# The role of galectin-1 in type 2 diabetes

## Clinical and experimental studies

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Cover illustration: *Thinking of galectin-1 and diabetes* by Emanuel Fryk

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To my family

*It is better to be vaguely right than exactly wrong*

C. Read (1898)

# ABSTRACT

**Aim:** The purpose of this thesis was to identify a new agent in the subcutaneous adipose tissue and assess its clinical potential in the context of type 2 diabetes.

**Study I:** Through a combination of microdialysis and mass-spectrometry, we found increased galectin-1 levels in the subcutaneous adipose tissue in a small experimental study of 15 men with and without type 2 diabetes.

**Study II and Study III:** Serum galectin-1 was also independently associated with type 2 diabetes and body-mass index in of 989 individuals from the cross-sectional population based SCAPIS pilot study. Furthermore, high serum-levels of galectin-1 predicted an increased risk of incident type 2 diabetes in 4022 individuals from the longitudinal Malmö Diet and Cancer Study - Cardiovascular Cohort, after adjustment for known risk factors.

**Study IV:** In addition, serum levels of galectin-1 were associated with all major adipose tissue depots and presented a similar metabolic association profile as circulating galectin-3 in 502 individuals from the cross-sectional population-based POEM-study.

**Study V:** In a small experimental study of 25 individuals from the MD-Lipolysis study, fasting serum galectin-1 correlated with insulin, and the lipid metabolism markers glycerol and free fatty acids during an oral glucose tolerance test, and adipose tissue *LGALS1* expression correlated with markers of lipid metabolism. Modulation of galectin-1 activity in a cultured human preadipocyte cell-line indicated effects on triglyceride content, and genetic markers of lipid uptake, lipogenesis and glucose uptake during differentiation to mature adipocytes.

**Interpretation:** Galectin-1 is altered in the blood in type 2 diabetes, and may have a direct metabolic role in the adipose tissue and in type 2 diabetes development.

**Keywords**: galectin-1, type 2 diabetes, human, adipose tissue

# SAMMANFATTNING PÅ SVENSKA

Typ 2 diabetes är en progressiv sjukdom förknippad med ökad dödlighet och flera följdsjukdomar. Livsstil och fetma har en betydande roll i utvecklingen av typ 2 diabetes, eftersom fettväven har en central plats i kroppens energihantering. Det är idag känt att fettceller utsöndrar olika proteiner som ett svar på vad som sker i kroppen. Galektin-1 är ett protein som i djurförsök har visat på en möjlig betydelse för fettcellens energihantering. Kunskap om kopplingen mellan galektin-1 i fettet hos människa och typ 2 diabetes, samt dess funktionella aspekter i fettväven saknas dock. Syftet med det här projektet har varit att identifiera ett nytt protein i människa, utsöndrat i fettväven och med koppling till typ 2 diabetes, samt utforska eventuella funktionella aspekter.

Studie I jämförde därför proteinutsöndringsmönstret hos 7 män med nyupptäckt typ 2 diabetes och 8 män utan diabetes. Ett av de proteiner som upptäcktes var galektin-1. Proteinnivåerna av galektin-1 var förhöjda i extracellulärvätskan i fettet hos personer med typ 2 diabetes, och genuttrycket var också förhöjt i fettceller från samma område. Vidare kunde man i andra grupper se att genuttrycket påverkades vid förändringar i energiintag, och att stimulering med galektin-1 i fettceller minskade glukosupptaget hos cellerna.

Studie II undersökte vidare om galektin-1 i blodet kunde kopplas till fetma och typ 2 diabetes oberoende av varandra i en befolkningsstudie på 989 personer från SCAPIS-studien i Göteborg, vilket visade sig stämma. Vidare visade det sig att höga nivåer av galektin-1 i blodet kunde relateras till en ökad risk att insjukna i typ 2 diabetes många år senare i Studie III, där 4022 personer från Malmö Kost Cancer-studien undersöktes. I Studie III visade det sig också att galektin-1 förefaller skydda vissa personer med typ 2 diabetes från njurskador genom en Mendeliansk randomiseringsstudie på deltagare i ANDIS-kohorten i Skåne.

Studie IV undersökte kopplingarna mellan galektin-1, kroppsfett och ämnesomsättning i 502 personer från POEM-studien i Uppsala. Studien bekräftade ett nära samband mellan galektin-1 och fettväven, samt visade på kopplingar till blodsocker, insulinkänslighet och fettsyrametabolism. Där fanns också likheter mellan de metabola kopplingarna för galektin-1 och ett annat galectin-protein, galectin-3. I Studie V visade det sig också att genuttrycket för galektin-1 i fettväv korrelerade med genuttrycket för flera gener som styr fettvävens funktion, inklusive gener för fettupptag och fettnedbrytning hos 25 personer med och utan diabetes. Blodnivåerna av

galektin-1 kopplades också till omsättning av glycerol och fria fettsyror efter intag av en glukosbelastning. I laboratorieexperiment begränsades triglyceridinnehållet i fettceller som fått mogna fram med en blockerad galektin-1 signal, och genuttrycket för markörer för fettupptag, fettsyrasyntes och lipolys minskades.

