, meaning that the low level of glycogen synthase activity found in insulin-resistant states is a consequence of impaired insulin action, rather than reduced glucose disposal. Nevertheless, this assumption has been challenged in a more recent study in which adult patients with T1D exhibited both impaired glucose utilization and impaired insulin-induced non-esterified fatty acid suppression [36]. In addition, these patients showed IR in hepatic and skeletal muscle tissue, despite good glycemic control [37]. Another report [38] demonstrated that T1D adolescents had significantly impaired functional exercise capacity and decreased insulin sensitivity as compared to non-diabetic adolescents. In spite of their IR and reduced cardiovascular fitness, T1D youth showed paradoxically normal intramyocellular lipid content (IMCL). This finding contradicts the previously well- established theory that IMCL accumulation is a marker for insulin resistance in both T1D and T2D [39,40].

Metabolic flexibility defined as the ability to switch from fat to carbohydrate oxidation is usually impaired during a hyperinsulinemic clamp in insulin-resistant subjects. Thus, the phenomena of metabolic inflexibility mainly described in T2D and other insulin-resistant states could explain some of the alterations occurring in the machinery of lipid and glucose metabolism [41,42]. In addition, the inability to modify fuel oxidation in response to changes in nutrient availability has been implicated in the accumulation of intramyocellular lipids as well as in insulin resistance. Exercise represents a paradigm which requires a highly regulated coordination between fuel supply and oxidative machinery. The assumption that metabolic inflexibility may affect energy metabolism under exercise conditions in T1D patients remains to be elucidated.

Several studies involving T2D patients and their offspring have demonstrated the presence of mitochondrial dysfunction [43,44]. In T1D patients, mitochondrial dysfunction has also been described, contributing to abnormalities in the TCA cycle and fatty acid metabolism. A recent report [45] verified that the mitochondrial capacity of untrained women with T1D correlates positively with glycemic control. The hypothesis that mitochondrial abnormalities may be a primary cause of metabolic inflexibility and insulin resistance has been offered. Significant differences in mitochondrial number, structure and function have been described between insulin-resistant and insulin-sensitive subjects, but the causal link still remains unknown [46].

Although our study was not designed to analyze IR or metabolic flexibility, these factors might have influenced the metabolomic spectrum described in our T1D patients. In addition, the presence of hyperglucagonemia and impaired glucagon counterregulation, as reported in several studies [47], could affect lipolysis, gluconeogenesis and protein metabolism. Finally, other situations not present in our patients should be taken into account, such as autonomic dysfunction, which influences lypolitic responsiveness [48], cardiac dysfunction, which has been documented in T1D patients as affecting exercise responses [49,50], and abnormal blood flow during exercise, which could affect muscular vascular function, contributing toward metabolic disturbances [51].

In summary, we report that T1D patients have an attenuated metabolic response as compared to their healthy control counterparts after a short period of acute, intense exercise. We speculate that exercise could mobilize the subcutaneous exogenous insulin depot in adipose tissue. Furthermore, our data suggest that high insulinemia levels might play a role in the attenuated response in lipolysis, proteolysis, glycogenolysis, and oxidative metabolism observed in T1D patients following exercise. Whether the attenuation of metabolic response to acute and intense exercise might interfere in the training performance of T1D patients remains to be elucidated, and additional studies are required.

Supporting Information

Table S1 Metabolite identification parameters. **^{1}H-NMR**: proton nuclear magnetic resonance spectroscopy; **δ**: chemical shift; **GC-MS**: gas chromatography-mass spectrometry; **RT**: retention time
(DOC)

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Author Contributions

Conceived and designed the experiments: LB MV SM XC AN. Performed the experiments: LB SM MV SS MAR AB. Analyzed the data: MV LB SS MAR GD CL. Contributed reagents/materials/analysis tools: LB SM MV SS MAR AB. Wrote the paper: LB MV AN.

References

1. DCCT/EDIC - Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 353: 2643–2653.
2. DPP - Diabetes Prevention Program Research Group (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346: 393–403.
3. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, et al. (1997) Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 20: 537–544.
4. Tuomilehto J, Lindström J, Eriksson J, Valle TT, Hämäläinen H, et al. (2001) Prevention of type 2 diabetes by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344: 1343–1350.
5. Thompson PD, Buchner D, Piña IL, Balady GJ, Williams MA, et al. (2003) Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease - a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). Circulation 107: 3109–3116.
6. Leon AS, Rice T, Mandel S, Després JP, Bergeron J, et al. (2000) Blood lipid response to 20 weeks of supervised exercise in a large biracial population: the HERITAGE Family Study. Metabolism 49: 513–529.
7. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, et al. (2007) Physical activity and public health updated recommendation for adults. From the American College of Sports Medicine and the American Heart Association. Circulation 116: 1081–1093.
8. Valerio G, Spagnuolo MI, Lombardi F, Spadaro R, Siano M, et al. (2007) Physical activity and sports participation in children and adolescents with type 1 diabetes mellitus. Nutr Metab Cardiovasc Dis 17: 376–382.

9. Lindon JNJ, Holmes E (2007) The handbook of metabonomics and metabolomics. 1 ed. UK: Elsevier.
10. Mäkinen VP, Soininen P, Forsblom C, Parkkonen M, Ingman P, et al. (2006) Diagnosing diabetic nephropathy by 1H-NMR metabonomics of serum. Magn Reson Mater Phy 19: 281–296.
11. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muioi DM, et al. (2009) Metabolomics applies to diabetes research. Moving from information to knowledge. Diabetes, 58: 2429–2443.
12. Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, et al. (2010) Quantitative metabolomics by 1H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. PLoS ONE 5: e10538, 1–10.
13. Farrell L, Wood MJ, Martinovic M, Arany Z, et al. (2010) Metabolic signatures of exercise in human plasma. Sci Transl Med 2: 33ra37. doi:10.1126/scitranslmed.3001006.
14. Enea C, Seguin F, Petitpas-Mulliez J, Boildieu N, Boisseau N, et al. (2010) ^{1}H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise. Anal Bioanal Chem 396: 1167–1176.
15. Pechlivanis A, Kostidis S, Saraslanidis P, Petridou A, Tsalis G, et al. (2010) 1H NMR-Based metabonomic investigation of the effect of two different exercise sessions on the metabolic fingerprint of human urine. J Proteome Res 9: 6405–6516.
16. Zhang S, Zheng C, Lanza IR, Nair S, Raftery D, et al. (2009) Interdependence of signal processing and analysis of urine 1H NMR spectra for metabolomic profiling. Anal Chem, 81: 6080–6088.
17. Devlin J, Scrimgeour A, Brodsky I, Fuller S (1994) Decreased protein catabolism after exercise in subjects with IDDM. Diabetologia 37: 358–364.
18. Palmieri V, Pezzullo S, Arezzi E, D'Andrea C, Cassese S, et al. (2008) Cycle-ergometry stress testing and use of chronotropic reserve adjustment of ST depression for identification of significant coronary artery disease in clinical practice. Int J Cardiol 127: 390–392.
19. IPAQ - International Physical Activity Questionnaires, Available: http://www.ipaq.ki.se/ipaq.htm.
20. Palazoglu M, Fiehn O (2009) Metabolite identification in blood plasma using GC/MS and the agilent fiehn GC/MS metabolomics RTL Library. Agilent Application Note.
21. Albers M J, Butler TN, Rahwa I, Bao N, Keshari KR, et al. (2009) Evaluation of the ERETIC Method as an improved quantitative reference for H-1 HR-MAS spectroscopy of prostate tissue. Magnetic Resonance in Medicine 61, 525–532.
22. Storey JD, Tibshirani R (2003). Statistical significance for genomewide studies. Proc Nat Acad Sci USA 100: 9440–9445.
23. Sahlin K (1990) Muscle carnitine metabolism during incremental dynamic exercise in humans. Acta Physiol Scand, 138, 259–262.
24. Bowtell JL, Marwood S, Bruce M, Constantin-Teodosiu D, Greenhaff PL (2007) Tricarboxylic acid cycle intermediate pool size: functional importance for oxidative metabolism in exercising human skeletal muscle. Sports Medicine 37: 1071–1088.
25. Gibala MJ, MacLean DA, Graham TE, Saltin B (1998) Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. Am J of Physiol - End And Metab 275: E235–E242.
26. Chatzinikolaou A, Fatouros I, Petridou A, Jamurtas A, Avloniti A, et al. (2008) Adipose tissue lipolysis is upregulated in lean and obese men during acute resistance exercise. Diabetes Care 31: 1397–1399.
27. Jacob S, Hauer B, Becker R, Artzner S, Grauer P, et al. (1999) Lipolysis in skeletal muscle is rapidly regulated by low physiological doses of insulin. Diabetologia 42: 1171–1174.
28. Stumvoll M, Jacob S, Wahl HG, Hauer B, Löblein K, et al. (2000) Suppression of systemic, intramuscular, and subcutaneous adipose tissue lipolysis by insulin in humans J Clin Endocrinol Metab 85: 3740–3745.
29. Henriksson J (1991) Effect of exercise on amino acid concentrations in skeletal muscle and plasma J Exp Biol. 1991 Oct;160: 149–65.
30. Gelfand RA, Barrett EJ (1987) Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. J Clin Invest 80: 1–6.
31. Biolo G, Williams BD, Fleming RY, Wolfe RR (1999) Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. Diabetes 48: 949–957.
32. Lepore M, Pampanelli S, Fanelli C, Porcellati F, Bartocci L, et al. (2000) Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. Diabetes 49: 2142–2148.
33. Davison GW, George L, Jackson SK, Young IS, Davies B, et al. (2002) Exercise, free radicals, and lipid peroxidation in type 1 diabetes mellitus. Free Radic Biol Med 33: 1543–1551.
34. Yki-Järvinen H, Mott D, Young AA, Stone K, Bogardus C (1987) Regulation of glycogen synthase and phosphorylase activities by glucose and insulin in human skeletal muscle. J Clin Invest 80: 95–100.
35. Yki-Järvinen H, Helve E, Koivisto VA (1987) Hyperglycemia decreases glucose uptake in type I diabetes. Diabetes: 36: 892–896.
36. Schauer IE, Snell-Bergeon JK, Bergman BC, Maahs DM, Kretowski A, et al. (2011) Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: The CACTI study. Diabetes 60: 306–314.
37. Bergman BC, Howard D, Schauer IE, Maahs DM, Snell-Bergeon JK, et al. (2012) Features of Hepatic and Skeletal Muscle Insulin Resistance Unique to Type 1 Diabetes. J Clin Endocrinol Metab. 2012, doi: 10.1210/jc.2011–3172.
38. Nadeau KJ, Regensteiner JG, Bauer TA, Brown MS, Dorosz JL, et al. (2010) Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. J Clin Endocrinol Metab, 95: 513–521.
39. Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, et al. (2003) Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. Am J Physiol Endocrinol Metab 285: E1174–1181.
40. Levin K, Daa Schroeder H, Alford FP, Beck-Nielsen H (2001) Morphometric documentation of abnormal intramyocellular fat storage and reduced glycogen in obese patients with Type II diabetes. Diabetologia, 44: 824–833.
41. Kelley DE, Mandarino LJ (2000) Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 49: 677–683.
42. Galgani JE, Moro C, Ravussin E (2008) Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab 295: E1009–1017.
43. Mogensen M, Sahlin K, Fernström M, Glintborg D, Vind BF, et al. (2007) Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 56: 1592–1599.
44. Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, et al. (2007) Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes 56: 1376–1381.
45. Item F, Heinzer-Schweize S, Wyss M, Fontana P, Lehmann R, et al. (2011) Mitochondrial capacity is affected by glycemic status in young untrained women with type 1 diabetes but is not impaired relative to healthy untrained women Am J Physiol Regul Integr Comp Physiol 301: R60–R66.
46. Morino K, Petersen KF, Shulman GI (2006) Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 55 (Suppl 2): S9–S15.
47. Farhy LS, Chan A, Breton MD, Anderson SM, Kovatchev BP, et al. (2012) Association of Basal hyperglucagonemia with impaired glucagon counter-regulation in type 1 diabetes. Front Physiol.3:40, doi: 10.3389/fphys.2012.00040.
48. Divertie GD, Jensen MD, Cryer PE, Miles JM (1997) Lipolytic responsiveness to epinephrine in nondiabetic and diabetic humans. Am J Physiol, 272: E1130–1135.
49. Komatsu WR, Gabbay MA, Castro ML, Saraiva GL, Chacra AR, et al. (2005) Aerobic exercise capacity in normal adolescents and those with type 1 diabetes mellitus. Pediatr Diabetes 6: 145–149.
50. Gusso S, Hofman P, Lalande S, Cutfield W, Robinson E, et al. (2008) Impaired stroke volume and aerobic capacity in female adolescents with type 1 and type 2 diabetes mellitus. Diabetologia 51: 1317–1320.
51. Vervoort G, Wetzels JF, Lutterman JA, van Doorn LG, Berden JH, et al. (1999) Elevated skeletal muscle blood flow in noncomplicated type 1 diabetes mellitus: role of nitric oxide and sympathetic tone. Hypertension 34: 1080–1085.

Supporting Information

Table S1. Metabolite identification parameters

^{1}H-NMR Metabolites	δ(ppm)	multiplicity	Moieties
Glycerol	3.65	double doublet	Half (-CH_2-)
Citrate	2.52	doublet	Half (-CH_2-)
Lactate	1.33	doublet	-CH_3
Succinate	2.39	singlet	(-CH_2-)
Pyruvate	2.36	singlet	(-CH_2-)
Alanine	1.46	doublet	-CH_3

GC-MS Metabolites	RT(min)	Quantitative ion (m/z)
Malate	12.87	245
Lysine	17.02	200
Fumarate	11.0	245
α-ketoglutarate	13.88	198
Oleic acid	20.48	339
Linoleic acid	20.43	337
α-ketoisocaproic acid (2-KIC)	9.13	200
Leucine	8.33	170
Valine	9.22	144

Metabolite identification parameters. ^{1}**H-NMR**: proton nuclear magnetic resonance spectroscopy; **δ**: chemical shift; **GC-MS**: gas chromatography-mass spectrometry; **RT**: retention time

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References in the publication:

1. DCCT/EDIC, 2005 (Nathan et al., 2005)
2. DPP, 2002 (Knowler et al., 2002)
3. Pan et *al.,* 1997 (Pan et al., 1997)
4. Tuomilehto *et al*., 2001(Tuomilehto et al., 2001)
5. Thompson et al., 2003 (Thompson et al., 2003)
6. Leon *et al.,* 2000 (Leon et al., 2000)
7. Haskell *et al.,* 2007 (Haskell et al., 2007)
8. Valerio *et al.,* 2007 (Valerio et al., 2007)
9. Lindon *et al.,* 2007 (Lindon & Holmes, 2007)
10. Mäkinen *et al.,* 2006 (Mäkinen et al., 2006)
11. Bain *et al.,* 2009 (Bain et al., 2009)
12. Lanza *et al.,* 2010 (Lanza et al., 2010)
13. Lewis *et al.,* 2010 (Lewis et al., 2010)
14. Enea *et al.,* 2010 (Enea et al., 2010)
15. Pechlivanis *et al*., 2010 (Pechlivanis et al., 2010)
16. Zhang *et al.,* 2009 (Zhang et al., 2009)
17. Devlin *et al.,* 1994 (Devlin, Scrimgeour, Brodsky, & Fuller, 1994)
18. Palmieri *et al.,* 2008 (Palmieri et al., 2008)
19. IPAQ ("International Physical Activity Questionnaire," n.d.)
20. Palazoglu *et al.,* 2009 (Palazoglu & Fiehn, 2009)

21. Albers *et al*., 2009 (M. J. Albers et al., 2009)
22. Storey *et al.,* 2003 (Storey & Tibshirani, 2003)
23. Sahlin *et al.,* 1990 (Sahlin, Katz, & Broberg, 1990)
24. Bowtell *et al.,* 2007 (Bowtell, Marwood, Bruce, Constantin-teodosiu, & Greenhaff, 2007)
25. Gibala *et al.,* 1998 (Gibala, Maclean, Graham, & Saltin, 1998)
26. Chatzinikolaou *et al.*, 2008 (Chatzinikolaou et al., 2008)
27. Jacob *et al.,* 1999 (S Jacob et al., 1999)
28. Stumvoll *et al.*, 2000 (Stumvoll et al., 2000)
29. Henriksson *et al.,* 1991 (Henriksson, 1991)
30. Gelfand *et al.*, 1987 (Gelfand & Barrett, 1987)
31. Biolo *et al.,* 1999 (Biolo, Williams, Fleming, & Wolfe, 1999)
32. Lepore *et al.,* 2000 (Lepore et al., 2000)
33. Davison *et al*., 2002 (Davison et al., 2002)
34. Yki-Järvinen *et al.,* 1987 (Yki-Järvinen, Mott, Young, Stone, & Bogardus, 1987)
35. Yki-Järvinen *et al.,* 1987 (Yki-Järvinen, Helve, & Koivisto, 1987)
36. Schauer *et al.,* 2011 (Schauer et al., 2011)
37. Bergman *et al.,* 2012 (Bergman et al., 2012)
38. Nadeau *et al.,* 2010 (Nadeau et al., 2010)
39. Perseghin *et al.,* 2003 (Gianluca Perseghin et al., 2003)
40. Levin *et al.,* 2001 (Levin, Daa Schroeder, Alford, & Beck-Nielsen, 2001)
41. Kelley *et al.,* 2000 (David E Kelley & Mandarino, 2000)
42. Galgani *et al*., 2008 (Galgani, Moro, & Ravussin, 2008)
43. Mogensen *et al.,* 2007 (Mogensen et al., 2007)
44. Befroy *et al*., 2007 (Befroy et al., 2007)
45. Item *et al.,* 2011 (Item et al., 2011)
46. Morino *et al.,* 2006 (Morino et al., 2006)
47. Farhy *et al.,* 2012 (Farhy et al., 2012)
48. Divertie *et al.,* 1997 (Divertie, Jensen, Cryer, & JM, 1997)
49. Komatsu *et al*., 2005 (William Ricardo Komatsu et al., 2005)
50. Gusso *et al.,* 2008 (Gusso et al., 2008)
51. Vervoort *et al.,* 1999 (Vervoort et al., 1999)

AIM 3 – STUDY 3

Muscular lipids content in patients with type 1 diabetes in relation to their level of physical activity

In the third study, we proposed to analyze the muscular lipids content in patients with type 1 diabetes in relations to their level of physical activity, in order to understand components imbalances and/or some influence of insulin resistance.

For this study, we had the collaboration of Ana Isabel García and Jaume Pomes, from the Department of Radiology of Hospital Clínic de Barcelona; Guerau Fernandez and Susana Kalko, from Bioinformatics Core Facility from IDIBAPS; and Miguel Angel Rodriguez, Maria Vinaixa and Xavier Correig, from the Metabolomics Platform from Universitat Rovira i Virgili, in Reus.

Rationale

The skeletal muscle is one of the most important organs for the glucose metabolism and is directly related to insulin resistance and sensitivity (DeFronzo et al., 1979; Petersen et al., 2007). Some metabolic conditions, such as type 2 diabetes (T2D) and obesity, have been importantly associated with muscular insulin resistance (IR) (S E Kahn, 2003; Steven E Kahn, Hull, & Utzschneider, 2006). In subjects with type 1 diabetes (T1D), despite the growing knowledge about IR associated components, there are little information on muscle particularities and specific metabolism.

Imbalances of muscular composition, fatty acids intramyocellular accumulations and mitochondrial dysfunction have been implicated in muscular dysfunction (Petersen et al., 2004). The mitochondrial dysfunction, related to a reduction of mitochondrial content, mitochondrial biogenesis and/or electron transport chain (ETC) content, induces a reduction of the oxidative capacity, and is associated with lipid accumulation and reduction of insulin action (Befroy et al., 2007; Montgomery & Turner, 2015; Morino et al., 2006; Petersen et al., 2004), and, in parallel, the accumulation of intramyocellular lipids (IMCL) has been proposed as an important predictor of IR (White et al., 2006). In subjects with T2D and/or obesity, it was observed an excess of IMCL accumulation (He et al., 2001; D E Kelley et al., 1999) and this condition occurs in muscle with predominantly type I muscular fibers (oxidative slow-twitch) (Hwang, Pan, Heydari, Hetherington, & Stein, 2001).

In humans, two main muscular fiber types, the type I and the type II, are described. The type I fibers, in normal conditions, have a high capacity for fatty acid oxidation and insulin-stimulated glucose transport, present more mitochondria and GLUT4 content than type II fibers, and are mainly implicated in aerobic actions. These fibers are characterized by long period utilization. By the opposite, type II fibers, in their subclasses type IIa (oxidative fast-twitch) and specially type IIx (glycolytic fast-twitch), show less IMCL amounts, are more glycolytic, present lower mitochondrial and GLUT4 content, and are mainly implicated in anaerobic actions (Egan & Zierath, 2013; Schiaffino & Reggiani, 2011). Some studies have identified, by biopsies of vastus lateralis muscle, a less content of type I fibers in T2D and in obese subjects, less GLUT4 expression, and/or less oxidative enzyme capacity especially in type I fibers than in lean controls (Gaster et al., 2001; He et al., 2001; Oberbach et al., 2006; Stuart et al., 2013).

Recently, it has been recognized that patients with T1D could present different degrees of insulin resistance (Bergman et al., 2012; Liu et al., 2009). However, there is less information about changes in the muscle fibers, neither dysfunction

nor lipid accumulation in this group of subjects. One study identified decrease of the slow oxidative and increase of the glycolytic fibers fractions by biopsies in vastus lateralis muscle when compared with normal glucose tolerant subjects; and the study also showed significantly higher glycolytic enzyme activities in all fiber types, which were correlated with a worse glycemic control, expressed by HbA1c (Fritzsche et al., 2008). Another study (Gianluca Perseghin et al., 2003) verified higher accumulations of IMCL in calf muscles of adult population with T1D, mostly if there was an inadequate glycemic control, indicating also an assignment of this organ and probable changes on its functions.

Designed interventions of physical activity in patients with T2D, or high-risk populations, were able to reduce the IMCL, demonstrating to be effective in the increase of mitochondria activity and in insulin sensitivity (Meex et al., 2010). Nevertheless, it is not clear if this beneficial effect of physical activity would have the same results in patients with T1D.

Proton magnetic resonance spectroscopy (1H MRS) is a specialized non-invasive approach used for the estimation, *in vivo,* of certain human muscle compounds. Recently, by this technique, it was described that carnosine could estimate fiber type composition (Baguet et al., 2011). Carnosine is a dipeptide present in high concentrations in skeletal muscle and is involved in many physiological processes. This dipeptide has an important role as an intracellular buffer and it is more abundant in fast-twitch (type II) muscle type, which has the higher capacity to generate energy from anaerobic glycolytic metabolism (Baguet et al., 2011; Boldyrev, Aldini, & Derave, 2013). Nowadays, a few studies are starting to use this resource for the elucidation of muscular composition in different conditions (Baguet et al., 2011; Gualano et al., 2012). Creatine is another stable dipeptide in muscle mainly present in type II fibers (Rico-Sanz et al., 1999). In parallel, 1H MRS is also a well-established method for the determination of IMCL, and even for the differentiation of saturated and unsaturated lipids into the cell, whose imbalance has been described as altered in processes such as osteoporosis and renal cell carcinoma (Katz-Brull et al., 2005; Yeung et al., 2005).

The aim of this study was to evaluate a possible muscle imbalance in patients with T1D through a non-invasive method (1H MRS), for the assessment of intramyocellular lipid (IMCL) accumulation and the influence of physical activity on these features. The identification of muscular components by a non-invasive method in patients with T1D and the comprehension of the associated clinical factors could help in the approach and in the better care of these subjects.

Research design and Methods

Participants

Sixteen men with T1D, recruited from the Department of Endocrinology (Hospital Clinic, Barcelona), and fourteen men without diabetes (control group), recruited from staff of Laboratory of Diabetes and Obesity (IDIBAPS), were enrolled in the study. From the subjects with T1D, 10 were highly trained competitor athletes (marathon, triathlon or cycling) and six were sedentary (less than once a week of structured physical activity); and in control group, nine were athletes and five were sedentary. The Research and Ethics committees of the Hospital Clínic of Barcelona approved the experimental protocol, in accordance to the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation.

The patients with T1D that participated in the study had diabetes diagnosis for more than 5 years. Estimated glucose disposal rate (eGDR), an indicator of insulin sensitivity in patients with T1D, was calculated (taking into account HbA1c, waist/hip ratio and presence of hypertension) (Trevor J Orchard et al., 2006; Williams et al., 2000, Waden 2005). Patients with chronic complications related to diabetes were excluded, except if presented incipient retinopathy. All patients presented microalbuminuria values below 30 mg/L; normal peripheral

neurologic evaluation by clinical exploration and biothesiometry (Biothesiometer, Bio-Medical Instrument Company, Newbury, OH, U.S.); and normal cardiologic evaluation by rest electrocardiogram (ECG) and by cycle-ergometer test. Hypertension and active smoking habit were registered. None of the participants was in use of lipid lowering drugs. At the time of testing, none of the participants was taking any form of prescription medication. Patients with T1D were in multiple daily injection (MDI) regimen.

Procedures

All subjects were invited to perform the tests in the Diabetes Research Clinical Unit of IDIBAPS/Hospital Clínic. Baseline clinical characteristics such as height, weight, BMI and total body composition where also obtained, and each subject was required to complete an evaluation of current physical activity using the short version of International Physical Activity Questionnaire, that provided an estimation of METs ("International Physical Activity Questionnaire," n.d.). They were also classified as athletes if performed routinely 4 or more sessions (1 or more hours) a week of moderate and/or intense exercises like running or cycling; and classified as sedentary if performed less than once a week of structured physical activity.

In addition to the conventional anthropometric parameters, total body composition was measured by densitometry using DXA (Lunar iDXA body composition, GE Healthcare). Maximal oxygen uptake (VO_2peak) was determined by using a maximal progressive incremental exercise test on a friction-braked cycle-ergometer (Monark 828E, Monark Sweden) as described elsewhere (Brugnara et al., 2012).