Sammantaget kan man se att galektin-1 nivåerna avviker i blodet hos personer med med fetma och vid typ 2 diabetes. Galektin-1 är också kopplat till en ökad risk för typ 2 diabetes, och förefaller ha en direkt koppling till ämnesomsättningen i fettväven, både i experimentella studier och på befolkningsnivå.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Fryk E, Perman Sundelin J, Strindberg L, Pereira M J, Federici M, Marx N, Nyström F H, Schmelz M, Svensson P-A, Eriksson J W, Borén J, Jansson P-A. Microdialysis and proteomics of subcutaneous interstitial fluid reveals increased galectin-1 in type 2 diabetes patients.
Metabolism Clinical and Experimental 2016; 65: 998-1006

II. Fryk E, Strindberg L, Lundqvist A, Sandstedt M, Bergfeldt L, Mattsson Hultén L, Bergström G, Jansson P-A. Galectin-1 is inversely associated with type 2 diabetes independently of obesity - A SCAPIS pilot study.
Metabolism Open 2019; 4: 100017

III. Drake I & Fryk E, Strindberg L, Lundqvist A, Rosengren A H, Groop L, Ahlqvist E, Borén J, Orho-Melander M, Jansson P-A. The role of circulating galectin-1 in type 2 diabetes and chronic kidney disease: evidence from cross-sectional, longitudinal, and Mendelian randomisation analyses.
Diabetologia 2022; 65: 128–139

IV. Fryk E & Silva V, Strindberg L, Strand R, Fall T, Kullberg J, Lind L, Jansson P-A. Metabolic profiling of circulating galectin-1 and galectin-3 in a general population - A cross-sectional association study.
*Manuscript*

V. Silva V & Fryk E, Lembke-Ross K, Strindberg L, Bauzá Thorbrügge M, Zetterberg F, Wabitsch M, Mossberg K, Pereira M J, Wernstedt Asterholm I, Leffler H, Jansson P-A. Galectin-1 is a modulator of human adipose tissue function.
*Manuscript*

# CONTENT

# ABBREVIATIONS

| | |
|---|---|
| ADA | American Diabetes Association |
| ANDIS | All New Diabetics In Scania |
| BMI | Body mass index |
| CGL | Congenital generalized lipodystrophy |
| CHR | Carbohydrate-recognising domain |
| CKDgen | Chronic Kidney Disease Genetics |
| CRP | C-reactive protein |
| DIAGRAM | DIAbetes Genetics Replication And Meta-analysis |
| ELISA | Enzyme-linked immunosorbent assay |
| GLP-1 | Glucagon-like peptide-1 |
| HOMA | Homeostatic model assessment |
| LC-MS/MS | Liquid chromatography - tandem mass spectrometry |
| *LGALS1* | The gene encoding galectin-1 |
| MARD | Mild age-related diabetes |
| MOD | Mild obesity-related diabetes |
| OGTT | Oral glucose tolerance test |
| PEA | Proximity extension assay |
| PCR | Polymerase Chain Reaction |
| POEM | Prospective investigation of Obesity, Energy and Metabolism |
| PPAR-γ | Peroxisome proliferator-activated receptor gamma |

| | |
|---|---|
| qPCR | Quantitative PCR |
| RNAseq | RNA-sequencing |
| SAID | Severe autoimmune diabetes |
| SCAPIS | Swedish CArdioPulmonary bioImage Study |
| SGBS | Simpson-Golabi-Behmel syndrome |
| SGLT2 | Sodium-glucose transport protein 2 |
| SIDD | Severe insulin-deficient diabetes |
| SIRD | Severe insulin-resistant diabetes |
| siRNA | Small inhibitory RNA |
| SNP | Single-nucleotide polymorphism |
| TNF-α | Tumour necrosis factor alpha |

# 1 INTRODUCTION

This thesis will examine the possible role of galectin-1 in type 2 diabetes, and in the adipose tissue. Therefore, the following is a brief overview of some important aspects of type 2 diabetes, adipose tissue, and galectin-1.

## 1.1 DIABETES

Diabetes has likely been known to man since ancient times. The greatest scientific advances in the treatment of diabetes, including the importance of early diagnostics, a restricted caloric diet, and the important role of insulin for maintaining glucose control, were all discovered more than a century ago (1-3). Definitions have changed over the years, and the definition used today is adopted from the World Health Organization statement in 1998 (4). Additional criteria with HbA1c measurements (5), and randomly measured elevated glucose levels combined with typical symptoms of hyperglycaemia were later added (6).

### 1.1.1 THE CURRENT DEFINITION OF DIABETES ACCORDING TO THE AMERICAN DIABETES ASSOCIATION (ADA) (7):

- Fasting plasma glucose ≥ 7.0 mmol/l[1]
- 2-hour plasma glucose after a 75 g glucose load ≥ 11.1 mmol/l[1]
- HbA1c ≥ 48 mmol/mol[1]
- Plasma glucose ≥ 11.1 mmol/l and clinical symptoms of hyperglycemia