Blood determinations

Fasting blood samples were obtained. Standard biochemical analyses were determined at Hospital Clinic laboratory: fasting glycemia (glucose-oxidase method, Advia 2400 Siemens Diagnostics, Deerfield, IL, U.S.), glycated hemoglobin (HbA1c) (high-performance liquid chromatography [HPLC]), albuminuria (immunoturbidimetric assay), total-cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) (molecular absorption spectrometry) and LDL-cholesterol (LDL-C) (Friedewald equation).

1H MRS studies

Subjects were scanned using a 3T whole body MRI system (Siemens Trio, Erlangen, Germany), positioned the right leg in the magnet bore, using a transmit/receive extremity coil and a 4-channel flexible coil for calf and thigh musculature, respectively. Axial T1-weighted spin-echo (SE) MR images were obtained at the proximal two thirds of the calf and the thigh (TR 450ms, TE 18ms, slice thickness 5mm, interslice gap 0mm, two acquisitions, 166 x 256 to 320 x 260 matrix, and field of view 220 to 400 mm). Single voxel point-resolved spectral selection (PRESS) spectroscopy pulse sequence (TR 3000ms, TE 30ms, 128 average in the acquisition with water suppression, 60 average in the acquisition without water suppression of the same voxel for each scan, 1024 data points over 2000 kHz spectral width, TA: 6´) with manual shimming, was used to obtain spectra from the soleus (predominant slow-twitch), tibialis anterior (predominant fast-twitch) and vastus intermedius (mixed fast-twitch) muscles (Edgerton, Smith, & Simpson, 1975; Yang & Yoo, 1986), with very careful positioning of the voxel (size 3.6mL to 4.5mL) in muscles without any visible intermuscular fat, blood vessels and bone in the images. The PRESS box was prescribed on the axial T1-weighted SE images of the calf and thigh in the cross-sectional area with greater muscles volumes (Figure 3.1).

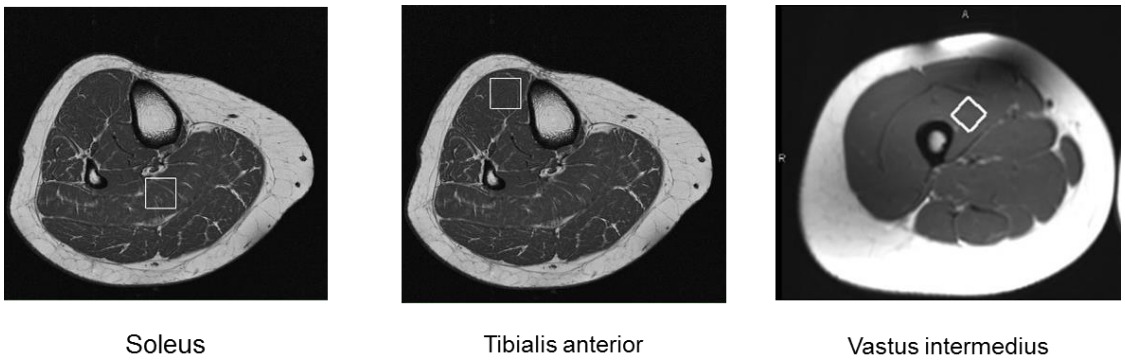


Figure 3.1. Example of the three muscles studied by 1H MRS and the voxel positioning (white square in each muscle).

The spectra were analyzed using jMRUI software (v. 4.0 for Java). The metabolite peaks were fitted with AMARES using lorentzian line shapes and soft constraints for the peak position and linewidth. Area under the curve of peak arising at δ ~8 ppm was used to estimate the carnosine and at δ ~3 ppm was used to estimate the creatine skeletal muscle levels. The lipid pool was obtained with the area under the curve from methylene protons (in saturated lipids) within IMCL (at 1.2 ppm) and EMCL (at 1.4 ppm), and olefinic protons (in unsaturated lipids, or UFA) within IMCL (at 5.2 ppm) and EMCL (at 5.4 ppm). Carnosine, creatine and lipid content were scaled to the water peak at 4.6 ppm position of the spectra. (Figure 3.2)

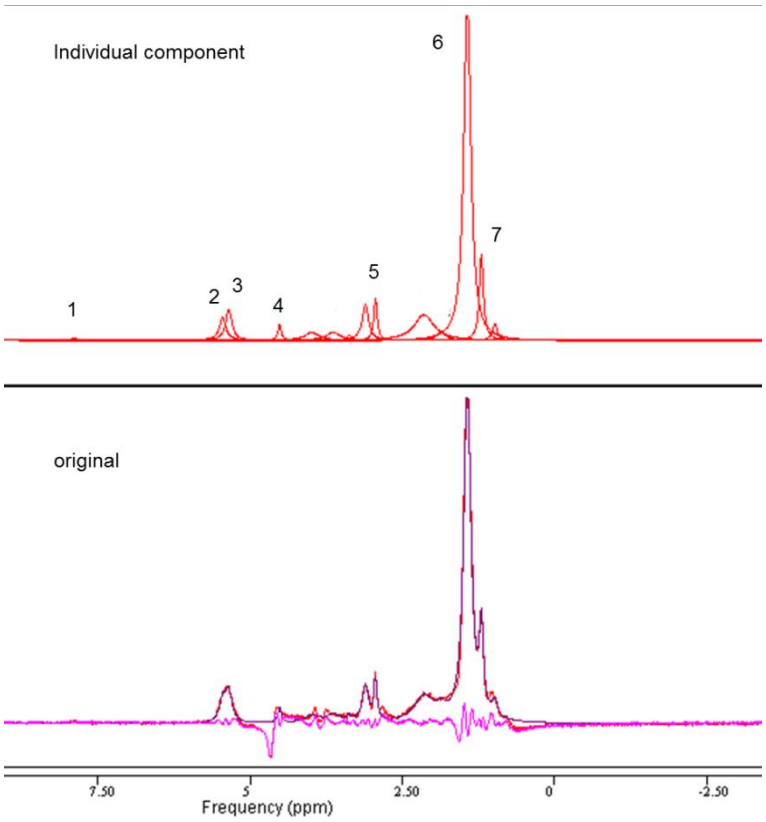


Figure 3.2. Example of a muscular espectrum in tibialis anterior from one patient. In the upper part of the figure, individual component, the peaks are represented as follows: 1. carnosine; 2. olefinic protons EMCL; 3. olefinic protons IMCL; 4. water; 5. creatine; 6. methylene protons EMCL; 7. methylene protons IMCL. In the botton part it is represented the original raw spectrum.

Lipid unsaturation index was calculated according the ratio of the olefinic to methylene protons in intramyocellular and extramyocellular compartments (signal at 5.2 / 1.2 ppm within IMCL, and 5.4 / 1.4 ppm within EMCL) (Katz-Brull et al., 2005), here called as UI_IMCL and UI_EMCL, respectively.

Statistical analysis

Categorical data are expressed as frequencies and percentages, and continuous data as means ± SD or 95% confidence interval (95% CI) following normal distribution, and median and interquartile ranges (IQRs) following non normal distribution.

We ran an exploratory covariance PCA on Partek to identified outliers. Statistical differences of clinical as well as muscle data by group, subgroup or by exercise were analyzed using Fisher exact test for categorical data and ANOVA and FDR Step Up as multiple test correction method for continuous data. We also tested exercise as interaction with grouping. We performed Wilcoxon test for variables years of DM (years of evolution of diabetes), eGDR and HbA1c. We applied Kruskal-Wallis test and post-hoc Dunn test with Bonferroni p-adj to detect differences of metabolites between muscles.

Statistical analyses were conducted in R v3.2.2 (R Core Team, 2015) and significance was assumed at $p \leq 0.05$.

Pearson Correlation and Networks

To determine the relationship between clinic variables, standard serum laboratory, muscular fatty acids, carnosine and creatine, Pearson correlations were computed at p-value < 0.05 in subjects with T1D and controls separately, and for different muscles.

Integration of datasets was accomplished with the corrplot and qgraph R packages, which constructs and displays correlation networks between components (seen in Figure 3.3 with thresholds – in p-value < 0.05, in R > 0.5).

RESULTS

Subject characteristics and clinical data

Clinical data, body composition and standard laboratory analysis of the participants in the study are presented in Table 3.1. Subjects with T1D had diabetes diagnosis for a mean of 16.5 ± 9 years, were older ($p = 0.01$), did not smoke ($p = 0.035$), and presented higher glycemia ($p < 0.001$) and higher HDL-C ($p = 0.042$) than control subjects. Six patients with T1D presented incipient diabetic retinopathy.

The VO_2peak and METs were higher in all athletes than in all sedentaries ($p < 0.001$, for both parameters), higher in athletes with T1D than sedentary with T1D ($p < 0.001$, for both parameters) and higher in athlete controls than in sedentary controls ($p < 0.001$ and $p < 0.05$, respectively), confirming a better fitness and higher amount of physical activity of athletes in general. Total body fat and abdominal fat percentages, estimated by DXA, were lower in athletes than in sedentary subjects ($p = 0.03$, for both measurements). Subjects with T1D had higher HDL-cholesterol ($p = 0.03$) than control ones. The other parameters were not statistically different.

Table 3.1 – Clinical characteristics of type 1 diabetes and control subjects

	Type 1 diabetes *(16)*		***Controls*** *(14)*		***p***
	Athlete	Sedentary	Athlete	Sedentary	
	10	6	9	5	
Age (years)	41 ± 8	41 ± 7.5	35.8 ± 7.88	30.8 ± 1.9	a
Evolution of diabetes (years)	15.5 ± 9.5	18.1 ± 8.6	-	-	ns
Body composition					
Weight (kg)	78 ± 9	83 ± 9.3	76.6 ± 8.2	79.7 ± 10.8	ns
BMI (kg/m^2)	25 ± 2.5	25.6 ± 2.3	25.1± 2.1	23.4 ± 4.3	ns
WHR (waist/hip ratio)	0.86 ± 0.06	0.88 ± 0.04	0.86 ± 0.05	0.86 ± 0.06	ns
Total fat (% - by DXA)	20.6 ± 6.9	29.9 ± 4.6	22.3 ± 5.2	26.7 ± 7.9	c
Abdominal fat (% - by DXA)	24.6 ± 6.9	37.8 ± 5.3	28.4 ± 10	36.6 ± 12.6	c
Physical fitness					
SF-IPAQ **(METs/**week)	3512 ± 1604	619 ± 427	5940 ± 4032	832 ± 699	d, e, g
VO_2peak (mL/kg/min)	41.3 ± 10.4	22.4 ± 6.2	46.3 ± 16.1	26.6 ± 7.2	d, e, f
Other characteristics					
Hypertension (yes)	1	3	0	0	-
Dyslipidemia (yes)	1	1	0	1	-
Active smoking (yes)	0	0	2	2	-
Incipient retinopathy (yes)	5	1	-	-	-
eGDR	8.52 ± 1.59	7.25 ± 1.6	-	-	ns
Laboratory analysis					
Fasting glycemia (mg/dl)	160 ± 57	141 ± 30.2	91.8 ± 5.8	91.5 ± 12	a
HbA1c (%)	7.2 ± 1.1	8.2 ± 1.8	-	-	ns
Total cholesterol (mg/dl)	175.3 ± 34.8	190.5 ± 25.8	190.5 ± 42.2	157 ± 21.2	ns
HDL cholesterol (mg/dl)	56.8 ± 5.6	50.1 ± 4.1	49.6 ± 15.9	43.5 ± 20.5	b
LDL cholesterol (mg/dl)	105.6 ± 28.8	125.5 ± 23.3	127.8 ± 39.5	131 ± 71.1	ns
Triglycerides (mg/dl)	64 ± 27.5	71.8 ± 24.3	63.3 ± 22.9	111.5 ± 95.4	ns
Creatinine (mg/dl)	0.93 ± 0.08	1.01 ± 0.08	0.99 ± 0.16	1.03 ± 0.1	ns

T Student and chi-square tests

a) p< 0.01 between T1D and CT; b) p< 0.05 between T1D and CT c) p < 0.05 between sedentary and athletes; d) p < 0.001 between sedentary and athletes ; e) p <0.001 between T1D sedentaries and athletes; f) p <0.05 between CT sedentaries and athletes; g) p <0.001 between CT sedentaries and athletes; ns – non significant. Obs.: T1D with retinopathy do not present clinical differences vs. without retinopathy

1H MRS Studies

Analyzing the three muscles, when separated by the groups T1D and control, it could be evidenced that IMCL (saturated, methylene protons) was higher in soleus muscle (2.21±1.21 U.A. for T1D and 2.42±1.62 for controls) when compared with tibialis anterior (0.57±0.29 U.A. for T1D and 0.43±0.15 for controls) ($p = 0.001$ for T1D and $p < 0.001$ for controls) and with vastus intermedius (1.1±0.71 U.A. for T1D and 1.04±0.37 for controls) ($p = 0.035$ for T1D and $p = 0.018$). The estimation of olefinic fatty acids was not different among the muscles.

The estimation of carnosine, creatine, IMCL (saturated fatty acids, methylene protons), olefinic fatty acids (unsaturated fatty acids, or UFA), UI_IMCL and UI_EMCL were not different between the subjects with T1D and control, as well as no differences were identified when compared all the subjects differentiating them by the physical activity (athlete and sedentary) criteria. All the comparisons took in account the three different muscles.

Network analysis, data integration

Figure 3.3 presents the network layouts of the significant relationships observed between the clinical variables, carnosine and creatine estimations, intramyocellular fatty acids and unsaturated ratios ($p < 0.05$; $R > 0.5$), determined separately in six networks: subjects with T1D and control ones, and their three respective muscles (soleus, tibialis anterior, vastus intermedius).

In the six networks presented (Fig 3.3: a, soleus in T1D; b, soleus in controls; c, tibialis anterior in T1D; d, tibialis anterior in control; e, vastus intermedius in T1D; f, vastus intermedius in control), a consistent positive correlation was identified in clinical variables of body composition, as follows; BMI, waist/hip ratio (WHR), total fat and abdominal fat. EGDR, the index for insulin sensitivity in subjects with T1D, presented and inverse correlation with BMI, but with no other clinical variable, standard blood lipoproteins, nor with muscle variables.

VO_2peak, variable that indicates the physical fitness, was positively correlated with HDL-C in subjects with T1D, but not in control ones. VO_2peak was also

negatively correlated with abdominal fat in all subjects and, in control ones, also with total body fat. In soleus muscle of control subjects, VO_2peak was also negatively correlated with carnosine.

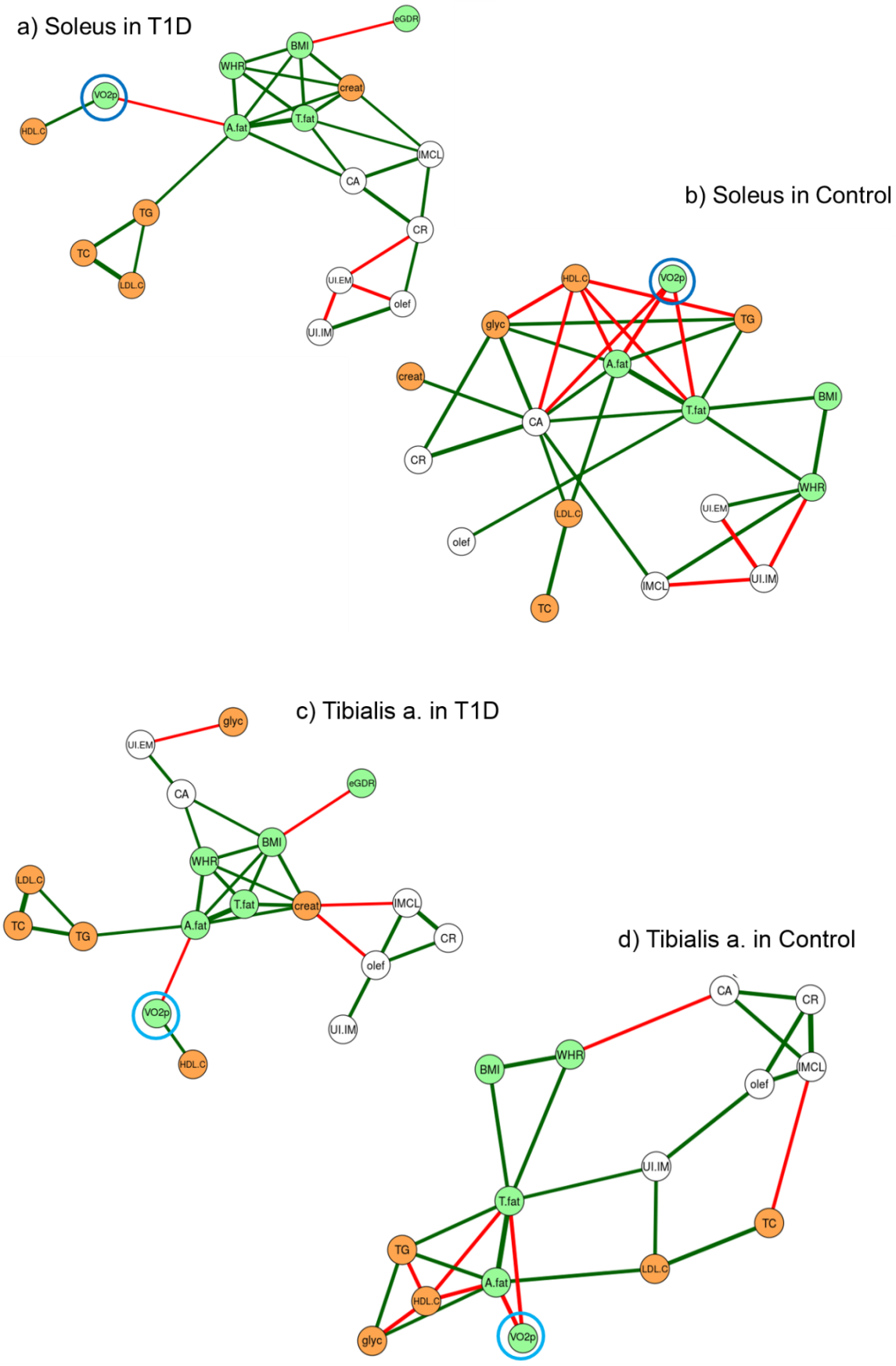
LDL-C was positively correlated with abdominal fat and TG with abdominal and total fat, as well as HDL-C was inversely connected with body fat composition in control subjects. In T1D, TG was associated with abdominal fat, while HDL-C was associated with VO_2peak. Serum creatinine had a noted hub role in networks of T1D, condition that did not occur in control subjects. Serum creatinine participated in different connections, depending on the muscle studied.

Carnosine appears positively associated with total and abdominal fat in soleus muscle of both T1D and control groups (Fig 3.3a and b), positively associated with BMI and WHR in tibialis anterior muscle in T1D (Fig 3.3c), but had a negative correlation with WHR in the same muscle in control subjects (Fig 3.3d). Interestingly, in soleus muscle, the more oxidative one, carnosine is negatively associated with VO_2peak, as referred before, but also positively with glycemia, LDL-C and negatively with HDL-C, in control subjects.

A constant positive cluster in almost all networks was identified among carnosine, creatine and IMCL. In the case of the muscles from subjects with T1D, olefinic fatty acids were also connected in this cluster. It is worth noting that there was an inverse correlation between UI_IMCL and UI_EMCL in soleus and muscle vastus intermedius muscles of both T1D and control groups.

In soleus and tibialis anterior muscles from control subjects (Fig 3.3b and d), compact lattices corresponding to the main classes of variables may be observed with large positive correlations inside the muscular clusters. In the case of the same muscles in subjects with T1D (Figure 3.3a and c), lattices from the main classes lose density, and cluster especially on body composition variables.

In vastus intermedius muscle, that has mixed fast-twitch characteristics, different muscle lattices were evidenced, in especial in subjects with T1D.


a) Soleus in T1D
eGDR
BMI
WHR
creat
VO2p
HDL.C
A.fat
T.fat
IMCL
CA
TG
CR
TC
LDL.C
UI.EM
olef
UI.IM
b) Soleus in Control
HDL.C
VO2p
glyc
TG
A.fat
creat
BMI
T.fat
CA
CR
WHR
UI.EM
LDL.C
olef
IMCL
UI.IM
TC
c) Tibialis a. in T1D
glyc
UI.EM
CA
eGDR
BMI
WHR
LDL.C
T.fat
creat
IMCL
TC
TG
A.fat
CR
olef
VO2p
UI.IM
HDL.C
d) Tibialis a. in Control
CA
CR
WHR
BMI
IMCL
olef
UI.IM
T.fat
TC
TG
LDL.C
A.fat
HDL.C
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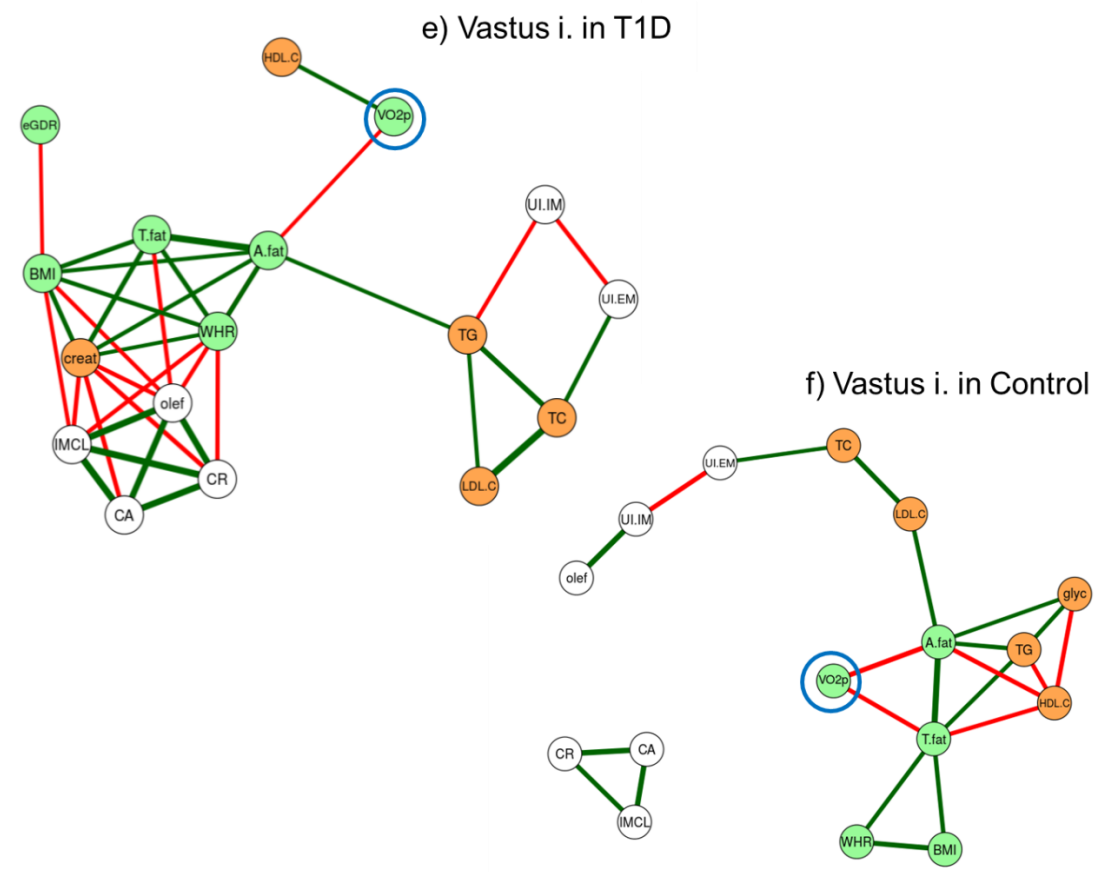

Figure 3.3 - Network models of clinical variables, serum determinations and intramuscular variables of subjects with T1D and control subjects, in soleus, tibialis anterior and vastus internus. *WHR*, waist/hip ratio; *T.fat*, total fat; *A.fat*, abdominal fat; *VO2p*, VO_2peak; *glyc*, glycemia, *TC*; total-cholesterol; *HDL.C*, HDL-cholesterol; *LDL.C*, LDL-cholesterol; *TG*, triglycerides; *creat*, creatinina; *CA*, carnosine; *CR*, creatine; *olef*, olefinic; *IMCL*, methylene intramyocellular lipids; *UI.IM*, UI_IMCL; *UI.EM*, UI_EMCL. **a)** Soleus in T1D; **b)** Soleus in controls; **c)** Tibialis anterior in T1D; **d)** Tibialis anterior in control; **e)** Vastus intermedius in T1D; **f)** Vastus intermedius in control. Integration of datasets was accomplished with the corrplot and qgraph R packages which constructs and displays correlation networks between components (p-value < 0.01 and thresholds R > 0.5). Green lines represent positive correlations, and red lines represent negative associations. Green spheres represent clinical variables; in orange, standard laboratory analysis; in white, intramuscular variables. Blue circles highlight the component VO_2peak

DISCUSSION

The current study showed that men with T1D presented similar muscle compounds than men without diabetes, as well as athletes and sedentary subgroups did not presented muscle component differences. This study analyzed similar healthy men, having the diabetes or physical activity levels as the basic differences. The subjects presented normal body fat compositions range by BMI, and, in the ones with T1D, few presented incipient chronic complications (retinopathy) and had no other pathology that could confound the results. These characteristics were intentionally chosen to seek for a better control of both aspects in focus, the T1D and the physical activity.

Gualano and col. (Gualano et al., 2012) described an experiment with subjects with T1D, T2D and respective matched controls, using also a 1H MRS, similar to the present one. Compared to controls, a lower carnosine content in gastrocnemius muscle of patients with T2D was identified and no differences were identified in carnosine in soleus muscle, as well as no differences were observed in those muscles in patients with T1D. Our findings are comparable to that study. We did not identify differences in carnosine content between subjects with T1D and controls in the three muscles studied.

Our study brought information about muscular compounds identified by 1H MRS and integrated them with clinical and laboratory variables. Considering the muscular variables studied, carnosine, as described by Boldyrev (Boldyrev et al., 2013), has a buffer activity during high-intensity muscle contractions, when the anaerobic glycolysis leads to the production of lactic acid which immediately dissociates into protons (H^+) and lactate ions, processes more typical of type II muscular fibers. Carnosine also has an antioxidant activity mediated by different mechanisms involving metal ion chelation, scavenging reactive oxygen species (ROS) and peroxyl radicals. Other experimental studies described the potential benefits of carnosine for the prevention or delay of diabetic nephropathy or retinopathy in animals (Pfister et al., 2011; Riedl et al., 2011). Carnosine, in our networks, was positively associated with IMCL in both groups of T1D and control.

The accumulation of IMCL has been proposed as an important predictor of IR (White, Ferguson, McCoy, Kim, & Castellano, 2006); however, it is also recognized a paradoxical increase of IMCL in athlete subjects (Moro, Bajpeyi, & Smith, 2008). It is referred that high IMCL predicted low insulin sensitivity, but mainly in untrained individuals (Machann, Häring, Shick, & Stumvoll, 2004), and that IMCL can even increase in obese subjects submitted to an exercise program, in parallel with an improvement of insulin sensitivity (Dubé et al., 2008). Soleus muscle is a typical deep muscle of sustaining that withstands prolonged time utilization, and is characterized by high proportion of oxidative fibers (type I). These oxidative fibers contain higher concentration of mitochondria and GLUT-4, and are the preferential fiber for the accumulation of IMCL. In our study, IMCL of soleus muscle was positively correlated with total fat in subjects with T1D and positively correlated with waist/hip ratio in control subjects, indicating association with inadequate body composition compounds, but not with physical fitness. As referred above, the carnosine was positively associated with IMCL in our networks; regarding so, it could be speculated that in the presence of higher levels of IMCL, and a probable oxidative dysfunction (related to IR), more carnosine is expressed as a buffer and/or antioxidant agent in glycolytic and other processes. Moreover, our study identified that this increased soleus carnosine in soleus muscle of T1D and controls was associated with poorer clinical characteristics, like total fat and abdominal fat, variables associated with IR.

VO_2peak presented a negative correlation with abdominal fat in all subjects and with total fat in control subjects, indicating that men that presented a better fitness also presented a lower fat accumulations, and probable less risk for IR. VO_2peak was not associated with any muscular fatty acid compound in our study. Nevertheless, an interesting negative association was detected between VO_2peak and carnosine in soleus muscle of control subjects, suggesting some association of better fitness and less carnosine amount and/or expression. This association, however, was not present in subjects with T1D.

It is worth to mention that EMCL was not used in this study as an isolate

variable because its estimations may vary even with discrete changes in the intra-individual coil positioning (Torriani et al., 2005). UI_EMCL ratio, nevertheless, has its interest due to an intrinsically balance of the different kind of lipids in the extramyocellular space. We could identify in the networks an inverse association between unsaturated indexes of intra and extramyocellular environment (UI_IMCL and UI_EMCL), suggesting an imbalance: in the presence of higher proportion of ratio olefinic/IMCL in the intramyocellular space, lower proportion of olefinic/EMCL in the extramyocellular space. This is a novel observation, not described previously.

We could not identify muscular differences concerning the different groups of athletes and sedentary. Two possible explanations are the small number of subjects that participated and the fact that this is a cross-sectional observational study. An interventional study would perhaps demonstrate some intra-individual changes of lipid content. Another limitation is the type of the 1H MRS study acquired, whereas there are other new sequences recently developed, as 2D-localized correlated spectroscopy (L-COSY), for the determination of the unsaturated lipid compounds, differencing monounsaturated fatty acids from polyunsaturated fatty acids. On this topic, it has been observed that patients with T2D, obese and overweight subjects present altered monounsaturated/polyunsaturated ratios, reflecting a dysregulation also in lipid metabolism (Velan et al., 2008).

The use of networks for understanding the interconnections of clinical and biochemical variables has been used by other authors and by our group (Brugnara et al., 2015; Mäkinen et al., 2009). This approach could give us the pattern of the main links between body composition, blood determinations and muscle variables, which in a traditional correlation analysis would be difficult to integrate. Slight differences could be observed between the patterns of T1D and control subjects, fact that could be explained by differences in metabolic status; moreover, some links differ among the muscles, which could be explained by the differences of fiber compositions and/or intrinsic metabolism.

This study demonstrates that patients with T1D with weight in normal range and without advanced chronic complications related to diabetes presented similar values of IMCL, unsaturated lipid ratios, carnosine and creatine than control subjects. However, despite the weight in normal range, slight alterations in BMI and/or body fat composition may be enough to establish alterations in IMCL amounts. Improvement in body composition, in terms of BMI, waist/hip ratio and body fat values, may be determinant for a more favorable muscle components profile, and prospective studies in this model of patients will help to elucidate the role of muscle components and its functionality.

AIM 4 – STUDY 4

Improving assessment of cardio-protective lipoprotein profile in type 1 diabetes by ^{1}H NMR spectrometry

In the fourth study, we proposed to analyze the lipoprotein profile in subjects with type 1 diabetes, in order to understand lipoprotein subfractions, the main clinical aspects involved and if there were differences compared to subjects without diabetes.

The volunteers were selected from the ones that already participated in the baseline evaluation in de Diabetes Research Clinical Unit, from the series explained in Study 1. Thirty persons with T1D and 30 without diabetes participated in Study 4. It was performed a careful selection for matching both groups concerning gender, age, body mass index (BMI), body fat percentage by DXA (dual-energy X-ray absorptiometry) and similar physical activity and cardiorespiratory fitness levels (VO_2peak).

To obtain a comprehensive profile of lipid and lipoprotein parameters in both groups of subjects, two different 1H NMR methods were used. In the first analysis, it was possible to estimate the lipoprotein particle concentrations and the average particle size for every main fraction, provided by the NMR LipoProfile test commercialized by LipoScience, Inc. (Raleigh, USA). The second analysis was performed at the Metabolomics Platform (URV), and 1H NMR spectroscopy was employed to determine the concentrations of the cholesterol and triglycerides content of 9 lipoprotein subclasses and, for this purpose, partial least square (PLS) regression was used.

The results are described in the following publication:

Brugnara L, Mallol R, Ribalta J, Vinaixa M, Murillo S, Casserras T, Guardiola M, Vallvé JC, Kalko SG, Correig X, Novials A (2015) Improving Assessment of Lipoprotein Profile in Type 1 Diabetes by 1H NMR Spectroscopy. PLoS ONE 10(8): e0136348. doi:10.1371/journal.pone.0136348

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PLOS ONE

RESEARCH ARTICLE

Improving Assessment of Lipoprotein Profile in Type 1 Diabetes by 1H NMR Spectroscopy

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Abstract

Patients with type 1 diabetes (T1D) present increased risk of cardiovascular disease (CVD). The aim of this study is to improve the assessment of lipoprotein profile in patients with T1D by using a robust developed method 1H nuclear magnetic resonance spectroscopy (1H NMR), for further correlation with clinical factors associated to CVD. Thirty patients with T1D and 30 non-diabetes control (CT) subjects, matched for gender, age, body composition (DXA, BMI, waist/hip ratio), regular physical activity levels and cardiorespiratory capacity (VO_{2peak}), were analyzed. Dietary records and routine lipids were assessed. Serum lipoprotein particle subfractions, particle sizes, and cholesterol and triglycerides subfractions were analyzed by 1H NMR. It was evidenced that subjects with T1D presented lower concentrations of small LDL cholesterol, medium VLDL particles, large VLDL triglycerides, and total triglycerides as compared to CT subjects. Women with T1D presented a positive association with HDL size ($p<0.005$; $R = 0.601$) and large HDL triglycerides ($p<0.005$; $R = 0.534$) and negative ($p<0.005$; $R = -0.586$) to small HDL triglycerides. Body fat composition represented an important factor independently of normal BMI, with large LDL particles presenting a positive correlation to total body fat ($p<0.005$; $R = 0.505$), and total LDL cholesterol and small LDL cholesterol a positive correlation ($p<0.005$; $R = 0.502$ and $R = 0.552$, respectively) to abdominal fat in T1D subjects; meanwhile, in CT subjects, body fat composition was mainly associated to HDL subclasses. VO_{2peak} was negatively associated ($p<0.005$; $R = -0.520$) to large LDL-particles only in the group of patients with T1D. In conclusion, patients with T1D with adequate glycemic control and BMI and without chronic complications presented a more favourable lipoprotein profile as compared to control counterparts. In addition, slight alterations in BMI and/or body fat composition showed to be relevant to provoking alterations in lipoproteins profiles. Finally, body fat composition appears to be a determinant for cardioprotector lipoprotein profile.

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Competing Interests: The authors have declared that no competing interests exist.

Introduction

Dyslipidemia is one of the most important risk factors involved in cardiovascular disease (CVD) [1] in addition to cigarette smoking, hypertension, family history of premature coronary heart disease, age and diabetes. CVD is the leading cause of mortality in patients with type 1 diabetes (T1D) [2–4]. Early observations of lipids and lipoprotein profile in patients with T1D, revealing pro-atherogenic features such as hypercholesterolemia and hypertriglyceridemia, were particularly associated with poor glycemic control [5,6] and nephropathy [7,8]. In the nineties, studies from Europe and US identified similar rates of cardiovascular disease in T1D subjects, but with different pattern of dyslipidemia: low high density lipoprotein cholesterol (HDL-C) in EURODIAB and hypertriglyceridemia in the US group [8].

Considering the well-known evidences that intensive treatment for glycemic control in T1D patients prevents and/or delays micro and macrovascular complications [9,10], international guidelines of diabetes care were designed mainly to establish goals of good glycemic control [11]. Nowadays, with the optimization of insulin treatment, it has been possible to corroborate a decrease in chronic complications related to T1D and also a reduction in cardiovascular mortality [10,12–14]. Furthermore, current epidemiological data have shown evidences that lipids and lipoprotein profiles are optimal in T1D subjects when they exhibit good glycemic control in absence of microalbuminuria or clinical nephropathy [7,15].

In parallel, the role of body composition in lipoprotein profile in T1D has been extensively analyzed [16,17]. In the Diabetes Control and Complications Trial (DCCT) [16], T1D patients receiving intensive insulin treatment showed greater weight gain than those with conventional treatment. Excessive weight gain in the intensive treatment group was associated to insulin resistance, higher blood pressure and worse lipid profile. The deterioration of these clinical parameters was accompanied by an increase in total triglycerides, total cholesterol, LDL-C, VLDL, IDL and denser LDL particles, and by a decrease in HDL-C. In the EURODIAB Prospective Complications Study, an increase in triglycerides and total cholesterol was identified along with a smaller improvement in HDL-C in the group that ameliorated glycemic control, but in parallel gained more weight, when compared with the group that was not so successful in glycemic control, but experienced less weight gain [17].

It is also well known that physical activity (PA) has protective effects on lipoprotein profile in the general population [18,19]. PA is associated with the prevention of cardiovascular disease and improvements in lipoprotein profile. It is known that HDL and HDL_2 (large) lipoproteins are firmly associated to protective factors for the risk of CVD in the non-diabetic population [20]. Few studies have analyzed the effect of increased PA on lipoprotein subfractions in T1D patients, using conventional methods such as routine clinical biochemistry or lipoprotein isolation by sequential ultracentrifugation. In T1D patients, an improvement in HDL/LDL ratio and in ApoB and triglyceride levels [21] was observed after performing a 16-week program of aerobic exercise. Another report also demonstrated an increase in HDL-C levels after implementing an exercise program in T1D patients [22].

Nuclear magnetic resonance spectroscopy (1H NMR) is, at present, a standard technique for the determination of advanced lipoprotein profile in serum and/or plasma samples. 1H NMR is able to measure the particle number and size of several subfractions of lipoproteins [23], which has been helpful in demonstrating a wider spectrum of CV risk factors in different populations [24]. Moreover, other 1H NMR approaches based on regression methods also allow for the determination of the cholesterol and triglycerides concentrations of several lipoprotein classes and subclasses [25]. Our group has, in the context of other pathologies, already used this methodology [26,27]. Therefore, the aim of the present study was to analyze and improve the knowledge of lipoprotein subclasses in T1D patients by using two complementary

methods based on 1H NMR spectroscopy [28] and by comparing the obtained lipoprotein profiles with age-matched, non-diabetic counterparts. From these data, we have detected the clinical factors with the largest correlations with lipoprotein profile in each population.

Participants, Material and Methods

Participants

Thirty patients with T1D, recruited by the Department of Endocrinology and Nutrition of the Hospital Clinic de Barcelona, and 30 subjects without diabetes, recruited by the staff of the IDIBAPS Diabetes and Obesity Research Laboratory, were enrolled in the study. Control (CT) subjects were matched with T1D patients for gender, age, body mass index (BMI), body fat percentage by DXA (dual-energy X-ray absorptiometry) and for similar physical activity and fitness levels (VO_{2peak}). The experimental protocol was approved by the Research and Ethics committees of the Hospital Clínic de Barcelona, in accordance to the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation (CEIC Register n°: 2009/4933).

T1D subjects participating in the study had diabetes for a mean of 11.9 ± 10.1 years, undetectable C-peptide levels and acceptable glycemic control, as determined by glycated hemoglobin A1c (7.05% ± 1.1). Estimated glucose disposal rate (eGDR), an indicator of insulin resistance in T1D patients, was calculated (taking into account HbA1c, waist/hip ratio and presence of hypertension) [29,30]. Patients with chronic complications related to diabetes were excluded, except for five who presented incipient retinopathy. All patients presented microalbuminuria values below 30 mg/L. Peripheral neurologic evaluation, assessed by clinical exploration and biothesiometry (Bio-thesiometer, Bio-Medical Instrument Company, Newbury, OH, U.S.), was normal in all subjects. In addition, all of them presented a normal cardiac evaluation, assessed by electrocardiogram (ECG) at rest and during bicycle ergometry test. Hypertension (systolic blood pressure $\geq$ 135 mmHg and/or diastolic blood pressure $\geq$ 85) and active smoking were registered. Patients with T1D were following multiple daily injections (MDI) regimens. None of the participants was using lipid-lowering drugs or any other medication.

Material and Methods

All subjects were invited to the Diabetes Research Clinical Unit of IDIBAPS/Hospital Clínic de Barcelona to perform the tests. Clinical history and baseline clinical characteristics, such as height, weight, BMI and total body composition, were obtained. Each subject was required to complete an evaluation of current physical activity using the Short-form of the International Physical Activity Questionnaire [31], estimating energetic expenditure for vigorous, moderate, and walking activities, expressed as METs-min/week. They were also classified as sedentary or physically active, defined as routinely performing three or more sessions of moderate and/or intense exercise (of one or more hours per session) per week. Participants were questioned regarding dietary habits using a four-day dietary at-home record (3 workdays and 1 non-workday) and the results were analyzed by the program PCN 1.0, CESNID-University of Barcelona [32].

Total body composition was measured by densitometry using DXA (Lunar iDXA body composition, GE Healthcare). Maximal oxygen uptake (VO_{2peak}) was determined by using a maximal progressive incremental exercise test on a friction-braked cycle-ergometer (Monark 828E, Monark Sweden). After a 3 min warm-up period at a power output of 25-W, workload was increased by 25-W each minute until exhaustion. Oxygen uptake was monitored during exercise using a computerized, open circuit gas-collection system (Vmax Spectra, version v12.0, Sensor Medics Corp, VIASYS Healthcare Inc, Yorba Linda, CA, U.S.), and VO_{2peak} was

determined at the point of highest oxygen consumption over a 15-s period. VO_{2peak} was confirmed using established physiological criteria, including a respiratory exchange ratio above 1.15, oxygen uptake reaching a plateau despite an increased work rate, and a heart rate near 95% of the age-predicted maximum value.

Blood determinations. Fasting blood samples were obtained for analysis. Glycemia (glucose-oxidase method, Advia 2400 Siemens Diagnostics, Deerfield, IL, U.S.) and glycated hemoglobin (high-performance liquid chromatography [HPLC]) were determined in the Hospital Clinic laboratory. For the conventional determination of lipoproteins and metabolomic measurements, serum was obtained once blood had been allowed to clot at room temperature for 30 min and after centrifugation at 4°C at 5000 rpm for 10 min. Samples were kept at -80°C until further analysis. Standard laboratory methods were used to determine total cholesterol, triglycerides and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula. Remnant lipoprotein cholesterol (RLPc) was measured by immuno-affinity chromatography.

Serum lipoprotein profile. To obtain a comprehensive profile of lipid and lipoprotein parameters in both groups of subjects, two different 1H NMR methods were used. In the first analysis, we obtained the distribution of lipoprotein subclasses as provided by the NMR LipoProfile test commercialized by LipoScience, Inc. (Raleigh, USA). The procedure simultaneously estimates the lipoprotein particle concentrations and the average particle size for every main fraction. NMR was performed on plasma samples collected in EDTA tubes and stored at -80°C [24]. The LipoProfile test measured 15 variables: (a) total VLDL and chylomicron particle concentrations (total VLDL-P) and chylomicron; large, medium and small VLDL-P (nmol/L); (b) total LDL particle concentrations (total LDL-P); IDL particles (IDL-P); large LDL-P; total small LDL-P (expressed by medium, small and very small LDL particles)–in (nmol/L); (c) total HDL particle concentrations (total HDL-P); large, medium and small HDL-P particles–in (μmol/L); and (d) mean particle sizes: VLDL, LDL and HDL size–in (nm).

In the second analysis, performed at the Metabolomics Platform (URV), 1H NMR spectroscopy was employed to determine the concentrations of the cholesterol and triglycerides content of 9 lipoprotein subclasses. For this purpose, partial least square (PLS) regression was used to calibrate the regression models as proposed in published method [25]. NMR spectra were acquired using the longitudinal eddy-current delay (LED) sequence, and cholesterol and triglycerides concentrations were obtained using high performance liquid chromatography (HPLC) as reference values. To calibrate these PLS regression models, 61 plasma samples were used.

In addition, these analyses complemented the information provided by LipoProfile by offering another 20 determinations: (a) total LDL-C; large, medium and small LDL-C (mg/dL); (b) total HDL-C; large, medium and small HDL-C (mg/dL); (c) total VLDL-TG (mg/dL); large, medium and small VLDL-TG (mg/dL); (d) total LDL-TG; large, medium and small LDL-TG (mg/dL); (e) total HDL-TG; large, medium and small HDL-TG (mg/dL).

Remnant lipoprotein cholesterol (RLPc) was measured in plasma using the method described by Nakajima et al., using RLP-Cholesterol Assay Kits (Jimro-II, Japan Immuno research Laboratories, Japan) [33]. The remnant lipoprotein particles were separated from plasma by immuno-affinity chromatography with a gel containing monoclonal antibodies raised against epitopes of apoB100 and apoA1.

Statistical analysis. A comprehensive profile of 40 lipid and lipoprotein parameters (20 measured by PLS regression, 15 measured by LipoProfile, and another 5 conventional determinations and remnant lipoprotein cholesterol) were studied in the 60 subjects (30 T1D subjects and 30 age-matched control subjects).

A linear model analysis of variance (ANOVA) adjusted for 6 covariates (age, gender, active smoking habits, waist/hip ratio, percentage of total body fat and VO_2peak) was used to

compare lipoprotein abundance between T1D versus control subjects. Lipoproteins with p-values < 0.05 were considered significant.

Pearson Correlation and Networks. To determine the relationship between lipoprotein abundance and clinic variables, or diet components, Pearson correlations were computed at p-value < 0.005 in patients and controls separately. In the case of diet components, variable correlations were analyzed in only 19 patients and 19 controls.

Integration of datasets was accomplished with the Cytoscape tool (www.cytoscape.org), which constructs and displays correlation networks between components (seen in Fig 1 with thresholds–in p-value < 0.005, in $R > \pm 0.5$ for clinical variables and $R > \pm 0.7$ for lipoprotein-lipoprotein variables). Besides, heatmaps were plotted for the correlations of all clinical and lipid and lipoprotein variables using handmade software.

Results

Clinical and dietary data

Table 1 describes the clinical characteristics of the participants. As previously described, the two groups were similar in terms of gender, age, BMI, physical activity habits (regular physical activity or nonphysical activity expressed in METs-min/week) and cardiorespiratory fitness (VO_{2peak}). Moreover, there were no differences in the usual dietary habits of T1D and CT groups concerning proteins, cholesterol, saturated or unsaturated fat intake. Only a higher intake of total carbohydrates in the control group ($p = 0.047$), and a tendency of higher consumption of simple carbohydrates and alcoholic beverages, also in the CT group, were observed (Table 2).

Main differences between T1D and CT subjects

Certain variables were established as covariates in the ANOVA comparative statistical analysis between T1D and CT subjects: age, gender, active smoking, waist/hip ratio, total fat percentage by DXA, and VO_{2peak}. Controlling for these covariates, a moderate number of lipid and lipoprotein parameters were identified as significantly different between the two groups. T1D subjects presented a lower concentration of small LDL-C, medium VLDL-P particles, large VLDL-TG, and total triglycerides ($p < 0.05$) (Table 3). Using the Benjamini & Hochberg [34] methodology for taking into account the multiple comparisons, no single variable was deemed significant, as fdr was 0.389552 for all of them. The change in abundance levels was very low in all cases; we are dealing with a dataset with no clear magnitude fingerprint, and this fact guided us to search for a correlation signature.

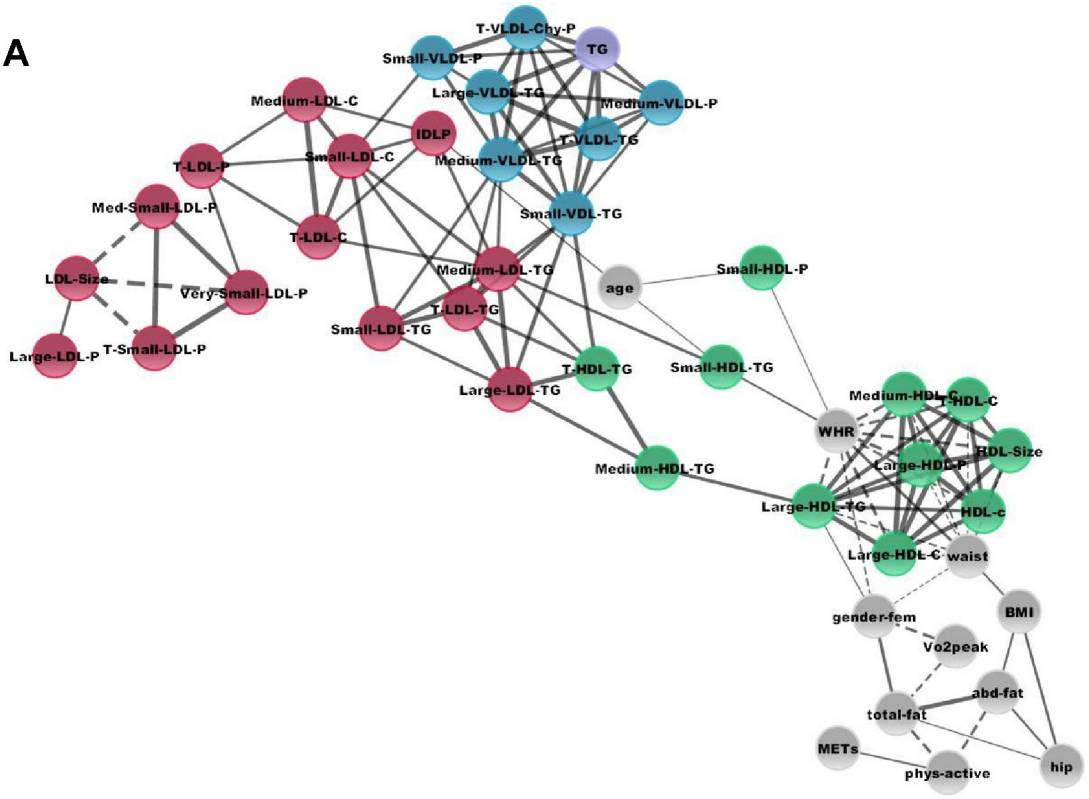
Correlations between lipoprotein profile and clinical variables

Gender. The most important findings resulting from the correlation analysis between lipoprotein profiles and clinical variables are presented in Tables 4 and 5. Women showed a positive correlation with HDL size, in addition to a positive correlation to large HDL-TG concentration and a negative correlation to small HDL-TG concentration in the T1D group (Table 4). The positive correlation between large HDL-TG concentration and the female gender was also identified in the control group (Table 5).

Physical activity. Physical activity had a significant negative correlation with cardiorespiratory capacity, as determined by VO_{2peak}, and large LDL-P in the T1D group. There was no evidence of correlations between physical fitness and other lipoprotein particles (Table 4).

Age. An increase in age showed a positive correlation with the concentration of small HDL particle (small-HDL-P), only in the control group (Table 5).

1A


T-VLDL-Chy-P
TG
Small-VLDL-P
Large-VLDL-TG
Medium-VLDL-P
Medium-LDL-C
IDLP
T-VLDL-TG
Small-LDL-C
Medium-VLDL-TG
T-LDL-P
Med-Small-LDL-P
Small-VDL-TG
T-LDL-C
Medium-LDL-TG
LDL-Size
Very-Small-LDL-P
age
Small-HDL-P
T-LDL-TG
Small-LDL-TG
Large-LDL-P
T-Small-LDL-P
T-HDL-TG
Small-HDL-TG
Large-LDL-TG
Medium-HDL-C
T-HDL-C
WHR
HDL-Size
Large-HDL-P
Medium-HDL-TG
Large-HDL-TG
HDL-c
Large-HDL-C
waist
gender-fem
BMI
Vo2peak
abd-fat
total-fat
METs
phys-active
hip

1B

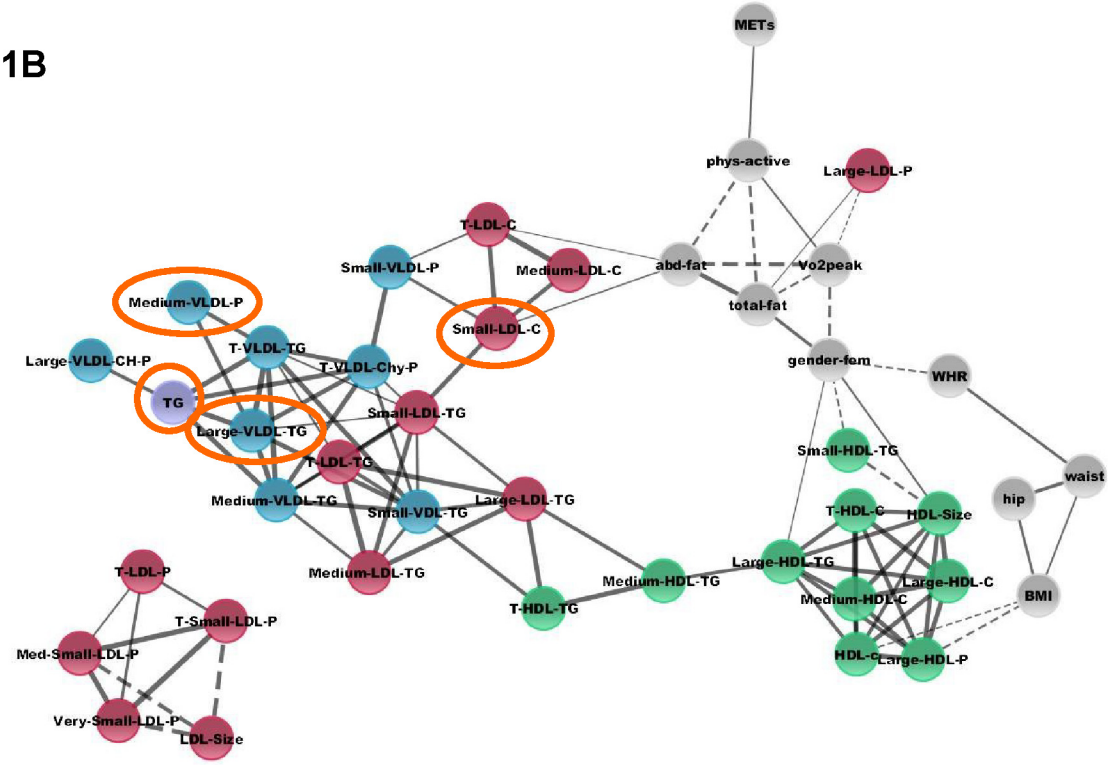

METs
phys-active
Large-LDL-P
T-LDL-C
Small-VLDL-P
Medium-LDL-C
abd-fat
Vo2peak
Medium-VLDL-P
total-fat
Small-LDL-C
T-VLDL-TG
Large-VLDL-CH-P
gender-fem
WHR
T-VLDL-Chy-P
TG
Small-LDL-TG
Large-VLDL-TG
Small-HDL-TG
T-LDL-TG
waist
hip
Medium-VLDL-TG
Small-VDL-TG
Large-LDL-TG
T-HDL-C
HDL-Size
T-LDL-P
Medium-LDL-TG
Large-HDL-TG
Medium-HDL-TG
Large-HDL-C
T-HDL-TG
BMI
T-Small-LDL-P
Medium-HDL-C
Med-Small-LDL-P
HDL-c
Large-HDL-P
Very-Small-LDL-P
LDL-Size

Fig 1. Network models of lipoproteins and clinical variables in control subjects and in T1D subjects. Control (1A) and T1D subjects (1B). Integration of datasets was accomplished with the Cytoscape tool (www.cytoscape.org), which constructs and displays correlation networks between components (p-value < 0.005 and thresholds R > ± 0.5 for clinical variables, and R > ± 0.7 for lipoprotein variables). Continuous lines represent positive correlations, and dashed lines represent negative associations. Grey spheres represent clinical variables; in red, LDL related lipoproteins; in green, HDL lipoproteins; in blue, VLDL lipoproteins; and in purple, total triglycerides. Orange circles mark the variables that are reduced in T1D subjects.

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Body composition. The most important findings on lipoprotein profiles as related to body composition are presented in Tables 4 and 5. In the T1D group, large LDL-P showed a positive correlation with total body fat, as determined by DXA ($R \geq 0.5$ and $p < 0.005$); and abdominal fat percentage (by DXA) presented a positive correlation to total LDL-C and small LDL-C (Table 4).

In the control group, large HDL-P and HDL size presented a negative correlation with waist and waist/hip ratio, while small HDL-P correlated positively with waist/hip ratio. A negative correlation was also identified between waist and/or waist/hip ratio for the following lipoprotein parameters: total HDL-C; large HDL-C; medium HDL-C; large HDL-TG; and conventional determination of HDL-C. The small HDL-TG presented a positive correlation with waist/hip ratio (Table 5).

Dietary habits and other type 1 diabetes specific factors. It is worth noting that only 19 T1D patients and 19 controls completed the 4-day dietary record. Few differences in dietary habits were identified between the two groups, mainly in total carbohydrates (g/day) (Table 2). Associations between different dietary compounds and lipoproteins can be verified in the supplemental tables (S1 and S2 Tables). In addition, neither the time elapsed, expressed as years of diabetes progression, nor the incipient retinopathy present in five of the patients with T1D, conditioned the lipoprotein profiles. Furthermore, indicators of diabetes control or insulin resistance, represented by Hb1Ac and eGDR respectively, were not associated to any lipoprotein parameter in the present study.

Table 1. Clinical characteristics of subjects with type 1 diabetes and controls.

	Type 1 diabetes (n = 30)	Controls (n = 30)
Gender (women/men)	10 / 20	10 / 20
Age (years)	34.8 ± 9.6	35.8 ± 8.8
Body Composition		
BMI (kg/m^2)	23.7 ± 2.8	23.2 ± 2.4
Waist (cm)	81.5 ± 9.1	82 ± 8.4
Waist/hip ratio	0.82 ± 0.06	0.82 ± 0.08
Total body fat (%—by DXA)	24.7 ± 9.2	26 ± 8.8
Abdominal fat (%—by DXA)	27.1 ± 11.8	29.8 ± 10.4
Physical fitness		
Physically active (yes)	15	21
METs (per week–SF-IPAQ)	3008 ± 2230	2115 ± 1728
VO_{2peak} (mL/kg/min)	33.5 ± 11.3	33.1 ± 9.1
Other characteristics		
Hypertension (yes)	5	3
Active smoking (yes)	8	4
Duration diabetes (years)	11.9 ± 10.1	-
Incipient retinopathy (yes)	5	-
HbA1c (%)	7.05 ± 1.1	-

MET: Metabolic Equivalent of Task. 1 MET = 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. No statistical differences between groups, based on Chi-square and Student T-test.

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Table 2. Diet components by dietary records in subjects with type 1 diabetes and controls.

	Type 1 diabetes (n = 19)	Controls (n = 19)	p
Gender (women/men)	5 / 14	6 / 13	ns
Energy (kcal)	2215 ± 489	2430 ± 641	ns
Total proteins (g/day)	101.2 ± 22.1	100.1 ± 28.9	ns
Total lipids (g/day)	112 ± 27.1	112.5 ± 33.2	ns
Saturated fatty acids(g/day)	33.6 ± 10.3	35.4 ± 14.5	ns
Monounsaturated fatty acids (g/day)	52.4 ± 13.7	51.3 ± 13.5	ns
Polyunsaturated fatty acids (g/day)	15.8 ± 5.5	16.6 ± 5.3	ns
Cholesterol (mg/d)	327.1 ± 122.8	351.7 ± 175.3	ns
Total carbohydrates (g/day)	193.2 ± 61.9	238.1 ± 72.4	**0.047**
Simple carbohydrates (sugars) (g/day)	83.6 ± 32.3	103.4 ± 37.2	ns (0.089)
Total fiber (g/day)	21.9 ± 7.1	22.2 ± 10.3	ns
Alcohol (g/day)	3.7 ± 7	9 ± 10	ns (0.069)

No statistical differences between groups, based on Student T-test, except for total carbohydrate intake.

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Network analysis

Network layouts of the significant ($p < 0.005$) relationships observed between the lipoproteins ($R > \pm 0.7$) and clinical variables ($R > \pm 0.5$) (Tables 4 and 5) are presented in Fig 1, determined separately in control subjects (Fig 1A) and T1D subjects (Fig 1B).

In control subjects (Fig 1A), compact lattices corresponding to the main classes of variables may be observed with large positive correlations inside the lipoprotein clusters. In the case of T1D subjects (Fig 1B), lattices from the main classes lose density, especially in the case of LDL-lipoproteins and clinical variables. A new sub-cluster of LDL-lipoproteins disconnected from the rest of components may be observed.

TG is the only biochemical parameter appearing in the networks, and highly connected to VLDL-lipoproteins in both groups.

For supplementary information about all the correlations of clinical, lipid and lipoprotein profile, heatmaps are available as supplementary figures for control subjects (S1 Fig) and subjects with T1D (S2 Fig).

Table 3. Statistical analysis of covariance contrasting lipid and lipoprotein data from subjects with type 1 diabetes and control subjects.

Lipoprotein	FC *	p-value
Medium VLDL-P (medium VLDL particles) [a]	-1.44	0.0216477
Small LDL-C (small LDL cholesterol) [b]	-1.26	0.0289017
Large VLDL-TG (large VLDL, fraction triglyceride) [b]	-1.21	0.0327273
TG (triglyceride) [c]	-1.06	0.0490274

[a] lipoprotein subfraction determined by LipoProfile

[b] lipoproteins subfraction determined PLS regression

[c] lipoprotein determined by conventional determinations

Covariates considered: age, gender, active smoking, waist/hip ratio, total fat percentage by DXA and VO_{2peak}

* FC: Fold change. Negative values correspond to lower concentrations found in T1D.

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Table 4. Association of clinical characteristics and lipoproteins features in subjects with type 1 diabetes (T1D).

Clinical variables R	Gender (+ for women)	BMI (kg/m^2)	Abdominal fat % (DXA)	Total fat % (DXA)	VO_{2peak} (mL/kg/min)
LipoProfile					
Large LDL-P	-	-	-	0.505	-0.520
Large HDL-P	-	-0.627	-	-	-
HDL size	0.601	-	-	-	-
PLS regression					
Total LDL-C	-	-	0.502	-	-
Small LDL-C	-	-	0.552	-	-
Large HDL-TG	0.534	-	-	-	-
Small HDL-TG	-0.586	-	-	-	-
Conventional					
HDL-C	-	-0.564	-	-	-

Summary of the main clinical variables and lipoprotein features that reached R $\geq$ ± 0.5 and p value < 0.005. Other clinical variables did not reach R $\geq$ ± 0.5: age, physically active profile, METs, active smoking, HbA1c or eGDR.

doi:10.1371/journal.pone.0136348.t004

Discussion

In the present study, we aim to improve the characterization of lipoprotein particles in patients with T1D by using two complementary methods based on 1H NMR spectroscopy, in order to find a better correlation of lipid and lipoprotein parameters with clinical phenotype. In the current context of improving diabetes care, patients with T1D selected to participate presented an adequate metabolic control and BMI, and no developed chronic complications related to diabetes or other drug except insulin. The subjects with T1D were paired with carefully selected subjects without diabetes, as control subjects, with the intention of matching their clinical characteristics within the extent possible. Matching was performed concerning gender, age, BMI, body fat percentage, physical activity and cardiorespiratory fitness, to avoid any interference by these factors in the differences in lipoproteins between the two groups. Nevertheless, some of

Table 5. Association of clinical characteristics and lipoproteins in control subjects.

Clinical variables R	Age (years)	Gender (+ for women)	Waist (cm)	Waist/Hip ratio
LipoProfile				
Large HDL-P	-	-	-0.525	-0.724
Small HDL-P	0.517	-	-	0.500
HDL size	-	-	-0.570	-0.719
PLS regression				
Total HDL-C	-	-	-0.554	-0.683
Large HDL-C	-	-	-0.600	-0.737
Medium HDL-C	-	-	-0.583	-0.711
Large HDL-TG	-	0.524	-0.598	-0.680
Small HDL-TG	-	-	-	0.595
Conventional				
HDL-C	-	-	-	-0.656

Summary of the clinical variables and lipoprotein features that reached R $\geq$ ± 0.5 and p value < 0.005. Other clinical variables did not reach R $\geq$ ± 0.5: Physically active profile, METs, VO_{2peak}, active smoking, BMI, total fat percentage (DXA), abdominal fat (DXA).

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these variables were identified as intrinsically significant covariates for analysis, requiring special attention in the proper evaluation of the lipoprotein profiles.

The main findings of this study were attributed to having diabetes, whereas the effect of physical activity per se in each population was independent of this status. We observed that the concentration of small LDL cholesterol subfraction was reduced in T1D as compared to controls. However, this finding is controversial, as it may depend on the method used and the clinical characteristics of the population under study. As noticed, Guy and colleagues [35] identified that young T1D patients presented higher levels of small LDL particles than subjects without diabetes, independent of their glycemic control. Different findings were described by Alberts and cols., in which poor glycemic control was related to more dense LDL particles [36]. On the other hand, Caixàs et al. observed that, after intensification of insulin treatment for the optimization of glycemic control in patients with T1D, VLDL particles, triglycerides, total LDL-C and HDL-C reached the levels of the control group, while the pattern of small LDL-C was also similar to that of the non-diabetic group, with no changes after optimization [37]. Regarding VLDL lipoproteins, we also identified that in T1D subjects the concentration of medium VLDL-P and large VLDL-TG were reduced as compared to the control group. A possible explanation could be attributed to treatment with insulin. Patients with T1D were using subcutaneous insulin treatment, thus hyperinsulinemia pharmacologically induced by insulin must be taken into consideration. It is known that insulin has an anti-lipolytic action, promoting the storage of triglycerides in the adipocytes while reducing the release of free fatty acids from adipose tissue into the circulation [5]. Insulin reduces VLDL production by diminishing circulating free fatty acids, which are substrates for VLDL, but also by a direct inhibitory effect on the liver [5]. Our patients presented a better glycemic control than those described in other studies. Furthermore, they exhibited lower levels of triglycerides, which can be explained by the effect of insulin, as this hormone is a potent activator of lipoprotein lipase, which promotes the catabolism of triglyceride-rich lipoproteins and reduces plasma triglyceride levels [5].

It is well established that physical activity is a protective factor for cardiovascular disease and promotes beneficial effects on lipid and lipoprotein profiles in non-diabetic subjects. For instance, cholesterol increases in the HDL_2-C subfraction have been reported [38], also in T1D subjects [22]. Concerning levels of LDL-C as measured by conventional methods, Rigla and cols. [22] identified a decrease in LDL-C in T2D patients when they participated in a structured aerobic exercise program. In the present study, we found a negative association of VO_{2peak}, indicator of physical fitness, with large LDL-P in the T1D group (Table 4). There is evidence that small dense LDL (sdLDL) remain in circulation longer than large LDL before being cleared by the LDL receptor; it is hypothesized [39] that the delayed catabolism results in modifications of the lipid composition and size of the sdLDL particles triggered by the cholesteryl ester transfer protein (CETP)-mediated exchange of cholesteryl ester and triglycerides between LDL and VLDL and/or HDL. An interesting point of debate is whether CETP activity could be altered by the effect of physical activity. Some studies showed an increased in reverse cholesterol transport in athletes [40], while others demonstrated a decrease in CETP levels after an exercise program [41], and even more others did not evidence any changes in CEPT levels with exercise [42].

In the present study, increased age showed a positive association with small HDL-P, but only in the control group. It has been described that ageing may affect the composition of HDL lipoproteins and their ability to promote cholesterol efflux via reverse cholesterol transport, which is linked to a loss of the potentially anti-atherogenic properties of HDL particles [43,44].

Increments in body weight, waist and/or waist-hip ratio are clinical characteristics of metabolic syndrome and risk factors for CVD in populations general population [45]. The BMIs of our groups were in the normal range (23.7 ± 2.8 kg/m^2 in T1D subjects, and 23.2 ± 2.4 kg/m^2

in control ones). Despite this observation, we have detected some associations with anthropometric and body composition measurements. In the group with diabetes, BMI was negatively associated to large HDL-P and conventional HDL-C; abdominal fat percentage was found positively associated to total LDL-C and small LDL-C by 1H NMR. Analyzing total body fat percentage, large LDL-P showed a positive correlation in the group with T1D. It has been described that a reduction in insulin sensitivity, assessed by hyperinsulinemic-euglucemic clamps, was observed in young people and adults with T1D. These subjects presented higher levels of triglycerides and a higher triglyceride/HDL ratio, while the younger group also presented lower levels of HDL [46]. Based on these results, with the goal of finding correlations with lipoprotein subclasses and certain degrees of insulin resistance in our population, we calculated the estimated glucose disposal rate (eGDR). Nevertheless, we did not find any correlation in the present study, which might be explained by the small number of T1D patients affected by hypertension and by the slight variation in HbA1c and in waist/hip ratio observed, which represent the three clinical criteria needed for calculating eGDR. Interestingly, in our study, the methods that evidenced the correlations of body fat composition with lipoproteins were different for T1D and control groups: BMI and DXA were the better methods for the T1D group and waist and waist/hip ratio for the control group. The subjects that participated in this study presented adequate waist and waist/hip ratios, but probably slight alterations were enough to modify lipoprotein profile.

As already cited before, HDL and HDL_2 (large) lipoproteins are associated to protective factors for CVD [20]. However, the exact role of HDLs still must be investigated, as the direct clinical effect of cardioprotection has been recently discussed, based, for example, on drugs that increase HDL concentrations but fail to prove CV protection [47]. In the particular case of patients with T1D, the expected protection effect of HDLs profile on patients carrying coronary artery calcification was not so determinant as observed in control subjects [48]; nevertheless, in a prospective study on T1D patients, an association of coronary artery disease with lower levels of large HDL and higher levels of medium HDL particles was identified, measured by 1H NMR [49]. Recent evidences are shedding light on HDL functionality, concerning its components and sizes [47,50]. In HDLs, particles of distinct molecule species of lipids have been identified, as well as several proteins compounds, characteristics that confer antioxidative, anti-inflammatory, cytoprotective, vasodilatatory, antithrombotic and anti-infection actions [50,51]. These aspects are raising new concepts and opening new fields of research and could better explain the association of lipoprotein profile and cardiovascular disease in patients with T1D.

Correlation networks constructed in our study helped in the integration of lipid, lipoprotein and clinical data and in the visualization of the global interconnections among them, similar to the way in which physical networks have helped in the understanding of the global interconnections of omics variables [52]. The FinnDiane Study also used similar features to explain the complex correlation between clinical, lipoprotein and other biochemical variables and diabetic kidney disease [53]. Overall, CT subjects show highly coordinated modules corresponding to the three main lipoprotein groups (VLDL, LDL and HDL) and connected to the clinical variables (mainly through HDL group components). T1D subjects, however, show a partial uncoupling of the LDL group and new interrelations between clinical variables and its components, particularly in the case of large LDL-P, small LDL-C, and the absence of IDL-P correlations (see Fig 1B). Other studies, for example, identified that, in nondiabetic subjects, lower average HDL particle size, lower LDL size, and higher VLDL size were associated with coronary calcification, but this association between particle size and calcification in T1D patients was not so clear [48]. It could be speculated that a reduction in certain variables that are usually associated with atherogenesis (small LDL-C, medium VLDL-P, large VLDL-T and TG) [48], in the case of T1D subjects identified in our study, could lead to a perturbation in the interconnection of

LDL lipoproteins and clinical variables, opening up new approaches to be explored in future studies.

From the comprehensive results obtained through 1H NMR techniques, we conclude that patients with T1D present a lipoprotein profile classically associated to cardio-protection compared to control counterparts. Nevertheless, slight alterations in BMI and/or body fat composition may be enough to establish alterations in lipoprotein profile, especially concerning HDL and its subfractions. Improved body composition, in terms of waist, waist/hip ratio, BMI and body fat values, is a determinant for a more favorable lipoprotein profile, and prospective studies in this model of patients will help elucidate the role of this lipoprotein profile.

Supporting Information

S1 Fig. Clinical and lipoproteins variables correlation heatmap in control subjects.
(TIF)

S2 Fig. Clinical and lipoproteins variables correlation heatmap in subjects with type 1 diabetes.
(TIF)

S1 Table. Association of lipoproteins and dietary habits in subjects with type 1 diabetes.
(DOC)

S2 Table. Association of lipoproteins and dietary habits in control subjects.
(DOC)

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Author Contributions

Conceived and designed the experiments: LB RM JR XC AN. Performed the experiments: LB RM MV SM MG JCV. Analyzed the data: RM LB JR TC SGK. Contributed reagents/materials/analysis tools: LB RM JR MV SM MG JCV. Wrote the paper: LB RM JR AN.

References

1. American Hearth Association. Third report of the National Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Bloos Cholesterol in Adults (Adult Treatment Panell III) Final Report. Circulation. 2002; 106:3143–3421. PMID: 12485966
2. Laing SP, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia. 2003; 46:760–5. PMID: 12774166
3. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. All-cause mortality rates in patients with type 1 diabetes mellitus compared with a non-diabetic population from the UK general practice research database, 1992–1999. Diabetologia. 2006; 49:660–6. PMID: 16432708
4. Lind M, Garcia-Rodriguez LA, Booth GL, Cea-Soriano L, Shah BR, Ekeroth G et al. Mortality trends in patients with and without diabetes in Ontario, Canada and the UK from 1996 to 2009: a population-based study. Diabetologia. 2013; 56:2601–8. doi: 10.1007/s00125-013-3063-1 PMID: 24114114
5. Vergès B. Lipid disorders in type 1 diabetes. Diabetes Metab. 2009; 35:353–60. doi: 10.1016/j.diabet.2009.04.004 PMID: 19733492

6. Patti L, di Marino L, Maffetone A, Romano G, Annuzzi G, Riccardi G et al. Very low density lipoprotein subfraction abnormalities in IDDM patients: any effect of blood glucose control? Diabetologia. 1995; 38:1419–1424. PMID: 8786015
7. Jenkins AJ, Lyons TJ, Zheng D, Otvos JD, Lackland DT, McGee D et al. Lipoproteins in the DCCT / EDIC cohort : associations with diabetic nephropathy. Kidney Int. 2003; 64:817–828. PMID: 12911531
8. Orchard TJ, Stevens LK, Forrest KY, Fuller JH. Cardiovascular disease in insulin dependent diabetes mellitus: similar rates but different risk factors in the US compared with Europe. Int J Epidemiol. 1998; 27:976–983. PMID: 10024191
9. The Diabetes Control and Complications Trial Research Group. The effect of the intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993; 329:977–986. PMID: 8366922
10. Nathan DM, Cleary PA, Backlund JY, Grenuth SM, Lachin JM, Orchard TJ et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005; 353:2643–2653. PMID: 16371630
11. American Diabetes Association. Glycemic Targets—Position Statment. Diabetes Care. 2015; 38(Supplement_1):S33–S40.
12. Nordwall M, Bojestig M, Arnqvist HJ, Ludvigsson J. Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of Type 1 diabetes-the Linköping Diabetes Complications Study. Diabetologia. 2004; 47(7):1266–72. PMID: 15235773
13. Hovind P, Tarnow L, Rossing K, Rossing P, Eising S, Larsen N et al. Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. Diabetes Care. 2003; 26(4):1258–64. PMID: 12663607
14. Lind M, Svensson A-M, Kosiborod M, Gudbjörnsdottir S, Pivodic A, Wendel H et al. Glycemic Control and Excess Mortality in Type 1 Diabetes. N Engl J Med. 2014; 371:1972–1982. doi: 10.1056/NEJMoa1408214 PMID: 25409370
15. Lehmann R, Kaplan V, Bingisser R, Bloch KE, Spinas G. Impact of Physical Activity on Cardiovascular Risk Factors in IDDM. Diabetes Care. 1997; 20:1603–1611. PMID: 9314643
16. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of Excessive Weight Gain With Intensive Therapy of Type 1 Diabetes on Lipid Levels and Blood Pressure. JAMA. 1998; 280:140–146. PMID: 9669786
17. Ferriss JB, Webb D, Fuller JH, The EURODIAB Prospective Complications Group. Weight gain is associated with improved glycaemic control but with adverse changes in plasma lipids and blood pressure in Type 1 diabetes. Diabetes Med. 2006; 23:557–564.
18. Paulweber B, Valensi P, Lindström J, Lalic NM, Greaves CJ, McGee M et al. A European evidence-based guideline for the prevention of type 2 diabetes. Horm Metab Res. 2010; 42 Suppl 1:S3–S36. doi: 10.1055/s-0029-1240928 PMID: 20391306
19. World Health Organization. Global Strategy on Diet, Physical Activity and Health. 2004;(May). Available: http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf.
20. Salonen JT, Salonen R, Seppanen K, Rauramaa R, Tuomilehto J. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation. 1991; 84:129–139. PMID: 2060089
21. Laaksonen DE, Atalay M, Niskanen LK, Mustonen J, Sen CK, Lakka TA et al. Aerobic exercise and the lipid profile in type 1 diabetic men: a randomized controlled trial. Med Sci Sports Exerc. 2000; 32 (9):1541–8. PMID: 10994902
22. Rigla M, Sánchez-Quesada JL, Ordóñez-Llanos J, Prat T, Caixàs A, Jorba O et al. Effect of physical exercise on lipoprotein(a) and low-density lipoprotein modifications in type 1 and type 2 diabetic patients. Metabolism. 2000; 49:640–7. PMID: 10831176
23. Mallol R, Amigó N, Rodríguez MA, Heras M, Vinaixa M, Plana N et al. Liposcale : a novel advanced lipoprotein test based on 2D diffusion-ordered H NMR spectroscopy. J Lipid Res. 2015; 56:737–746. doi: 10.1194/jlr.D050120 PMID: 25568061
24. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein Particle Analysis by Nuclear Magnetic Resonance Spectroscopy. Clin Lab Med. 2006; 26:847–870. PMID: 17110242
25. Mihaleva VV, Schalkwijk DB, Graaf AA, van Duynhover J, van Dorsten FA, Vervoort J et al. A systematic approach to obtain validated partial least square models for predicting lipoprotein subclasses from serum NMR spectra. Anal Chem. 2014; 86:543–550. doi: 10.1021/ac402571z PMID: 24319989
26. Gonzàlez M, Ribalta J, Vives G, Iftimie S, Ferré R, Plana N et al. Nuclear magnetic resonance lipoprotein subclasses and the APOE genotype influence carotid atherosclerosis in patients with systemic lupus erythematosus. J Rheumatol. 2010; 37:2259–2267. doi: 10.3899/jrheum.091175 PMID: 20682673

27. Guardiola M, Plana N, Ibarretxe D, Cabré A, González M, Ribalta J et al. Circulating PCSK9 levels are positively correlated with NMR-assessed atherogenic dyslipidemia in patients with high cardiovascular risk. Clin Sci. 2015; 128:877–882. doi: 10.1042/CS20140832 PMID: 25649668

28. Mallol R, Rodríguez MA., Heras M, Vinaixa M, Cañellas N, Brezmes J et al. Surface fitting of 2D diffusion-edited 1H NMR spectroscopy data for the characterisation of human plasma lipoproteins. Metabolomics. 2011; 7:572–582.

29. Williams K V, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? Diabetes. 2000; 49(4):626–32. PMID: 10871201

30. Orchard TJ, Costacou T, Kretowski A, Nesto RW. Type 1 diabetes and coronary artery disease. Diabetes Care. 2006; 29:2528–38. PMID: 17065698

31. International Physical Activity Questionnaire. Available: http://www.ipaq.ki.se/; https://sites.google.com/site/theipaq/.

32. Centre D'Ensenyament Superior de Nutrició I Dietètica (CESNID). CESNID PCN 1.0. 2014. Available: biblioteca.universia.net.

33. Nakajima K, Saito T, Tamura A, Suzuki M, Adachi M, Tanaka A et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta. 1993; 223:53–71. PMID: 8143370

34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc. 1995; 57:289–300.

35. Guy J, Ogden L, Wadwa RP, Hamman RF, Mayer-Davis EJ, Liese AD et al. Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. Diabetes Care. 2009; 32:416–20. doi: 10.2337/dc08-1775 PMID: 19092167

36. Albers JJ, Marcovina SM, Imperatore G, Snively BM, Stafford J, Fujimoto WY et al. Prevalence and determinants of elevated apolipoprotein B and dense low-density lipoprotein in youths with type 1 and type 2 diabetes. J Clin Endocrinol Metab. 2008; 93:735–42. PMID: 18089692

37. Caixàs A, Ordóñes-Llanos J, de Leiva A, Payés A, Homs R, Peréz A. Optimization of glycemic control by insulin therapy decreases the proportion of small dense LDL particles in diabetic patients. Diabetes. 1997; 46:1207–1213. PMID: 9200657

38. Spate-Douglas T, Keyser RE. Exercise intensity: its effect on the high-density lipid profile. Arch Phys Med Rehabil. 1999; 80:691–695. PMID: 10378497

39. Diffenderfer MR, Schaefer EJ. The composition and metabolism of large and small LDL. Curr Opin Lipidol. 2014; 25:221–6. doi: 10.1097/MOL.0000000000000067 PMID: 24811298

40. Gupta A, Ross E, Myers J, Kashyap M. Increased reverse cholesterol transport in athletes. Metabolism. 1993; 42:684–690. PMID: 8510511

41. Seip RL, Moulin P, Cocke T, Tall A, Kohrt WM, Mankowitz K et al. Exercise training decreases plasma cholesteryl ester transfer protein. Arterioscler, Thromb Vasc Biol. 1993; 13:1359–1367.

42. Olchawa B, Kingwell BA, Hoang A, Schneider L, Miyazaki O, Nestel P et al. Physical fitness and reverse cholesterol transport. Arterioscler Thromb Vasc Biol. 2004; 24:1087–1091. PMID: 15072992

43. Holzer M, Trieb M, Konya V, Wadsack C, Heinemann A, Marsche G. Aging affects high-density lipoprotein composition and function. Biochim Biophys Acta. 2013; 1831:1442–8. doi: 10.1016/j.bbalip.2013.06.004 PMID: 23792422

44. Berrougui H, Isabelle M, Cloutier M, Grenier G, Khalil A. Age-related impairment of HDL-mediated cholesterol efflux. J Lipid Res. 2007; 48:328–336. PMID: 17093293

45. National Cholesterol Education Program (NCEP) Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation. 2002; 106:3143–3421. PMID: 12485966

46. Maahs DM, Nadeau K, Snell-Bergeon JK, Schauer I, Bergman B, West NA et al. Association of insulin sensitivity to lipids across the lifespan in people with type 1 diabetes. Diabetes Med. 2011; 28:148–155.

47. Hovingh GK, Rader DJ, Hegele RA. HDL re-examined. Curr Opin Lipidol. 2015; 26:127–132. doi: 10.1097/MOL.0000000000000161 PMID: 25692348

48. Colhoun HM, Otvos JD, Rubens MB, Taskinen MR, Underwood SR, Fuller J. Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. Diabetes. 2002; 51:1949–1956. PMID: 12031985

49. Soedamah-Muthu SS, Chang Y-F, Otvos J, Evans RW, Orchard TJ. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease

in Type 1 diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia. 2003; 46:674–82. PMID: 12743701

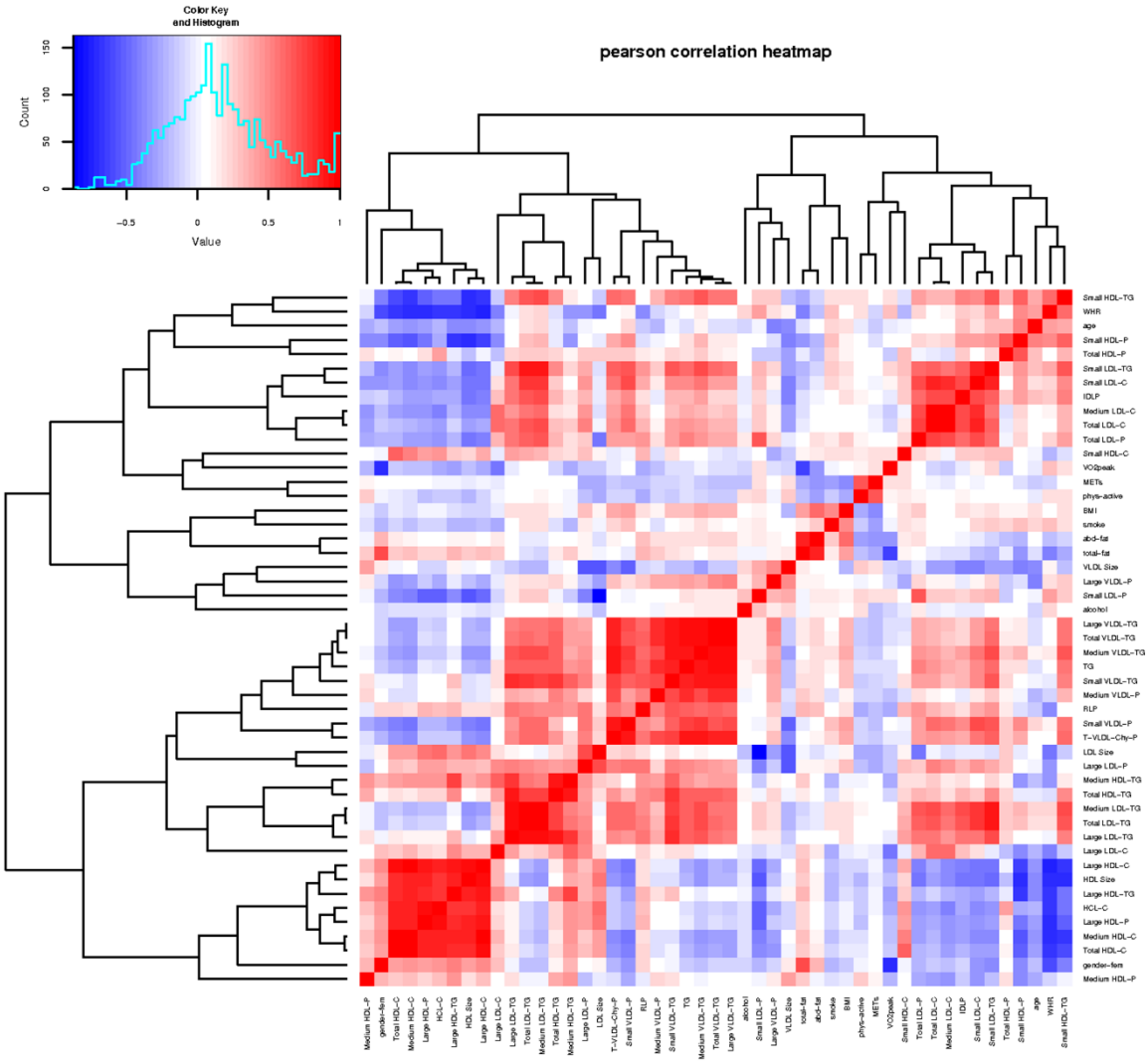
50. Soran H, Hama S, Yadav R, Durrington PN. HDL functionality. Curr Opin Lipidol. 2012; 23:353–366. doi: 10.1097/MOL.0b013e328355ca25 PMID: 22732521

51. Rached FH, Chapman MJ, Kontush A. HDL particle subpopulations: Focus on biological function. BioFactors. 2015; 41:67–77. doi: 10.1002/biof.1202 PMID: 25809447

52. Malpique R, Figueiredo H, Esteban Y, Rebuffat SA, Hanzu FA, Vinaixa M et al. Integrative analysis reveals novel pathways mediating the interaction between adipose tissue and pancreatic islets in obesity in rats. Diabetologia. 2014; 57:1219–1231. doi: 10.1007/s00125-014-3205-0 PMID: 24633677

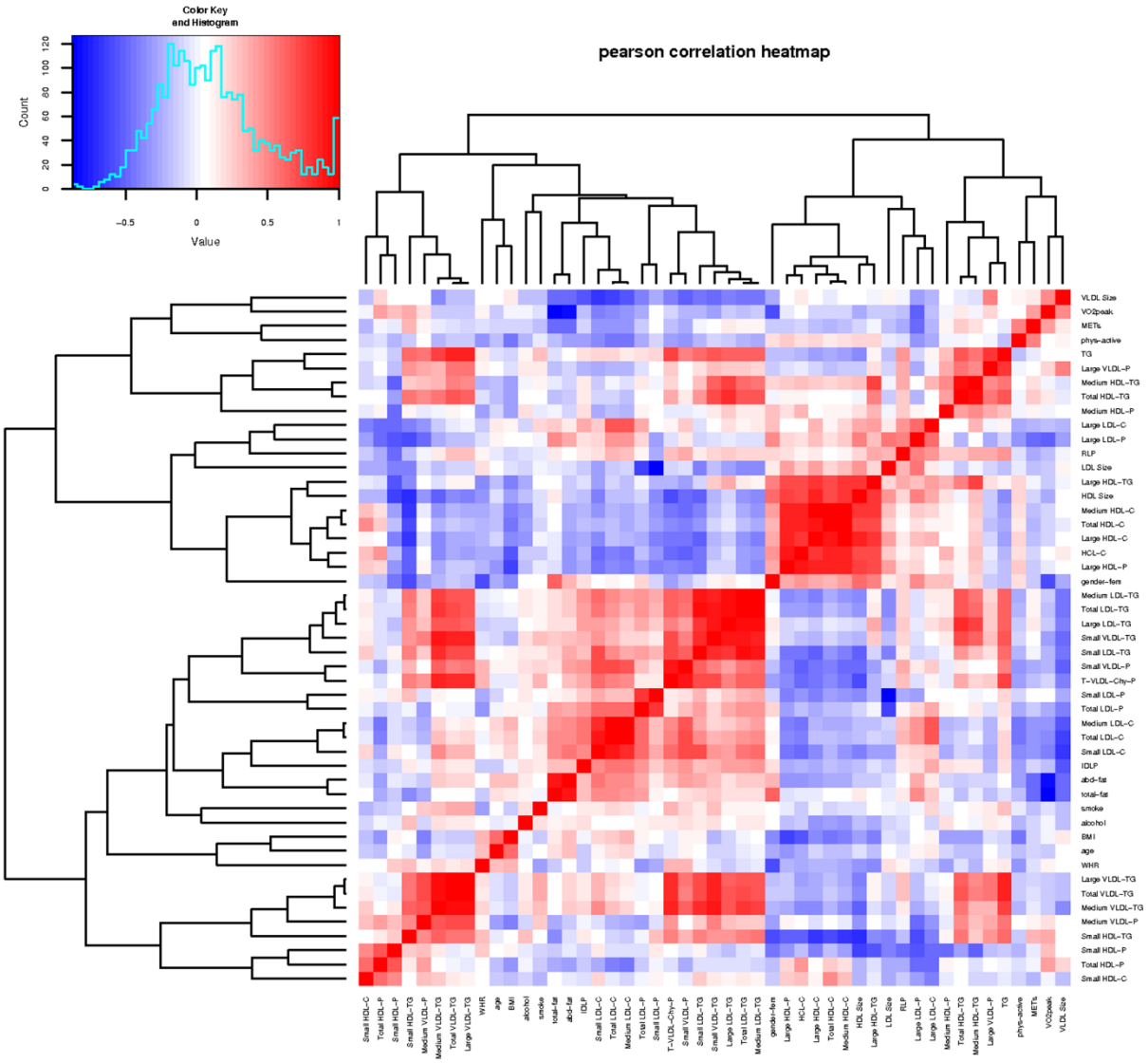
53. Mäkinen V-P, Forsblom C, Thorn LM, Wáden J, Kaski K, Ala-Korpela M et al. Network of vascular diseases, death and biochemical characteristics in a set of 4,197 patients with type 1 diabetes (the FinnDiane Study). Cardiovasc Diabetol. 2009; 8:54. doi: 10.1186/1475-2840-8-54 PMID: 19804653

Supporting information



S1 Fig. Clinical and lipoproteins variables correlation heatmap in control subjects.

doi:10.1371/journal.pone.0136348.s001



S2 Fig. Clinical and lipoproteins variables correlation heatmap in subjects with type 1 diabetes.

doi:10.1371/journal.pone.0136348.s002

S1 Table. Association of lipoproteins and dietary habits in subjects with type 1 diabetes.

doi:10.1371/journal.pone.0136348.s003

R	Energy (Kcal)	Total proteins (g/day)	Total lipids (g/day)	Saturated fatty acids (g/day)	Monouns. FA (g/day)	Polyuns. FA (g/day)	Cholesterol (mg/day)	Total carbohydrates (g/day)	Simple CH (g/day)	Total fiber (g/day)	Alcohol (g/day)
RLP	-	-	-	-	-	-	-	-	**-0.640**	**-0.600**	-
LipoProfile											
Large-VLDL-CH-P	-	-	-	-	-	-	-	-	-	-	0.512
Large LDL-P	-	-	-	-	-	-	-	-0.599	-0.524	-	-
Medium HDL-P	-	-	-	-	-	-	-	-	-0.562	-	-
Small HDL-P	**0.600**	-	0.506	-	-	**0.680**	-	0.556	0.518	0.540	-
HDL size	-0.503	-	-	-	-	-	-	-	-	-	-
PLS regression											
Small VLDL-TG	-	-	-	-	-	-	-	-	-0.549	-	-
Large HDL-TG	-	-	-	-	-	-	-	-	-0.538	-	-
Small HDL-TG	0.516	-	-	-	-	-	-	-	-	-	**0.615**

$p < 0.005$ and $R > 0.5$

S2 Table. Association of lipoproteins and dietary habits in control subjects.

doi:10.1371/journal.pone.0136348.s004

R	Energy (Kcal)	Total proteins (g/day)	Total lipids (g/day)	Saturated fatty acids (g/day)	Monouns. FA (g/day)	Polyuns. FA (g/day)	Cholesterol (mg/day)	Total carbohydrates (g/day)	Simple CH (g/day)	Total fiber (g/day)	Alcohol (g/day)
LipoProfile											
T-VLDL-Chy-P	-	-	-	-	-	-	-0.524	-	-	-	-0.519
Medium VLDL-P	-	-	-	-	-	-	-0.524	-	-	-	-
Small VLDL-P	-	-	-	-	-	-	-	-	-	0.529	-
Large HDL-P	-	-	-0.500	-	-0.530	-0.587	-	-	-	-	-
Medium HDL-P	-0.603	-0.514	-0.563	-0.517	-0.562	-	-	-0.551	-	-	-
Small HDL-P	0.578	**0.621**	-	-	-	-	-	0.525	-	-	-
VLDL size	-	-	-	-	-	-	-	-	-	-0.578	-
HDL size	-0.518	-	-	-	-0.511	-0.549	-	-	-	-	-
PLS regression											
Total HDL-C	-	-	-	-	-	**-0.575**	-	-	-	-	-
Large HDL-C	-0.534	-	-0.509	-	-0.588	**-0.651**	-	-	-	-	-
Medium HDL-C	-	-	-	-0.513	**-0.600**	-	-	-	-	-	-
Small VLDL-TG	-	-	-0.517	-	-	-	-	-	-	-	-0.500
Small LDL-TG	-	-	-	-	-	-	-	-	-	0.544	-
Total HDL-TG	-	-	-0.592	**-0.675**	-	-0.504	-	-	-	-	-
Large HDL-TG	**-0.671**	-	**-0.663**	-0.547	**-0.679**	**-0.753**	-	-0.532	-	-	-
Medium HDL-TG	**-0.623**	-0.529	**-0.662**	**-0.671**	-0.597	**-0.643**	-	-	-	-	-
Conventional HDL-C	-	-	-0.537	-	-0.601	**-0.638**	-	-	-	-	-

$p < 0.005$ and $R > 0.5$

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References in the publication:

1. Adult Treatment Panel III, 2002 (American Hearth Association, 2002)
2. Laing SP *et al.,* 2003 (Laing et al., 2003)
3. Soedamah-Muthu SS *et al*., 2006 (Soedamah-Muthu et al., 2006)
4. Lind M *et al.,* 2013 (M Lind et al., 2013)
5. Vergès B., 2009 (Vergès, 2009)
6. Patti L *et al*., 1995 (Patti et al., 1995)
7. Jenkins AJ *et al*., 2003 (Jenkins et al., 2003)
8. Orchard TJ *et al.,* 1998 (T J Orchard et al., 1998)
9. DCCT, 1993 (The Diabetes Control and Complications Trial Research Group, 1993)
10. Nathan DM *et al*., 2005 (Nathan et al., 2005)
11. ADA, 2015 (American Diabetes Association, 2015d)
12. Nordwall M *et al*., 2004 (Nordwall, Bojestig, Arnqvist, & Ludvigsson, 2004)
13. Hovind P *et al*., 2003 (Hovind et al., 2003)
14. Lind M *et al*., 2014 (Marcus Lind et al., 2014)
15. Lehmann *et al*., 1997 (Lehmann et al., 1997)
16. Purnell JQ *et al*.,1998 (Purnell et al., 1998)
17. Ferriss JB *et al.,* 2006 (Ferriss, Webb, Fuller, & The EURODIAB Prospective Complications Group, 2006)

18. Paulweber B *et al.,* 2010 (Paulweber et al., 2010)
19. WHO, 2004 (World Health Organization, 2004)
20. Salonen JT *et al*., 1991 (Salonen, Salonen, Seppanen, Rauramaa, & Tuomilehto, 1991)
21. Laaksonen DE *et al.,* 2000 (Laaksonen et al., 2000)
22. Rigla M *et al.,* 2000 (Rigla et al., 2000)
23. Mallol R *et al*., 2015 (Mallol et al., 2015)
24. Jeyarajah EJ *et al*., 2006 (Jeyarajah, Cromwell, & Otvos, 2006)
25. Mihaleva VV *et al.,* 2014 (Mihaleva et al., 2014)
26. González M *et al.,* 2010 (Gonzàlez et al., 2010)
27. Guardiola M *et al.,* 2015 (Guardiola et al., 2015)
28. Mallol R *et al.,* 2011 (Mallol et al., 2011)
29. Williams KV *et al*., 2000 (Williams et al., 2000)
30. Orchard TJ. *et al* 2006 (Trevor J Orchard et al., 2006)
31. IPAQ ("International Physical Activity Questionnaire," n.d.)
32. CESNID (Centre D'Ensenyament Superior de Nutrició I Dietètica (CESNID), 2014)
33. Nakajima K *et al.,* 1993 (Nakajima et al., 1993)
34. Benjamini Y *et al.,* 1995 (Benjamini & Hochberg, 1995)
35. Guy J *et al.,* 2009 (Guy et al., 2009)
36. Albers JJ *et al.,* 2008 (J. J. Albers et al., 2008)
37. Caixàs A *et al.,* 1997 (Caixàs et al., 1997)
38. Spade-Douglas T *et al.,* 1999 (Spate-Douglas & Keyser, 1999)
39. Diffenderfer MR *et al.,* 2014 (Diffenderfer & Schaefer, 2014)
40. Gupta A *et al.,* 1993 (Gupta, Ross, Myers, & Kashyap, 1993)
41. Seip RL *et al.,* 1993 (Seip et al., 1993)
42. Olchawa B *et al.,* 2004 (Olchawa et al., 2004)
43. Holzer M *et al.,* 2013 (Holzer et al., 2013)
44. Berrougui H *et al*., 2007 (Berrougui, Isabelle, Cloutier, Grenier, & Khalil, 2007)
45. Adult Treatment Panel III, 2002 (American Hearth Association, 2002)
46. Maahs DM *et al.,* 2011 (D M Maahs et al., 2011)
47. Hovingh GK *et al.,* 2015 (Hovingh et al., 2015)
48. Colhoun HM *et al.,* 2002 (Colhoun et al., 2002)
49. Soedamah-Muthu SS *et al.,* 2003 (Soedamah-Muthu et al., 2003)
50. Soran H *et al.,* 2012 (Soran et al., 2012)
51. Rached FH *et al.,* 2015 (Rached et al., 2015)
52. Malpique R *et al.,* 2014 (Malpique et al., 2014)
53. Mäkinen V-P *et al.,* 2009 (Mäkinen et al., 2009)

V. SUMMARY OF THE RESULTS

In the present work, different aspects related to physical activity in patients with T1D were studied.

In Study 1, the clinical characteristics of the large cohort of 265 patients and controls were described. A total of 129 subjects with T1D with no advanced chronic complications and with an adequate metabolic were compared with 136 control subjects. Women with T1D presented the same body composition and cardiorespiratory capacity (VO_2peak) than women without diabetes. Men with T1D also presented similar body composition to controls, but their VO_2peak was lower even when compared with the most active men of each group. Moreover, highly active men with T1D presented higher insulin sensitivity (eGDR) than the less active ones.

In Study 2, a careful design was performed to study differences in the metabolic response to an acute session of exercise in men with T1D and controls. A comprehensive 1H-NMR and GC-MS untargeted metabolomics approach was applied to serum samples taken from all participants before and after 30 min of static bicycle at 80% of individual VO_2peak. In response to exercise, both groups had increased concentrations of gluconeogenic precursors (alanine and lactate) and tricarboxylic acid cycle intermediates (citrate, malate, fumarate and succinate). The T1D group, however, showed attenuation in the response of these metabolites to exercise. Conversely to T1D, the control group also presented increases in α-ketoglutarate, alpha-ketoisocaproic acid, and lipolysis products (glycerol and oleic and linoleic acids), as well as a reduction in branched chain amino acid (valine and leucine) determinations. In this sense, an attenuated response in lipolysis, proteolysis, glycogenolysis, and oxidative metabolism in subjects with T1D patients following exercise was found. Exogenous insulin might play a role in these alterations.

In Study 3, an analysis of muscular components determined by 1H MRS in three different muscles was performed in T1D men and controls; each group included both athletes and sedentary subjects. The study showed that men with T1D presented similar amounts of IMCL, unsaturated ratios, carnosine and

creatine than men without diabetes. Also, athletes and sedentary subgroups did not present muscle component differences. The networks showed associations between IMCL (saturated methylene protons) and higher BMI or body fat composition in soleus muscle, the most oxidative one. Moreover, some repeated clusters of IMCL and carnosine (an intramyocellular buffer) were identified. Physical fitness was associated with body composition, but not with the muscular components featured in this study. Despite the normal weight range of the subjects, small alterations in BMI and/or body fat composition may have been enough to establish alterations in IMCL deposits.

In Study 4, an accurate lipid and lipoprotein analysis was performed using two different NMR platforms to characterize lipoprotein sizes and components in carefully matched T1D and control groups. It was possible to identify lower concentrations of small LDL cholesterol, medium VLDL particles, large VLDL-triglycerides and total triglycerides in subjects with T1D when compared with control subjects. Networks were constructed and different arrangements were identified in both groups. Patients with T1D with adequate glycemic control, normal BMI and without advanced chronic complications presented a more favorable lipoprotein profile as compared to their control counterparts. Physical fitness did not have effect on the lipoprotein profile in this study. In addition, slight alterations in BMI and/or body fat composition were relevant in promoting alterations in lipoproteins profiles.

VI. FINAL DISCUSSION

Nowadays, the pursuit of optimal glycemic control in T1D is essential, given its known benefits of delaying and/or avoiding chronic complications (Nathan et al., 2005; The Diabetes Control and Complications Trial Research Group, 1993). Nevertheless, the delicate balance of the requirements of external insulin is complex, with the dual goal of, on the one hand, preventing hyper- and hypoglycemic episodes, and, on the other, constantly adjusting to carbohydrate intake and physical activity expenditure. So, continuously fluctuations in insulin doses are required.

Some studies are identifying different degrees of insulin resistance in patients with T1D (Chillarón et al., 2011; Llauradó et al., 2012), although such occurrence is more typical of T2D and/or obesity cases. In one example, hyperinsulinemic-euglycemic clamps were performed in patients with T1D and controls for the study of lipoprotein subfractions (David M Maahs et al., 2010). As expected, the authors identified that subjects with T1D had a higher preclamp fasting glucose and insulin level than non-diabetic subjects, but there were no differences at the end of the clamp. However, subjects with T1D, in response to the test, were more insulin resistant than non-diabetic subjects.

The high prevalence and the growing incidence of T2D in these last decades, as well as the less than optimistic predictions for the future (International Diabetes Federation, 2013) represent a concern for public healthcare nowadays. The genetic factors in association with the increase in the obesity and sedentary lifestyle rates are the main causes for the increments in the cases of metabolic syndrome and T2D (International Diabetes Federation, 2006). It has been recognized that patients with T1D are also exposed to these factors (de Ferranti et al., 2014) and, in these subjects, insulin resistance may furthermore be aggravated by exogenous insulin administration, which may be associated with some degrees of hyperinsulinemia.

The subjects with T1D who participated in our studies presented no advanced chronic complications related to diabetes. Moreover, with different methods for the determination of body fat composition, most of them presented generally

good body fat composition, as well as few variations in eGDR index. To recall, eGDR is calculated using the waist/hip ratio, the presence of hypertension and glycated hemoglobin (HbA1c), variables that are similar for determining metabolic syndrome in the general population (International Diabetes Federation, 2006), that are obesity or increased waist circumference, hypertension, hyperglycemia and dyslipidemia. Few of our patients presented hypertension, and many of them presented adequate glycemic control. These observations make us believe that the patients that were evaluated might have fewer possibilities of developing insulin resistance, but this would probably be present to some degree.

Study 1 showed that women with or without T1D presented the same characteristics in body composition and physical fitness. Men with T1D, however, presented similar clinical characteristics to their control counterparts but with lower cardiorespiratory capacity (VO_2peak). As previously commented in the corresponding section, distinct causes could be involved in this reduction of cardiorespiratory capacity, such as metabolic control (Nguyen et al., 2014), reduced lung function (William Ricardo Komatsu et al., 2005; Niranjan et al., 1997) and even small differences in personal fitness trainings that were not registered in the studies. Other studies did not confirm this difference in VO_2peak (Stettler et al., 2006), shedding light on the need for a better understanding of this result. Another aspect of our study was that highly active men with T1D presented higher indicators of insulin sensitivity than less active ones (by eGDR), indicating a beneficial association of physical activity on insulin sensitivity. Other studies also addressed this topic by performing clamps before and after an exercise interventional study and identifying improvements in insulin sensitivity (Wallberg-Henriksson et al., 1982). However, another study did not confirm this finding (Ebeling et al., 1995), thus, further investigation is required.

Study 2 indicated an attenuated metabolic response to acute exercise in patients with T1D. We hypothesized that this response was an effect of insulin. It is known that the normal physiological response to exercise is a decrease of insulin levels (Powers & Howley, 2015). The identification of an increase in

insulin released into the blood stream after acute exercise in T1D was identified in Study 2, and it was also observed in a recent study by Mallad and colleagues (Mallad et al., 2015). In that study, subjects with T1D treated with insulin pump infusion presented plasma insulin concentrations that increased during exercise, despite no changes in insulin pump infusion rates; this phenomenon may be attributed to an increased mobilization of insulin from subcutaneous depots. A similar hypothesis was formulated in our study, and we suggest that this increase in insulin release might explain the observed attenuation in lipolysis, proteolysis, glycogenolysis, and oxidative metabolism response. Whether the attenuation of metabolic response to acute and intense exercise might interfere in the training performance of subjects with T1D remains to be elucidated.

Study 3 showed that subjects with T1D presented similar muscle components than their non-diabetic counterparts. Results from Study 1, as well as other studies (Bergman et al., 2012; Liu et al., 2009), suggested that subjects with T1D may present different degrees of insulin resistance. This insulin resistance may be reflected in muscle components, such as IMCL, and/or mitochondrial dysfunction (Stephan Jacob et al., 1999; Gianluca Perseghin et al., 1999; White et al., 2006). Study 3 was not able to demonstrate differences in the parameters studied in the muscles, probably because our subjects presented body composition in normal range and no other comorbidities. However, we were able to demonstrate that, in soleus muscle, IMCL was related to body fat composition and carnosine, an intramyocellular buffer. In our study, the cardiorespiratory capacity had no direct association with IMCL, as was the case in other studies (Coen & Goodpaster, 2012). However, it did have a negative association of cardiorespiratory fitness (VO_2peak) with body composition, and also a negative association of VO_2peak with carnosine in the soleus muscle of control subjects, suggesting some association of better fitness and less carnosine amount and/or expression.

In Study 4, we found that subjects with T1D presented a lipoprotein profile classically associated with cardio-protection as compared with the control group. Some studies described that subjects with T1D that present an adequate

glycemic control may present more cardioprotective aspects than the ones with inadequate control (Jenkins et al., 2003; Lehmann et al., 1997), which is probably related to the effects of insulin (Vergès, 2009). In our study, we were able to improve the knowledge about lipoprotein profile using 1H NMR techniques to achieve more information about its particles and sub-fractions. In this cross-sectional study, we were not able to identify lipoprotein association with cardiorespiratory capacity (VO_2peak), except for one single negative correlation with large LDL particles in T1D. Some clinical trials demonstrated reductions in LDL-C and Apo-B (Laaksonen et al., 2000) and increases in HDL-C (Rigla et al., 2000) after a 3 month program of aerobic exercise. Nonetheless, we found that the increase in body fat composition was associated with a less cardioprotective lipoprotein profile, especially related to LDL and HDL particles and subfractions (cholesterol components) in T1D and HDL particles and subfractions (cholesterol and triglyceride components) in controls, despite the normal body mass composition (BMI) of the subjects. Such worsening in cardioprotective lipid profile was observed in studies like EURODIAB (Ferriss et al., 2006) and DCCT (Purnell et al., 1998). In the EURODIAB study, cardioprotective lipid profile worsened in patients whose weight increased by 5 kg or more. In the DCCT study, this worsening occurred in patients undergoing intensive treatment who were in the fourth quartile of weight gain and whose BMI increased from 24 to 31 kg/m^2 by the end of the study. We again highlight that our patients with T1D presented normal BMI, but even their slightly higher values in weight and/or body fat composition were enough to change their lipoprotein profile.

VII. CONCLUSIONS

- The metabolic responses of lipolysis, proteolysis, glycogenolysis and oxidative metabolism to acute exercise in patients with T1D are attenuated. This phenomenon may be attributed to the exogenous administration of insulin.

- Subjects with T1D with good glycemic control and body fat composition presented a lipoprotein profile classically associated with cardio-protection.

- Small increases in BMI and/or body fat composition were enough to establish alterations in lipoprotein serum profiles and muscular lipid content.

- Subjects with highly active physical habits had better body fat composition, although physical activity did not correlate with lipoprotein profile or muscular lipid content.

VIII. REFERENCES

Aadland, E., Andersen, J. R., Anderssen, S. A., & Kvalheim, O. M. (2013). Physical Activity versus Sedentary Behavior: Associations with Lipoprotein Particle Subclass Concentrations in Healthy Adults. *PloS One*, *8*(12), e85223. doi:10.1371/journal.pone.0085223

Albers, J., Herman, W., Pop-Busui, R., Feldman, E., Marting, C., Cleary, P. a, … Lachin, J. (2010). Effect of prior intensive Insulin Treatment Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. *Diabetes Care*, *33*(5), 1090–96 doi:10.2337/dc09-1941

Albers, J. J., Marcovina, S. M., Imperatore, G., Snively, B. M., Stafford, J., Fujimoto, W. Y., … Dabelea, D. M. (2008). Prevalence and determinants of elevated apolipoprotein B and dense low-density lipoprotein in youths with type 1 and type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *93*, 735–42. doi:10.1210/jc.2007-2176

Albers, M. J., Butler, T. N., Rahwa, I., Bao, N., Keshari, K. R., Swanson, M. G., & Kurhanewicz, J. (2009). Evaluation of the ERETIC method as an improved quantitative reference for 1HR-MAS spectroscopy of prostate tissue. *Magnetic Resonance in Medicine*, *61*(3), 525–32. doi:10.1002/mrm.21808.Evaluation

Alvero Cruz, J. R., Diego Acosta, Á. M., Fernández Pastor, V. J., & Romero, J. G. (2005a). Métodos de evaluación de la composición corporal: tendencias actuales (I) / Body composition assessment methods: actual trends (I). *Archivos de Medicina Del Deporte*, *21*, 535–38

Alvero Cruz, J. R., Diego Acosta, Á. M., Fernández Pastor, V. J., & Romero, J. G. (2005b). Métodos de evaluación de la composición corporal: tendencias actuales (II) /Body composition assessment methods: actual trends (II). *Archivos de Medicina Del Deporte*, *22*, 45–49

Alvero Cruz, J. R., Diego Acosta, Á. M., Fernández Pastor, V. J., & Romero, J. G. (2005c). Métodos de evaluación de la composición corporal: tendencias actuales (III) / Body composition assessment methods: actual trends (III). *Archivos de Medicina Del Deporte*, *22*, 121–27

American Diabetes Association. (2014a). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *37 Suppl 1*(January), S81–90. doi:10.2337/dc14-S081

American Diabetes Association. (2014b). Standards of medical care in diabetes--2014. *Diabetes Care*, *37 Suppl 1*(October 2013), S14–80. doi:10.2337/dc14-S014

American Diabetes Association. (2015a). Cardiovascular Disease and Risk Management. *Diabetes Care*, *38*(Supplement_1), S49–S57. doi:10.2337/dc15-S011

American Diabetes Association. (2015b). Classification and diagnosis of diabetes. *Diabetes Care*, *38*, S8–S16. doi:10.2337/dc15-S005

American Diabetes Association. (2015c). Foundations of care: education, nutrition, physical activity, smoking cessation, psychosocial care, and immunization. *Diabetes Care*, *38*, S20–S30. doi:10.2337/dc15-S007

American Diabetes Association. (2015d). Glycemic Targets - Position Statment. *Diabetes Care*, *38*(Supplement_1), S33–S40. doi:10.2337/dc15-S009

American Diabetes Association. (2015e). Microvascular Complications and Foot Care. *Diabetes Care*, *38*(Supplement_1), S58–S66. doi:10.2337/dc15-S012

American Hearth Association. (2002). Third report of the National Education Program (NCEP) Expert Panel on Detection, Evaluatio, and Treatment of High Bloos Cholesterol in Adults (Adult Treatment Panell III) Final Report. *Circulation*, *106*, 3143–421

Aucouturier, J., Duché, P., & Timmons, B. W. (2011). Metabolic flexibility and obesity in children and youth. *Obesity Reviews*, *12*, e44–e53. doi:10.1111/j.1467-789X.2010.00812.x

Baguet, A., Everaert, I., Hespel, P., Petrovic, M., Achten, E., & Derave, W. (2011). A new method for non-invasive estimation of human muscle fiber type composition. *PloS One*, *6*(7), e21956. doi:10.1371/journal.pone.0021956; 10.1371/journal.pone.0021956

Bain, J. R., Stevens, R. D., Wenner, B. R., Ilkayeva, O., Muoio, D. M., & Newgard, C. B. (2009). Metabolomics applied to diabetes research: moving from information to knowledge. *Diabetes*, *58*(11), 2429–43. doi:10.2337/db09-0580

Barreiro, S. C., Rigual, M. R., Lozano, G. B., Siguero, J. P. L., Pelegrín, B. G., Val, M. P. R., & Dea, M. L. C. (2014). Epidemiología de la diabetes mellitus tipo 1 en menores de 15 años en España. *Anales de Pediatría*, *81*(3), 189.e1–189.e12

Battaglia, G. M., Zheng, D., Hickner, R. C., & Houmard, J. A. (2012). Effect of exercise training on metabolic flexibility in response to a high-fat diet in obese individuals. *Am J Physiol Endocrinol Metab*, *303*, E1440–45. doi:10.1152/ajpendo.00355.2012

Befroy, D. E., Petersen, K. F., Dufour, S., Mason, G. F., Graaf, R. A. De, Rothman, D. L., & Shulman, G. I. (2007). Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes*, *56*, 1376–81. doi:10.2337/db06-0783.COX

Benbassat, C. A., Stern, E., Kramer, M., Lebzelter, J., Blum, I., & Fink, G. (2001). Pulmonary Function in Patients with Diabetes Mellitus. *American Journal of the Medical Science*, *322*(3), 127–32

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, 57(1), 289-300. doi:10.2307/2346101

Beraki, A., Magnuson, A., Särnblad, S., Aman, J., & Samuelsson, U. (2014). Increase in physical activity is associated with lower HbA1c levels in children and adolescents with type 1 diabetes : Results from a cross-sectional study based on the Swedish pediatric diabetes quality registry (SWEDIABKIDS). *Diabetes Research and Clinical Practice*, *105*, 119–25. doi:10.1016/j.diabres.2014.01.029

Bergman, B. C., Howard, D., Schauer, I. E., Maahs, D. M., Snell-Bergeon, J. K., Eckel, R. H., … Rewers, M. (2012). Features of hepatic and skeletal muscle insulin resistance unique to type 1 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *97*(5), 1663–72. doi:10.1210/jc.2011-3172

Berrougui, H., Isabelle, M., Cloutier, M., Grenier, G., & Khalil, A. (2007). Age-related impairment of HDL-mediated cholesterol efflux. *Journal of Lipid Research*, *48*, 328–36. doi:10.1194/jlr.M600167-JLR200

Biolo, G., Williams, B. D., Fleming, R. Y., & Wolfe, R. R. (1999). Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes*, *48*(5), 949–57

Blazek, A., Rutsky, J., Osei, K., Maiseyeu, A., & Rajagopalan, S. (2013). Exercise-mediated changes in high-density lipoprotein: Impact on form and function. *American Heart Journal*, *166*(3), 392–400. doi:10.1016/j.ahj.2013.05.021

Boldyrev, A. a, Aldini, G., & Derave, W. (2013). Physiology and pathophysiology of carnosine. *Physiological Reviews*, *93*, 1803–45. doi:10.1152/physrev.00039.2012

Boulé, N. G., Haddad, E., Kenny, G. P., Wells, G. a, & Sigal, R. J. (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA : The Journal of the American Medical Association*, *286*(10), 1218–27

Boulé, N. G., Kenny, G. P., Haddad, E., Wells, G. a, & Sigal, R. J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia*, *46*(8), 1071–81. doi:10.1007/s00125-003-1160-2

Bowtell, J. L., Marwood, S., Bruce, M., Constantin-teodosiu, D., & Greenhaff, P. L. (2007). Tricarboxylic Acid Cycle Intermadeate Pool Size: Functional Importance for Oxidative Metabolism in Exercising Human Skeletal Muscle. *Sports Medicine*, *37*(12), 1071–88

Brazeau, A.-S., Rabasa-Lhoret, R., Strychar, I., & Mircescu, H. (2008). Barriers to Physical Activity Among Patients With Type 1 Diabetes. *Diabetes Care*, *31*(11), 2108–09. doi:10.2337/dc08-0720

Brugnara, L., Mallol, R., Ribalta, J., Vinaixa, M., Murillo, S., Casserras, T., … Novials, A. (2015). Improving assessment of lipoprotein profile in type 1 diabetes by 1H NMR spectroscopy. *PloS ONE*, *10*(8), e0136348. doi:10.1371/journal.pone.0136348

Brugnara, L., Vinaixa, M., Murillo, S., Samino, S., Rodriguez, M. A., Beltran, A., … Novials, A. (2012). Metabolomics Approach for Analyzing the Effects of Exercise in Subjects with Type 1 Diabetes Mellitus. *PLoS ONE*, *7*(7), e40600. doi:10.1371/journal.pone.0040600

Caixàs, A., Ordóñes-Llanos, J., Leiva, A. de, Payés, A., Homs, R., & Peréz, A. (1997). Optimization of glycemic control by insulin therapy decreases the proportion of small dense LDL particles in diabetic patients. *Diabetes*, *46*, 1207–13

Canivell, S., & Gomis, R. (2014). Diagnosis and classification of autoimmune diabetes mellitus. *Autoimmunity Reviews*, 1–5. doi:10.1016/j.autrev.2014.01.020

Centre D'Ensenyament Superior de Nutrició I Dietètica (CESNID). (2014). CESNID PCN 1.0. Retrieved from biblioteca.universia.net

Ceriello, A., Novials, A., Ortega, E., La Sala, L., Pujadas, G., Testa, R., … Giugliano, D. (2012). Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. *Diabetes*, *61*(11), 2993–97. doi:10.2337/db12-0224

Chatzinikolaou, A., Fatouros, I., Jamurtas, A., Avloniti, A., Douroudos, I., Mastorakos, G., … Vassilis, M. (2008). Adipose Tissue Lipolysis Is Upregulated in Lean and Obese Men During Acute Resistance Exercise. *Diabetes Care*, *31*(7), 7–9. doi:10.2337/dc08-0072

Chillarón, J. J., Goday, A., Flores-Le-Roux, J. a, Benaiges, D., Carrera, M. J., Puig, J., … Pedro-Botet, J. (2009). Estimated glucose disposal rate in assessment of the metabolic syndrome and microvascular complications in patients with type 1 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *94*(9), 3530–34. doi:10.1210/jc.2009-0960

Chillarón, J. J., Sales, M. P., Flores-Le-Roux, J. a, Murillo, J., Benaiges, D., Castells, I., … Pedro-Botet, J. (2011). Insulin resistance and hypertension in patients with type 1 diabetes. *Journal of Diabetes and Its Complications*, *25*(4), 232–36. doi:10.1016/j.jdiacomp.2011.03.006

Chimen, M., Kennedy, a, Nirantharakumar, K., Pang, T. T., Andrews, R., & Narendran, P. (2012). What are the health benefits of physical activity in type 1 diabetes mellitus? A literature review. *Diabetologia*, *55*(3), 542–51. doi:10.1007/s00125-011-2403-2

Christlieb, a R., Warram, J. H., Królewski, a S., Busick, E. J., Ganda, O. P., Asmal, a C., … Bradley, R. F. (1981). Hypertension: the major risk factor in juvenile-onset insulin-dependent diabetics. *Diabetes*, *30*(Suppl 2), 90–96

Coen, P. M., & Goodpaster, B. H. (2012). Role of intramyocelluar lipids in human health. *Trends in Endocrinology & Metabolism*, *23*(8), 391–98. doi:10.1016/j.tem.2012.05.009

Colhoun, H. M., Otvos, J. D., Rubens, M. B., Taskinen, M. R., Underwood, S. R., & Fuller, J. (2002). Lipoprotein subclasses and particle sizes and their relationship with coronary artery calcification in men and women with and without type 1 diabetes. *Diabetes*, *51*, 1949–56

Cuff, D. J., Meneilly, G. S., Martin, A., Ignaszewski, A., Tildesley, H. D., & Frohlich, J. J. (2003). Effective exercise modality to reduce insulin resistance in women with type 2 diabetes. *Diabetes Care*, *26*(11), 2977–82

Davison, G., George, L., Jackson, S. K., Young, I. S., Davies, B., Bailey, D. M., … Ashton, T. (2002). Exercise, free radicals, and lipid peroxidation in type 1 diabetes mellitus. *Free Radical Biology and Medicine*, *33*(11), 1543–51

De Ferranti, S. D., de Boer, I. H., Fonseca, V., Fox, C. S., Golden, S. H., Lavie, C. J., … Eckel, R. H. (2014). *Type 1 diabetes mellitus and cardiovascular disease: A scientific statement from the American Heart Association and American Diabetes Association*. *Circulation,* 130(13), 1110-30. doi:10.1161/CIR.0000000000000034

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *The American Journal of Physiology*, *237*, E214–23

Devlin, J. I., Scrimgeour, A., Brodsky, I., & Fuller, S. (1994). Decreased protein catabolism after exercise in subjects with IDDM. *Diabetologia*, *37*, 358–64

Diabetes Prevention Program Research Group. (2009). 10-year follow-up of diabetes incidence and weight loss in the diabetes prevention program outcomes study. *Lancet*, *374*(9702), 1677–86. doi:10.1016/S0140-6736(09)61457-4.10-year

Diffenderfer, M. R., & Schaefer, E. J. (2014). The composition and metabolism of large and small LDL. *Current Opinion in Lipidology*, *25*(3), 221–26 doi:10.1097/MOL.0000000000000067

Divertie, G., Jensen, M., Cryer, P., & JM, M. (1997). Lipolytic responsiveness to epinephrine in nondiabetic and diabetic humans. *American Journal of Physiology. Endocrinology and Metabolism*, *272*, E1130–35

Dorman, J. S., Laporte, R. E., Kuller, L. H., Cruickshanks, K. J., Orchard, T. J., Wagener, D. K., … Drash, a L. (1984). The Pittsburgh insulin-dependent diabetes mellitus (IDDM) morbidity and mortality study. Mortality results. *Diabetes*, *33*(3), 271–76

Dubé, J. J., Amati, F., Stefanovic-Racic, M., Toledo, F. G. S., Sauers, S. E., & Goodpaster, B. H. (2008). Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. *American Journal of Physiology. Endocrinology and Metabolism*, *294*, E882–88. doi:10.1152/ajpendo.00769.2007

Duke, J., McEvoy, M., Sibbritt, D., Guest, M., Smith, W., & Attia, J. (2007). Vibrotactile threshold measurement for detecting peripheral neuropathy: defining variability and a normal range for clinical and research use. *Diabetologia*, *50*, 2305–12. doi:10.1007/s00125-007-0813-y

Dyck, J. B., & Dyck, P. J. (1999). Diabetic Polyneuropathy - characteristics and diagnosis in brief. In P. J. Dyck & P. K. Thomas (Eds.), *Diabetic Neuropathy* (2nd ed., pp. 255–278). W.B. Saunders Company

Ebeling, P., Tuominen, J. a, Bourey, R., Koranyi, L., & Koivisto, V. a. (1995). Athletes with IDDM exhibit impaired metabolic control and increased lipid utilization with no increase in insulin sensitivity. *Diabetes*, *44*(4), 471–77

Eckel, R. H., Jakicic, J. M., Ard, J. D., De Jesus, J. M., Houston Miller, N., Hubbard, V. S., … Yanovski, S. Z. (2014). 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. *Journal of the American College of Cardiology*, *63*(25), 2960–84. doi:10.1016/j.jacc.2013.11.003

Edgerton, V., Smith, J., & Simpson, D. (1975). Muscle fibre type populations of human leg muscles. *Histochem J*, *7*, 259–66

Egan, B., & Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metabolism*, *17*(2), 162–84. doi:10.1016/j.cmet.2012.12.012

Enea, C., Seguin, F., Petitpas-Mulliez, J., Boildieu, N., Boisseau, N., Delpech, N., … Dugué, B. (2010). 1H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise. *Analytical and Bioanalytical Chemistry*, *396*(3), 1167–76. doi:10.1007/s00216-009-3289-4

Farhy, L. S., Chan, A., Breton, M. D., Anderson, S. M., Kovatchev, B. P., & McCall, A. L. (2012). Association of basal hyperglucagonemia with impaired glucagon counterregulation in type 1 diabetes. *Frontiers in Physiology*, *3*(February), 40. doi:10.3389/fphys.2012.00040

Ferriss, J. B., Webb, D., Fuller, J. H., & The EURODIAB Prospective Complications Group. (2006). Weight gain is associated with improved glycaemic control but with adverse changes in plasma lipids and blood pressure in Type 1 diabetes. *Diabetes Medicine*, *23*, 557–64

Fritzsche, K., Blüher, M., Schering, S., Buchwalow, I. B., Kern, M., Linke, A., … Punkt, K. (2008). Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes : Official Journal, German Society of Endocrinology [and] German Diabetes Association*, *116*(10), 606–13. doi:10.1055/s-2008-1073126

Galgani, J. E., Moro, C., & Ravussin, E. (2008). Metabolic flexibility and insulin resistance. *American Journal of Physiology. Endocrinology and Metabolism*, *295*, E1009–17. doi:10.1152/ajpendo.90558.2008

Gaster, M., Staehr, P., Beck-Nielsen, H., Schrøder, H. D., & Handberg, A. (2001). GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? *Diabetes*, *50*, 1324–29. doi:10.2337/diabetes.50.6.1324

Gelfand, R. A., & Barrett, E. J. (1987). Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *The Journal of Clinical Investigation*, *80*(1), 1–6. doi:10.1172/JCI113033

Giannini, C., Mohn, A., Chiarelli, F., & H, K. C. J. (2011). Macrovascular angiopathy in children and adolescents with type 1 diabetes. *Diabetes/metabolism Research and Reviews*, (March), 436–60. doi:10.1002/dmrr

Gibala, M. J., Maclean, D. A., Graham, T. E., & Saltin, B. (1998). Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *American Journal of Physiology. Endocrinology and Metabolism*, *275*, E235–42

Giménez, M., Gilabert, R., Monteagudo, J., Alonso, A., Casamitjana, R., Paré, C., & Conget, I. (2011). Repeated episodes of hypoglycemia as a potential aggravating factor for preclinical atherosclerosis in subjects with type 1 diabetes. *Diabetes Care*, *34*(1), 198–203. doi:10.2337/dc10-1371

Girgis, C. M., Scalley, B. D., & Park, K. E. J. (2012). Utility of the estimated glucose disposal rate as a marker of microvascular complications in young adults with type 1 diabetes. *Diabetes Research and Clinical Practice*, *96*(3), e70–e72. doi:10.1016/j.diabres.2012.02.004

Gonzàlez, M., Ribalta, J., Vives, G., Iftimie, S., Ferré, R., Plana, N., … Castro, A. (2010). Nuclear magnetic resonance lipoprotein subclasses and the APOE genotype influence carotid atherosclerosis in patients with systemic lupus erythematosus. *The Journal of Rheumatology*, *37*(11), 2259–67. doi:10.3899/jrheum.091175

Gregg, E. W., Li, Y., Wang, J., Burrows, N. R., Ali, M. K., Rolka, D., … Geiss, L. (2014). Changes in diabetes-related complications in the United States, 1990-2010. *The New England Journal of Medicine*, *370*(16), 1514–23. doi:10.1056/NEJMoa1310799

Gualano, B., Everaert, I., Stegen, S., Artioli, G. G., Taes, Y., Roschel, H., … Derave, W. (2012). Reduced muscle carnosine content in type 2, but not in type 1 diabetic patients. *Amino Acids*, *43*, 21–24. doi:10.1007/s00726-011-1165-y

Guardiola, M., Plana, N., Ibarretxe, D., Cabré, A., Gonzàlez, M., Ribalta, J., & Masana, L. (2015). Circulating PCSK9 levels are positively correlated with NMR-assessed atherogenic dyslipidemia in patients with high cardiovascular risk. *Clinical Science*, *128*(12), 877–82. doi:10.1042/CS20140832

Gupta, A., Ross, E., Myers, J., & Kashyap, M. (1993). Increased reverse cholesterol transport in athletes. *Metabolism*, *42*, 684–90

Gusso, S., Hofman, P., Lalande, S., Cutfield, W., Robinson, E., & Baldi, J. C. (2008). Impaired stroke volume and aerobic capacity in female adolescents with type 1 and type 2 diabetes mellitus. *Diabetologia*, *51*(7), 1317–20. doi:10.1007/s00125-008-1012-1

Guthold, R., Ono, T., Strong, K. L., Chatterji, S., & Morabia, A. (2008). Worldwide variability in physical inactivity a 51-country survey. *American Journal of Preventive Medicine*, *34*(6), 486–94. doi:10.1016/j.amepre.2008.02.013

Guy, J., Ogden, L., Wadwa, R. P., Hamman, R. F., Mayer-Davis, E. J., Liese, A. D., … Dabelea, D. (2009). Lipid and lipoprotein profiles in youth with and without type 1 diabetes: the SEARCH for Diabetes in Youth case-control study. *Diabetes Care*, *32*(3), 416–20. doi:10.2337/dc08-1775

Halverstadt, A., Phares, D. a., Wilund, K. R., Goldberg, A. P., & Hagberg, J. M. (2007). Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat

phenotypes in older men and women. *Metabolism: Clinical and Experimental*, *56*(4), 444–50. doi:10.1016/j.metabol.2006.10.019

Harchaoui, K. El, Arsenault, B. J., Frassen, R., Després, J.-P., Hoving, G. K., Stroes, E. S. G., … Boekholdt, S. M. (2009). High-density lipoprotein particle size and concentration and coronary risk. *Annals of Internal Medicine*, *150*, 84–93

Haskell, W. L., Lee, I.-M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. a, … Bauman, A. (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*, *116*(9), 1081–93. doi:10.1161/CIRCULATIONAHA.107.185649

He, J., Watkins, S., & Kelley, D. E. (2001). Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes*, *50*, 817–23. doi:10.2337/diabetes.50.4.817

Heady, J. a, Morris, J. N., Kagan, a, & Raffle, P. a. (1961). Coronary heart disease in London busmen. A progress report with particular reference to physique. *British Journal of Preventive & Social Medicine*, *15*, 143–53

Henriksson, J. (1991). Effect of exercise on amino acid concentrations in skeletal muscle and plasma. *The Journal of Experimental Biology*, *160*, 149–65

Herbst, A., Kordonouri, O., Scheab, K. O., Schmidt, F., & Holl, R. (2007). Impact of Physical Activity on Cardiovascular Risk Factors in Children With Type 1 Diabetes - A multicenter study of 23,251 patients. *Diabetes Care*, *30*(8), 2098–100. doi:10.2337/dc06-2636

Holzer, M., Trieb, M., Konya, V., Wadsack, C., Heinemann, A., & Marsche, G. (2013). Aging affects high-density lipoprotein composition and function. *Biochimica et Biophysica Acta*, *1831*, 1442–48. doi:10.1016/j.bbalip.2013.06.004

Hovind, P., Tarnow, L., Rossing, K., Rossing, P., Eising, S., Larsen, N., … Parving, H.-H. (2003). Decreasing incidence of severe diabetic microangiopathy in type 1 diabetes. *Diabetes Care*, *26*(4), 1258–64

Hovingh, G. K., Rader, D. J., & Hegele, R. a. (2015). HDL re-examined. *Current Opinion in Lipidology*, *26*, 127–32. doi:10.1097/MOL.0000000000000161

Howard, B. V. (1987). Lipoprotein metabolism in diabetes mellitus. *Journal of Lipid Research*, *28*(6), 613–28

Hwang, J.-H., Pan, J. W., Heydari, S., Hetherington, H. P., & Stein, D. T. (2001). Regional differences in intramyocellular lipids in humans observed by in vivo 1 H-MR spectroscopic imaging. *Journal of Applied Physiology*, *90*, 1267–74

Ibañez, J., Izquierdo, M., Argüelles, I., Forga, L., Larrión, J. L., García-Unciti, M., … Gorostiaga, E. M. (2005). Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. *Diabetes Care*, *28*, 662–67

Idzior-Walus, B., Mattock, M., Solnica, B., Stevens, L., Fuller, J., & Eurodiab, I. (2001). Factors associated with plasma lipid and lipoproteins in type 1 diabetes mellitus: The EURODIAB IDDM Complications Study. *Diabetes Medicine*, *18*, 786–96

International Diabetes Federation. (2006). The IDF consensus worldwide definition of the metabolic syndrome Part 1: Worldwide definition for use in clinical practice, 1–16. Retrieved from https://www.idf.org/webdata/docs/MetS_def_update2006.pdf

International Diabetes Federation. (2013). *IDF Diabetes Atlas - Sixth edition*. Retrieved from http://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf

International Physical Activity Questionnaire. (n.d.). *http://www.ipaq.ki.se/ ; https://sites.google.com/site/theipaq/*

Item, F., Heinzer-schweizer, S., Wyss, M., Fontana, P., Lehmann, R., Henning, A., … Toigo, M. (2011). Mitochondrial capacity is affected by glycemic status in young untrained women with type 1 diabetes but is not impaired relative to healthy untrained women. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *301*, R60–R66. doi:10.1152/ajpregu.00747.2010

Jaberi-Douraki, M., Liu, S. W. (Shalon), Pietropaolo, M., & Khadra, A. (2014). Autoimmune responses in T1DM: quantitative methods to understand onset, progression and prevention of disease. *Pediatric Diabetes*, *15*(3), 162–74. doi:10.1111/pedi.12148.Autoimmune

Jacob, S., Hauer, B., Becker, R., Artzner, S., Grauer, P., Löblein, K., … Häring, H.-U. (1999). Lipolysis in skeletal muscle is rapidly regulated by low physiological doses of insulin. *Diabetologia*, *42*, 1171–74

Jacob, S., Machann, J., Rett, K., Brechtel, K., Volk, A., Renn, W., … Haring, H.-U. (1999). Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetes subjects. *Diabetes*, *48*, 1113–19

Jenkins, A. J., Lyons, T. J., Zheng, D., Otvos, J. D., Lackland, D. T., McGee, D., … Group, D. R. (2003). Lipoproteins in the DCCT / EDIC cohort : associations with diabetic nephropathy. *Kidney International*, *64*, 817–28

Jessen, N., Pold, R., Buhl, E. S., Jensen, L. S., Schmitz, O., Lund, S., … Lund, S. (2003). Effects of AICAR and exercise on insulin-stimulated glucose uptake , signaling , and GLUT-4 content in rat muscles. *J Appl Physiol*, *94*, 1373–79

Jeyarajah, E. J., Cromwell, W. C., & Otvos, J. D. (2006). Lipoprotein Particle Analysis by Nuclear Magnetic Resonance Spectroscopy. *Clinics in Laboratory Medicine*, *26*, 847–70 doi:10.1016/j.cll.2006.07.006

Kahn, S. E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*, *46*, 3–19. doi:10.1007/s00125-002-1009-10

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, *444*, 840–46. doi:10.1038/nature05482

Katz-Brull, R., Rofsky, N. M., Morrin, M. M., Pedrosa, I., George, D. J., Michaelson, M. D., … Lenkinski, R. E. (2005). Decreases in free cholesterol and fatty acid unsaturation in renal cell carcinoma demonstrated by breath-hold magnetic resonance spectroscopy. *American Journal of Physiology. Renal Physiology*, *288*(4), F637–41. doi:10.1152/ajprenal.00140.2004

Kavey, R.-E. W., Allada, V., Daniels, S. R., Hayman, L. L., McCrindle, B. W., Newburger, J. W., … Steinberger, J. (2006). Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, . *Circulation*, *114*(24), 2710–38. doi:10.1161/CIRCULATIONAHA.106.179568

Kelley, D. E., Goodpaster, B., Wing, R. R., & Simoneau, J. a. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *The American Journal of Physiology*, *277*, E1130–41

Kelley, D. E., & Mandarino, L. J. (2000). Fuel selection in human skeletal muscle in insulin. A reexamination. *Diabetes*, *49*, 677–83

Kennedy, A., Nirantharakumar, K., Chimen, M., Pang, T. T., Hemming, K., Andrews, R. C., & Narendran, P. (2013). Does exercise improve glycaemic control in type 1 diabetes? A systematic review and meta-analysis. *PloS One*, *8*(3), e58861. doi:10.1371/journal.pone.0058861

Kennedy, J. W., Hirshman, M. F., Gervino, E. V, Ocel, J. V, Forse, R. A., Hoenig, S. J., … Horton, E. S. (1999). Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes*, *48*, 1192–97

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. a, & Nathan, D. M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine*, *346*(6), 393–03. doi:10.1056/NEJMoa012512

Komatsu, W. R., Barros Neto, T. L., Chacra, A. R., & Dib, S. A. (2010). Aerobic exercise capacity and pulmonary function in athletes with and without type 1 diabetes mellitus. *Diabetes Care*, *33*, 2555–57. doi:10.2337/dc10-0769

Komatsu, W. R., Gabbay, M. A. L., Castro, M. L., Saraiva, G. L., Chacra, A. R., de Barros Neto, T. L., & Dib, S. A. (2005). Aerobic exercise capacity in normal adolescents and those with type 1 diabetes mellitus. *Pediatric Diabetes*, *6*(3), 145–49. doi:10.1111/j.1399-543X.2005.00120.x

Krolewski, A. S., Warram, J. H., Leland, S., Asmal, A. C., Christlieb, A. R., Robert, F., … Rand, I. (1987). Magnitude and Determinants of Coronary Artery Disease Diabetes Mellitus. *American Journal of Cardiology*, *59*, 750–55

Laaksonen, D. E., Atalay, M., Niskanen, L. K., Mustonen, J., Sen, C. K., Lakka, T. a, & Uusitupa, M. I. (2000). Aerobic exercise and the lipid profile in type 1 diabetic men: a randomized controlled trial. *Medicine and Science in Sports and Exercise*, *32*(9), 1541–48

Laing, S. P., Swerdlow, A. J., Slater, S. D., Burden, A. C., Morris, A., Waugh, N. R., … Patterson, C. C. (2003). Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia*, *46*(6), 760–65. doi:10.1007/s00125-003-1116-6

Lampasona, V., Petrone, A., Tiberti, C., Capizzi, M., Spoletini, M., Di Pietro, S., … Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study Group. (2010). Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes. *Diabetes Care*, *33*, 104–108. doi:10.2337/dc08-2305.

Landt, K. W., Campaigne, B. N., James, F. W., & Sperling, M. A. (1985). Effects of exercise training on insulin sensitivity in adolescents with type I diabetes. *Diabetes Care*, *8*(5), 461–65

Lanza, I. R., Zhang, S., Ward, L. E., Karakelides, H., Raftery, D., & Nair, K. S. (2010). Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. *PloS One*, *5*(5), e10538. doi:10.1371/journal.pone.0010538

Lee, I.-M., Shiroma, J. E., Lobelo, F., Puska, P., Blair, S. N., & Katzmarzyk, P. T. (2012). Impact of Physical Inactivity on the World's Major Non-Communicable Diseases. *Lancet*, *380*(9838), 219–29. doi:10.1016/S0140-6736(12)61031-9.Impact

Lehmann, R., Kaplan, V., Bingisser, R., Bloch, K. E., & Spinas, G. (1997). Impact of Physical Activity on Cardiovascular Risk Factors in IDDM. *Diabetes Care*, *20*(10), 1603–11

Leon, a S., Rice, T., Mandel, S., Després, J. P., Bergeron, J., Gagnon, J., … Bouchard, C. (2000). Blood lipid response to 20 weeks of supervised exercise in a large biracial population: the HERITAGE Family Study. *Metabolism: Clinical and Experimental*, *49*(4), 513–20

Lepore, M., Pampanelli, S., Fanelli, C., Porcellati, F., Bartocci, L., Di Vincenzo, a, … Bolli, G. B. (2000). Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. *Diabetes*, *49*(12), 2142–48

Lerner, D. J., & Kannel, W. B. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes : a 26-year follow-up of the Framingham population. *American Heart Journal*, *111*, 383–90. doi:10.1016/0002-8703(86)90155-9

Levin, K., Daa Schroeder, H., Alford, F. P., & Beck-Nielsen, H. (2001). Morphometric documentation of abnormal intramyocellular fat storage and reduced glycogen in obese patients with Type II diabetes. *Diabetologia*, *44*(7), 824–33. doi:10.1007/s001250100545

Lewis, G. D., Farrell, L., Wood, M. J., Martinovic, M., Arany, Z., Rowe, G. C., … Gerszten, R. E. (2010). Metabolic signatures of exercise in human plasma. *Science Translational Medicine*, *2*(33), 33ra37. doi:10.1126/scitranslmed.3001006

Lind, M., Garcia-Rodriguez, L. A., Booth, G. L., Cea-Soriano, L., Shah, B. R., Ekeroth, G., & Lipscombe, L. L. (2013). Mortality trends in patients with and without diabetes in Ontario, Canada and the UK from 1996 to 2009: a population-based study. *Diabetologia*, *56*(12), 2601–08. doi:10.1007/s00125-013-3063-1

Lind, M., Svensson, A.-M., Kosiborod, M., Gudbjörnsdottir, S., Pivodic, A., Wedel, H., … Rosengren, A. (2014). Glycemic Control and Excess Mortality in Type 1 Diabetes. *The New England Journal of Medicine*, *371*(21), 1972–82. doi:10.1056/NEJMoa1408214

Lindon, J., & Holmes, E. (2007). *The handbook of metabonomics and metabolomics* (1st edition). U.K.: Elsevier

Lindström, J., Louheranta, A., Mannelin, M., Rastas, M., Salminen, V., Eriksson, J., … Tuomilehto, J. (2003). The Finnish Diabetes Prevention Study (DPS) - Lifestyle intervention and 3-year results on diet and physical activity. *Diabetes Care*, *26(12)*, 3230-36

Lindström, J., Peltonen, M., Eriksson, J. G., Ilanne-Parikka, P., Aunola, S., Keinänen-Kiukaanniemi, S., … Tuomilehto, J. (2013). Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia*, *56*(2), 284–93. doi:10.1007/s00125-012-2752-5

Liu, H. Y., Cao, S. Y., Hong, T., Han, J., Liu, Z., & Cao, W. (2009). Insulin is a stronger inducer of insulin resistance than hyperglycemia in mice with type 1 diabetes mellitus (T1DM). *The Journal of Biological Chemistry*, *284*(40), 27090–27100. doi:10.1074/jbc.M109.016675; 10.1074/jbc.M109.016675

Livingstone, S. J., Looker, H. C., Hothersall, E. J., Wild, S. H., Lindsay, R. S., Chalmers, J., … Colhoun, H. M. (2012). Risk of cardiovascular disease and total mortality in adults with type 1 diabetes: Scottish registry linkage study. *PLoS Medicine*, *9*(10), e1001321. doi:10.1371/journal.pmed.1001321

Llauradó, G., Gallart, L., Tirado, R., Megia, A., Simón, I., Caixàs, A., … González-Clemente, J. M. (2012). Insulin resistance, low-grade inflammation and type 1 diabetes mellitus. *Acta Diabetologica*, *49*(1), 33–39. doi:10.1007/s00592-011-0257-1

Maahs, D. M., Hokanson, J. E., Wang, H., Kinney, G. L., Snell-Bergeon, J. K., East, A., … Eckel, R. H. (2010). Lipoprotein subfraction cholesterol distribution is proatherogenoc in women with type 1 diabetes and insulin resistance. *Diabetes*, *59*, 1771–79. doi:10.2337/db09-1626.

Maahs, D. M., Nadeau, K., Snell-Bergeon, J. K., Schauer, I., Bergman, B., West, N. A., … Dabelea, D. (2011). Association of insulin sensitivity to lipids across the lifespan in people with type 1 diabetes. *Diabetes Medicine*, *28*, 148–55. doi:10.1111/j.1464-5491.2010.03143.x.Association

Machann, J., Häring, H., Shick, F., & Stumvoll, M. (2004). Intramyocellular lipids and insulin resistance. *Diabetes, Obesity and Metabolism*, *6*, 239–248. doi:10.1111/j.1462-8902.2004.00339.x

Mäkinen, V.-P., Forsblom, C., Thorn, L. M., Wadén, J., Kaski, K., Ala-Korpela, M., & Groop, P.-H. (2009). Network of vascular diseases, death and biochemical characteristics in a set of 4,197 patients with type 1 diabetes (the FinnDiane Study). *Cardiovascular Diabetology*, *8*, 54. doi:10.1186/1475-2840-8-54

Mäkinen, V.-P., Soininen, P., Forsblom, C., Parkkonen, M., Ingman, P., Kaski, K., … Ala-Korpela, M. (2006). Diagnosing diabetic nephropathy by 1H NMR metabonomics of serum. *Magma (New York, N.Y.)*, *19*(6), 281–96. doi:10.1007/s10334-006-0054-y

Mallad, A., Hinshaw, L., Schiavon, M., Man, C. D., Dadlani, V., Basu, R., … Carter, R. (2015). Exercise effects on postprandial glucose metabolism in type 1 diabetes: a triple-tracer approach. *Am J Physiol Endocrinol Metab*, *308*, E1106–15. doi:10.1152/ajpendo.00014.2015

Mallol, R., Amigó, N., Rodríguez, M. A., Heras, M., Vinaixa, M., Plana, N., … Correig, X. (2015). Liposcale : a novel advanced lipoprotein test based on 2D diffusion-ordered H NMR spectroscopy. *Journal of Lipid Research*, *56*, 737–46. doi:10.1194/jlr.D050120

Mallol, R., Rodriguez, M. A., Brezmes, J., Masana, L., & Correig, X. (2013). Progress in Nuclear Magnetic Resonance Spectroscopy human serum/plasma lipoprotein analysis by NMR: Application to the study of diabetic dyslipidemia. *Progress in Nuclear Magnetic Resonance Spectroscopy*, 1–24. doi:10.1016/j.pnmrs.2012.09.001

Mallol, R., Rodríguez, M. a., Heras, M., Vinaixa, M., Cañellas, N., Brezmes, J., … Correig, X. (2011). Surface fitting of 2D diffusion-edited 1H NMR spectroscopy data for the characterisation of human plasma lipoproteins. *Metabolomics*, *7*(4), 572–82. doi:10.1007/s11306-011-0273-8

Malpique, R., Figueiredo, H., Esteban, Y., Rebuffat, S. A., Hanzu, F. A., Vinaixa, M., … Gomis, R. (2014). Integrative analysis reveals novel pathways mediating the interaction between adipose tissue and pancreatic islets in obesity in rats. *Diabetologia*, *57*, 1219–31. doi:10.1007/s00125-014-3205-0

Masding, M. G., Stears, A. J., Burdge, G. C., Wootton, S. A., & Sandeman, D. D. (2003). Premenopausal advantages in postprandial lipid metabolism are lost in women with type 2 diabetes. *Diabetes Care*, *26*, 3243–49

Mason, N. J., Jenkins, A. J., Best, J. D., & Rowley, K. G. (2006). Exercise frequency and arterial compliance in non-diabetic and type 1 diabetic individuals. *European Journal of Cardiovascular Prevention and Rehabilitation : Official Journal of the European Society of*

Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology, *13*(4), 598–603. doi:10.1097/01.hjr.0000216546.07432.b2

McGee, S. L., & Hargreaves, M. (2006). Exercise and skeletal muscle glucose transportes 4 expression: molecular mechanisms. *Clinical and Experimental Pharmachology and Physiology*, *33*, 395–99

Meex, R. C., Schrauwen-Hinderling, V. B., Moonen-Kornips, E., Schaart, G., Mensink, M., Phielix, E., … Hesselink, M. K. (2010). Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes*, *59*(3), 572–79. doi:10.2337/db09-1322; 10.2337/db09-1322

Mena, N. A., Sea, E. A., & Lucia, S. (2014). IDF Diabetes Atlas 6th edn. 2014 update. Retrieved from www.idf.org/diabetesatlas

Mihaleva, V. V, Schalkwijk, D. B., Graaf, A. A., Duynhoven, J., Dorsten, F. A., Vervoort, J., … Jacobs, D. M. (2014). A systematic approach to obtain validated partial least square models for predicting lipoprotein subclasses from serum NMR spectra. *Analytical Chemistry*, *86*, 543–50

Miller, M., Ginsberg, H. N., & Schaefer, E. J. (2008). Relative atherogenicity and predictive value of non-high-density lipoprotein cholesterol for coronary heart disease. *American Journal of Cardiology*, *101*(7), 1003–08. doi:10.1016/j.amjcard.2007.11.046

Mogensen, M., Sahlin, K., Fernström, M., Glintborg, D., Vind, B. F., Beck-Nielsen, H., & Højlund, K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes*, *56*, 1592–99. doi:10.2337/db06-0981.ETC

Montgomery, M. K., & Turner, N. (2015). Mitochondrial dysfunction and insulin resistance: an update. *Endocrine Connections*, *4*(1), R1–R15. doi:10.1530/EC-14-0092

Mora, S., Cook, N., Buring, J. E., Ridker, P. M., & Lee, I. M. (2007). Physical activity and reduced risk of cardiovascular events: Potential mediating mechanisms. *Circulation*, *116*, 2110–18. doi:10.1161/CIRCULATIONAHA.107.729939

Morino, K., Petersen, K. F., & Shulman, G. I. (2006). Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*, *55*(December). doi:10.2337/db06-S002

Moro, C., Bajpeyi, S., & Smith, S. R. (2008). Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *American Journal of Physiology. Endocrinology and Metabolism*, *294*, E203–13. doi:10.1152/ajpendo.00624.2007

Morris, P. B., Ballantyne, C. M., Birtcher, K. K., Dunn, S. P., & Urbina, E. M. (2014). Review of clinical practice guidelines for the management of LDL-related risk. *Journal of the American College of Cardiology*, *64*(2), 196–206. doi:10.1016/j.jacc.2014.05.015

Nadeau, K. J., Regensteiner, J. G., Bauer, T. a, Brown, M. S., Dorosz, J. L., Hull, A., … Reusch, J. E. B. (2010). Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. *The Journal of Clinical Endocrinology and Metabolism*, *95*(2), 513–21. doi:10.1210/jc.2009-1756

Nakajima, K., Saito, T., Tamura, A., Suzuki, M., Nakano, T., Adachi, M., … Campos, E. (1993). Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. *Clinical Chimical Acta*, *223*, 53–71

Nathan, D. M., Cleary, P. A., Backlund, J. Y., Genuth, S. M., Lachin, J. M., Orchard, T. J., … Group, T. D. C. and C. T. of D. I. and C. (DCCT/EDIC) S. R. (2005). Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *New England Journal of Medicine*, *353*, 2643–53

National Center for Chronic Disease Prevention and Health Promotion. (2014). National Diabetes Statistics Report , 2014 Estimates of Diabetes and Its Burden in the Epidemiologic estimation methods. *National Diabetes Statistics Report*, 2009–12

Nguyen, T., Obeid, J., Walker, R. G., Krause, M. P., Hawke, T. J., McAssey, K., … Timmons, B. W. (2014). Fitness and physical activity in youth with type 1 diabetes mellitus in good or poor glycemic control. *Pediatric Diabetes*, 1–10. doi:10.1111/pedi.12117

Niranjan, V., McBrayer, D. G., Ramirez, L. C., Raskin, P., & Hsia, C. C. (1997). Glycemic control and cardiopulmonary function in patients with insulin-dependent diabetes mellitus. *The American Journal of Medicine*, *103*, 504–513. doi:10.1016/S0002-9343(97)00251-9

Nordestgaard, B. G., & Varbo, A. (2014). Triglycerides and cardiovascular disease. *The Lancet*, *384*(9943), 626–635. doi:10.1016/S0140-6736(14)61177-6

Nordwall, M., Bojestig, M., Arnqvist, H. J., & Ludvigsson, J. (2004). Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of Type 1 diabetes-the Linköping Diabetes Complications Study. *Diabetologia*, *47*(7), 1266–72. doi:10.1007/s00125-004-1431-6

Oberbach, A., Bossenz, Y., Lehmann, S., Niebauer, J., Adams, V., Paschke, R., … Punkt, K. (2006). Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. *Diabetes Care*, *29*(4), 895–900. doi:10.2337/diacare.29.04.06.dc05-1854

Olchawa, B., Kingwell, B. A., Hoang, A., Schneider, L., Miyazaki, O., Nestel, P., & Sviridov, D. (2004). Physical fitness and reverse cholesterol transport. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*, 1087–91. doi:10.1161/01.ATV.0000128124.72935.0f

Olson, J. C., Erbey, J. R., Williams, K. V, Becker, D. J., Edmundowicz, D., Kelsey, S. F., … Orchard, T. J. (2002). Subclinical atherosclerosis and estimated glucose disposal rate as predictors of mortality in type 1 diabetes. *Annals of Epidemiology*, *12*(5), 331–37

Orchard, T. J., Costacou, T., Kretowski, A., & Nesto, R. W. (2006). Type 1 diabetes and coronary artery disease. *Diabetes Care*, *29*(11), 2528–38. doi:10.2337/dc06-1161

Orchard, T. J., Forrest, K. Y.-Z., Kuller, L. H., & Becker, D. J. (2001). Lipid and blood pressure treatment goals for type 1 diabetes. *Diabetes Care*, *24*(6), 1053–59

Orchard, T. J., Olson, J. C., Erbey, J. R., Williams, K., Forrest, K. Y.-Z., Kinder, L. S., … Becker, D. J. (2003). Insulin resistance-related factors , but not glycemia, predict coronary artery disease in type 1 diabetes. *Diabetes Care*, *26*, 1374–79

Orchard, T. J., Stevens, L. K., Forrest, K. Y., & Fuller, J. H. (1998). Cardiovascular disease in insulin dependent diabetes mellitus: similar rates but different risk factors in the US compared with Europe. *International Journal of Epidemiology*, *27*(6), 976–83

Ortega, E., Amor, A. J., Rojo-Martínezı, G., Castell, C., Giménez, M., & Conget, I. (2015). Enfermedad cardiovascular en pacientes con diabetes mellitus tipo 1 y tipo 2 en España. *Medicina Clinica (Barc.)*, *145*, 233–38. doi:10.1016/j.medcli.2014.10.017

Palazoglu, M., & Fiehn, O. (2009). Metabolite identification in blood plasma using GC/MS and the agilent fiehn GC/MS metabolomics RTL Library. Agilent Application Note.

Palmieri, V., Pezzullo, S., Arezzi, E., D'Andrea, C., Cassese, S., Martino, S., & Celentano, A. (2008). Cycle-ergometry stress testing and use of chronotropic reserve adjustment of ST depression for identification of significant coronary artery disease in clinical practice. *International Journal of Cardiology*, *127*(3), 390–92. doi:10.1016/j.ijcard.2007.04.035

Pan, X.-R., Li, G.-W., Hu, Y.-H., Wang, J.-X., Yang, W.-Y., An, Z.-X., … Howard, B. V. (1997). Effects of Diet and Exercise in Preventing NIDDM in People With Impaired Glucose Tolerance. *Diabetes Care*, *20*(4), 537–44

Patterson, C. C., Gyürüs, E., Rosenbauer, J., Cinek, O., Neu, a, Schober, E., … Soltész, G. (2012). Trends in childhood type 1 diabetes incidence in Europe during 1989-2008: evidence of non-uniformity over time in rates of increase. *Diabetologia*, *55*(8), 2142–47. doi:10.1007/s00125-012-2571-8

Patti, L., Marino, L. di, Maffetone, A., Romano, G., Annuzzi, G., Riccardi, G., & Rivellese, A. A. (1995). Very low density lipoprotein subfraction abnormalities in IDDM patients: any effect of blood glucose control? *Diabetologia*, *38*, 1419–24

Paulweber, B., Valensi, P., Lindström, J., Lalic, N. M., Greaves, C. J., McKee, M., … Yilmaz, T. (2010). A European evidence-based guideline for the prevention of type 2 diabetes. *Hormone and Metabolic Research*, *42 Suppl 1*, S3–S36

Pechlivanis, A., Kostidis, S., Saraslanidis, P., Petridou, A., Tsalis, G., Mougios, V., … Theodoridis, G. A. (2010). H NMR-Based Metabonomic Investigation of the Effect of Two Different Exercise Sessions on the Metabolic Fingerprint of Human Urine research articles. *Journal of Proteome Research*, *9*, 6405–16

Perseghin, G., Lattuada, G., Danna, M., Sereni, L. P., Maffi, P., De Cobelli, F., … Luzi, L. (2003). Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *American Journal of physiology.Endocrinology and Metabolism*, *285*(6), E1174–81. doi:10.1152/ajpendo.00279.2003

Perseghin, G., Lattuada, G., Danna, M., Sereni, L. P., Maffi, P., De Cobelli, F., … Luzi, L. (2003). Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *American Journal of Physiology. Endocrinology and Metabolism*, *285*(6), E1174–81. doi:10.1152/ajpendo.00279.2003

Perseghin, G., Scifo, P., De Cobelli, F., Pagliato, E., Battezzati, A., Arcelloni, C., … Luzi, L. (1999). Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans. *Diabetes*, *48*, 1600–06

Peters, S. A. E., Huxley, R. R., & Woodward, M. (2014). Diabetes as risk factor for incident coronary heart disease in women compared with men : a systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. *Diabetologia*, *57*, 1542–1551. doi:10.1007/s00125-014-3260-6

Petersen, K. F., Dufour, S., Befroy, D., Garcia, R., & Shulman, G. I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*, *350*, 664–71. doi:10.1056/NEJMoa031314

Petersen, K. F., Dufour, S., Savage, D. B., Bilz, S., Solomon, G., Yonemitsu, S., … Shulman, G. I. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(31), 12587–94. doi:10.1073/pnas.0705408104

Pfister, F., Riedl, E., Wang, Q., Hagen, F., Deinzer, M., Harmsen, M. C., … Hammes, H. (2011). Cellular Physiology and Biochemistr y Biochemistry Oral Carnosine Supplementation Prevents Vascular Damage in Experimental Diabetic Retinopathy. *Cell Physiol Biochem*, *28*, 125–36

Powers, S., & Howley, E. (2015). *Exercise physiology* (9th edition). USA: Mc Graw Hill.

Purnell, J. Q., Hokanson, J. E., Marcovina, S. M., Steffes, M. W., Cleary, P. A., & Brunzell, J. D. (1998). Effect of Excessive Weight Gain With Intensive Therapy of Type 1 Diabetes on Lipid Levels and Blood Pressure. *JAMA : The Journal of the American Medical Association*, *280*(2), 140–46. doi:10.1001/jama.280.2.140

Quirk, H., Blake, H., Tennyson, R., Randell, T. L., & Glazebrook, C. (2014). Physical activity interventions in children and young people with Type 1 diabetes mellitus: a systematic review with meta-analysis. *Diabetic Medicine*, *31*, 1163–1173. doi:10.1111/dme.12531

R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *https://www.R-project.org/*.

Rached, F. H., Chapman, M. J., & Kontush, A. (2015). HDL particle subpopulations: Focus on biological function. *BioFactors*, *41*, 67–77. doi:10.1002/biof.1202

Rader, D. J., & Hovingh, G. K. (2014). HDL and cardiovascular disease. *The Lancet*, *384*(9943), 618–25. doi:10.1016/S0140-6736(14)61217-4

Ramachandran, A., Snehalatha, C., Mary, S., Mukesh, B., Bhaskar, a D., & Vijay, V. (2006). The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia*, *49*(2), 289–97. doi:10.1007/s00125-005-0097-z

Richter, E. a, & Hargreaves, M. (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews*, *93*(3), 993–1017. doi:10.1152/physrev.00038.2012

Rico-Sanz, J., Thomas, E. L., Jenkinson, G., Sarka, M., Iles, R., & Bell, J. D. (1999). Diversity in levels of intracellular total creatine and triglycerides in human skeletal muscles observed by 1 H-MRS. *J Appl Physiol*, *87*, 2068–72

Ridker, P. M. (2014). LDL cholesterol: controversies and future therapeutic directions. *Lancet*, *384*(9943), 607–17. doi:10.1016/S0140-6736(14)61009-6

Riedl, E., Pfister, F., Braunagel, M., Brinkkötter, P., Sternik, P., Deinzer, M., … Koeppel, H. (2011). Carnosine prevents apoptosis of glomerular cells and podocyte loss in stz diabetic rats. *Cellular Physiology and Biochemistry*, *28*, 279–88. doi:10.1159/000331740

Rigla, M., Sánchez-Quesada, J. L., Ordóñez-Llanos, J., Prat, T., Caixàs, a, Jorba, O., … Pérez, a. (2000). Effect of physical exercise on lipoprotein(a) and low-density lipoprotein modifications in type 1 and type 2 diabetic patients. *Metabolism: Clinical and Experimental*, *49*(5), 640–47

Russell, R. D., Kraemer, R. R., & Nelson, A. G. (2013). Metabolic dysfunction in diabetic offspring: deviations in metabolic flexibility. *Med Sci Sports Exerc*, *45*, 8–15. doi:10.1249/MSS.0b013e31826909d3

Sahlin, K., Katz, a, & Broberg, S. (1990). Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *The American Journal of Physiology*, *259*(5 Pt 1), C834–41

Salonen, J. T., Salonen, R., Seppanen, K., Rauramaa, R., & Tuomilehto, J. (1991). HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. *Circulation*, *84*(1), 129–39. doi:10.1161/01.CIR.84.1.129

Schauer, I. E., Snell-Bergeon, J. K., Bergman, B. C., Maahs, D. M., Kretowski, A., Eckel, R. H., & Rewers, M. (2011). Insulin resistance , defective insulin-mediated fatty acid suppression , and coronary artery calcification in subjects with and without type 1 diabetes - The CACTI Study. *Diabetes*, *60*, 306–14. doi:10.2337/db10-0328.

Schiaffino, S., & Reggiani, C. (2011). Fiber Types in Mammalian Skeletal Muscles. *Physiological Reviews*, *91*(4), 1447–531. doi:10.1152/physrev.00031.2010

Schmid, H., Neumann, C., & Brugnara, L. (2003). O diabetes melito e a desnervação dos membros inferiores: a visão do diabetólogo. *J Vasc Br*, *2*(1), 37–48

Seip, R. L., Moulin, P., Cocke, T., Tall, A., Kohrt, W. M., Mankowitz, K., … Schonfeld, G. (1993). Exercise training decreases plasma cholesteryl ester transfer protein. *Arteriosclerosis , Thrombosis and Vascular Biology*, *13*, 1359–67. doi:10.1161/01.ATV.13.9.1359

Sjöström, M., Oja, P., Hagströmer, M., Smith, B. J., & Bauman, A. (2006). Health-enhancing physical activity across European Union countries: the Eurobarometer study. *Journal of Public Health*, *14*(5), 291–300. doi:10.1007/s10389-006-0031-y

Skrivarhaug, T., Bangstad, H.-J., Stene, L. C., Sandvik, L., Hanssen, K. F., & Joner, G. (2006). Long-term mortality in a nationwide cohort of childhood-onset type 1 diabetic patients in Norway. *Diabetologia*, *49*(2), 298–305. doi:10.1007/s00125-005-0082-6

Snowling, N. J., & Hopkins, W. G. (2006). Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients. *Diabetes Care*, *29*, 2518–27. doi:10.2337/dc06-1317

Soedamah-Muthu, S. S., Chang, Y.-F., Otvos, J., Evans, R. W., & Orchard, T. J. (2003). Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in Type 1 diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia*, *46*(5), 674–82. doi:10.1007/s00125-003-1094-8

Soedamah-Muthu, S. S., Fuller, J. H., Mulnier, H. E., Raleigh, V. S., Lawrenson, R. A., & Colhoun, H. M. (2006). All-cause mortality rates in patients with type 1 diabetes mellitus compared with a non-diabetic population from the UK general practice research database, 1992-1999. *Diabetologia*, *49*(4), 660–66. doi:10.1007/s00125-005-0120-4

Soran, H., Hama, S., Yadav, R., & Durrington, P. N. (2012). HDL functionality. *Current Opinion in Lipidology*, *23*(4), 353–66. doi:10.1097/MOL.0b013e328355ca25

Soriguer, F., Goday, a, Bosch-Comas, a, Bordiú, E., Calle-Pascual, a, Carmena, R., … Vendrell, J. (2012). Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. *Diabetologia*, *55*(1), 88–93. doi:10.1007/s00125-011-2336-9

Spate-Douglas, T., & Keyser, R. E. (1999). Exercise intensity: its effect on the high-density lipid profile. *Archives of Physical Medicine and Rehabilitation*, *80*, 691–95

Stettler, C., Jenni, S., Allemann, S., Steiner, R., Hoppeler, H., Trepp, R., … Diem, P. (2006). Exercise capacity in subjects with type 1 diabetes mellitus in eu- and hyperglycaemia. *Diabetes/metabolism Research and Reviews*, *22*(4), 300–06. doi:10.1002/dmrr.608

Storey, J. D., & Tibshirani, R. (2003). Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9440–45. doi:10.1073/pnas.1530509100

Storlien, L., Oakes, N. D., & Kelley, D. E. (2004). Adipose tissue-liver-muscle interactions leading to insulin resistane: Metabolic flexibility. *The Proceedings of the Nutrition Society*, *63*(2), 363–68. doi:10.1079/PNS2004349

Stuart, C. a., McCurry, M. P., Marino, A., South, M. a., Howell, M. E. a, Layne, A. S., … Stone, M. H. (2013). Slow-twitch fiber proportion in skeletal muscle correlates with insulin responsiveness. *Journal of Clinical Endocrinology and Metabolism*, *98*(May), 2027–36. doi:10.1210/jc.2012-3876

Stumvoll, M., Jacob, S., Wahl, H. G., Hauer, B., Löblein, K., Grauer, P., … Häring, H. (2000). Suppression of Systemic, Intramuscular, and Subcutaneous Adipose Tissue Lipolysis by Insulin in Humans. *Journal of Clinical Endocrinology & Metabolism*, *85*(10), 3740–45. doi:10.1210/jc.85.10.3740

Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., … Valensi, P. (2010). Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care*, *33*(10), 2285–93. doi:10.2337/dc10-1303

The Diabetes Control and Complications Trial Research Group. (1993). The effect of the intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New England Journal of Medicine*, *329*, 977–86

The Diabetes Control and Complications Trial Research Group. (1995a). Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial. *Kidney International*, *47*(6), 1703–20. doi:10.1038/ki.1995.236

The Diabetes Control and Complications Trial Research Group. (1995b). Progression of retinopathy with intensive versus conventional treatment in the Diabetes Control and Complications Trial. *Ophthalmology*, *102*(4), 647–61. doi:10.1016/S0161-6420(95)30973-6

The Diabetes Control and Complications Trial Research Group. (1995c). The effect of intensive diabetes therapy on the development and progression of neuropathy. *Annals of Internal Medicine*, *122*(8), 561–68

The Diabetes Control and Complications Trial Research Group. (1998). The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). *Diabetologia*, *41*(4), 416–23

Thomas, D., Elliott, E. J., & Naughton, G. A. (2006). Exercise for type 2 diabetes mellitus. *Cochrane Database Os Systematic Review*, (3), CD002968.pub2. doi:10.1002/14651858

Thompson, P. D., Buchner, D., Pina, I. L., Balady, G. J., Williams, M. a, Marcus, B. H., … Wenger, N. K. (2003). Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical. *Circulation*, *107*(24), 3109–16. doi:10.1161/01.CIR.0000075572.40158.77

Thyfault, J. P., Cree, M. G., Zheng, D., Zwetsloot, J. J., Tapscott, E. B., Koves, T. R., … Dohm, G. L. (2007). Contraction of insulin-resistant muscle normalizes insulin action in association with increased mitochondrial activity and fatty acid catabolism. *American Journal of Physiology. Cell Physiology*, *292*, 729–39. doi:10.1152/ajpcell.00311.2006.

Torriani, M., Thomas, B. J., Halpern, E. F., Jensen, M. E., Rosenthal, D. I., & Palmer, W. E. (2005). Intramyocellular lipid quantification: repeatability with 1H MR Spectroscopy. *Radiology*, *236*, 609–14

Toth, P. P., Grabner, M., Punekar, R. S., Quimbo, R. A., Cziraky, M. J., & Jacobson, T. A. (2014). Cardiovascular risk in patients achieving low-density lipoprotein cholesterol and particle targets. *Atherosclerosis*, *235*(2), 585–91. doi:10.1016/j.atherosclerosis.2014.05.914

Trejo-Gutierrez, J. F., & Fletcher, G. (2007). Impact of exercise on blood lipids and lipoproteins. *Journal of Clinical Lipidology*, *1*(3), 175–81. doi:10.1016/j.jacl.2007.05.006

Trost, S. G., & Loprinzi, P. D. (2008). Exercise - Promoting healthy lifestyles in children and adolescents. *Journal of Clinical Lipidology*, *2*(3), 162–68. doi:10.1016/j.jacl.2008.03.001

Tuomilehto, J., Lindström, J., Eriksson, J. G., Valle, T. T., Hämäläinen, H., Ilanne-Parikka, P., … Uusitupa, M. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine*, *344*(18), 1343–50

UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet*, *352*, 837–53

Valdés, S., Rojo-Martínez, G., & Soriguer, F. (2007). Evolución de la prevalencia de la diabetes tipo 2 en población adulta española. *Medicina Clínica*, *129*(9), 352–55. doi:10.1157/13109554

Valerio, G., Spagnuolo, M. I., Lombardi, F., Spadaro, R., Siano, M., & Franzese, A. (2007). Physical activity and sports participation in children and adolescents with type 1 diabetes mellitus. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD*, *17*(5), 376–82. doi:10.1016/j.numecd.2005.10.012

Velan, S. S., Said, N., Durst, C., Frisbee, S., Frisbee, J., Raylman, R. R., … Alway, S. E. (2008). Distinct patterns of fat metabolism in skeletal muscle of normal-weight, overweight, and obese humans. *American Journal of physiology.Regulatory, Integrative and Comparative Physiology*, *295*(4), R1060–65. doi:10.1152/ajpregu.90367.2008; 10.1152/ajpregu.90367.2008

Vergès, B. (2009). Lipid disorders in type 1 diabetes. *Diabetes & Metabolism*, *35*(5), 353–60. doi:10.1016/j.diabet.2009.04.004

Vermeulen, I., Weets, I., Asanghanwa, M., Ruige, J., Van Gaal, L., Mathieu, C., … The Belgian Diabetes Registry. (2011). Contribution of antibodies against IA-2 b and Zinc transporter 8 to classification of diabetes diagnosed under 40 years of age. *Diabetes Care*, *34*, 1760–65. doi:10.2337/dc10-2268

Vervoort, G., Wetzels, J. F., Lutterman, J. a., van Doorn, L. G., Berden, J. H., & Smits, P. (1999). Elevated skeletal muscle blood flow in noncomplicated type 1 diabetes mellitus: role of nitric oxide and sympathetic tone. *Hypertension*, *34*(5), 1080–85. doi:10.1161/01.HYP.34.5.1080

Vinik, A. I., Maser, R. E., Mitchell, B. D., & Freeman, R. (2003). Diabetic Autonomic Neuropathy. *Diabetes Care*, *26*, 1553–79

Wåden, J., Tikkanen, H., Forsblom, C., Fagerudd, J., Pettersson-Fernholm, K., Lakka, T., … Groop, P.-H. (2005). Leisure Time Physical Activity is Associated With Pooor Glycemic Control in Type 1 Diabetic Women - The FinnDiane study. *Diabetes Care*, *28*(4), 777–82

Wallberg-Henriksson, H., Gunnarsson, R., Henriksson, J., DeFronzo, R., Felig, P., Ostman, J., & Wahren, J. (1982). Increased peripheral insulin sensitivity and muscle mitochondrial enzymes but unchanged blood glucose control in type I diabetics after physical training. *Diabetes*, *31*(12), 1044–50

White, L. J., Ferguson, M. A., McCoy, S. C., Kim, H. W., & Castellano, V. (2006). Cardiovascular/non-insulin-dependent diabetes mellitus risk factors and intramyocellular lipid in healthy subjects: a sex comparison. *Metabolism: Clinical and Experimental*, *55*(1), 128–34. doi:10.1016/j.metabol.2005.08.026

Williams, K. V, Erbey, J. R., Becker, D., Arslanian, S., & Orchard, T. J. (2000). Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes*, *49*(4), 626–32

Wilson, J. M., Loenneke, J. P., Jo, E., Wilson, G. J., Zourdos, M. C., & Kim, J.-S. (2012). The effects of endurance, strength, and power training on muscle fiber type shifting. *Journal of Strength and Conditioning Research*, *26*, 1724–29

Wing, R. R., Bolin, P., Brancati, F. L., Bray, G. a, Clark, J. M., Coday, M., … Yanovski, S. Z. (2013). Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. *The New England Journal of Medicine*, *369*(2), 145–54. doi:10.1056/NEJMoa1212914

World Health Organization. (2004). Global Strategy on Diet, Physical Activity and Health, (May). Retrieved from http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf

World Health Organization. (2014). Global Status Report On Noncommunicable Diseases 2014. Retrieved from http://www.who.int/nmh/publications/ncd-status-report-2014/en/

Yang, J. C., & Yoo, J. Y. (1986). Histochemical muscle fiber types of autopsied human gastrocnemius, soleus, peroneus longus and tibialis anterior muscles. *J Pathol Transl Med*, *20*(4), 413–26

Yardley, J. E., Hay, J., Abou-Setta, A. M., Marks, S. D., & McGavock, J. (2014). A systematic review and meta-analysis of exercise interventions in adults with type 1 diabetes. *Diabetes Research and Clinical Practice*, *106*(3), 393–400. doi:10.1016/j.diabres.2014.09.038

Yeung, D. K. W., Griffith, J. F., Antonio, G. E., Lee, F. K. H., Woo, J., & Leung, P. C. (2005). Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: A proton MR spectroscopy study. *Journal of Magnetic Resonance Imaging*, *22*, 279–85. doi:10.1002/jmri.20367

Yki-Järvinen, H., Helve, E., & Koivisto, V. (1987). Hyperglycemia decreases glucose uptake in type 1 diabetes. *Diabetes*, *36*, 892–96

Yki-Järvinen, H., Mott, D., Young, a a, Stone, K., & Bogardus, C. (1987). Regulation of glycogen synthase and phosphorylase activities by glucose and insulin in human skeletal muscle. *The Journal of Clinical Investigation*, *80*(1), 95–100. doi:10.1172/JCI113069

Zhang, S., Zheng, C., Lanza, I. R., Nair, K. S., Raftery, D., & Vitek, O. (2009). Interdependence of signal processing and analysis of urine 1H NMR spectra for metabolomic profiling. *Analytical Chemistry*, *81*(15), 6080–88. doi:10.1021/ac900424c.Interdependence

Zinman, B., Zuniga-Guajardo, S., & Kelly, D. (1984). Comparison of the acute and long-term effects of exercise on glucose control in type I diabetes. *Diabetes Care*, *7*, 515–19

IX. ANNEXES

Spanish version

CUESTIONARIO INTERNACIONAL DE ACTIVIDAD FÍSICA

Estamos interesados en saber acerca de la clase de actividad física que la gente hace como parte de su vida diaria. Las preguntas se referirán acerca del tiempo que usted utilizó siendo físicamente activo(a) en los **últimos 7 días**. Por favor responda cada pregunta aún si usted no se considera una persona activa. Por favor piense en aquellas actividades que usted hace como parte del trabajo, en el jardín y en la casa, para ir de un sitio a otro, y en su tiempo libre de descanso, ejercicio o deporte.

Piense acerca de todas aquellas actividades **vigorosas** que usted realizó en los **últimos 7 días**. Actividades **vigorosas** son las que requieren un esfuerzo físico fuerte y le hacen respirar mucho más fuerte que lo normal. Piense *solamente* en esas actividades que usted hizo por lo menos 10 minutos continuos.

1. Durante los **últimos 7 días**, ¿Cuántos días realizó usted actividades físicas **vigorosas** como levantar objetos pesados, excavar, aeróbicos, o pedalear rápido en bicicleta?

   _____ días por semana

   ☐ Ninguna actividad física vigorosa → ***Pase a la pregunta 3***

2. ¿Cuánto tiempo en total usualmente le tomó realizar actividades físicas **vigorosas** en uno de esos días que las realizó?

   _____ horas por día

   _____ minutos por día

   ☐ No sabe/No está seguro(a)

Piense acerca de todas aquellas actividades **moderadas** que usted realizo en los **últimos 7 días.** Actividades **moderadas** son aquellas que requieren un esfuerzo físico moderado y le hace respirar algo más fuerte que lo normal. Piense *solamente* en esas actividades que usted hizo por lo menos 10 minutos continuos.

3. Durante los **últimos 7 días**, ¿Cuántos días hizo usted actividades físicas **moderadas** tal como cargar objetos livianos, pedalear en bicicleta a paso regular, o jugar dobles de tenis? No incluya caminatas.

   _____ días por semana

   ☐ Ninguna actividad física moderada → ***Pase a la pregunta 5***

4. Usualmente, ¿Cuánto tiempo dedica usted en uno de esos días haciendo actividades físicas **moderadas**?

_____ **horas por día**

_____ **minutos por día**

☐ No sabe/No está seguro(a)

Piense acerca del tiempo que usted dedicó a caminar en los **últimos 7 días**. Esto incluye trabajo en la casa, caminatas para ir de un sitio a otro, o cualquier otra caminata que usted hizo únicamente por recreación, deporte, ejercicio, o placer.

5. Durante los **últimos 7 días**, ¿Cuántos días caminó usted por al menos 10 minutos continuos?

_____ **días por semana**

☐ No caminó → ***Pase a la pregunta 7***

6. Usualmente, ¿Cuánto tiempo gastó usted en uno de esos días **caminando**?

_____ **horas por día**

_____ **minutos por día**

☐ No sabe/No está seguro(a)

La última pregunta se refiere al tiempo que usted permaneció **sentado(a)** en la semana en los **últimos 7 días**. Incluya el tiempo sentado(a) en el trabajo, la casa, estudiando, y en su tiempo libre. Esto puede incluir tiempo sentado(a) en un escritorio, visitando amigos(as), leyendo o permanecer sentado(a) o acostado(a) mirando televisión.

7. Durante los **últimos 7 días**, ¿Cuánto tiempo permaneció **sentado(a)** en un **día en la semana**?

_____ **horas por día**

_____ **minutos por día**

☐ No sabe/No está seguro(a)

- English version

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____days per week

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____days per week

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____**days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure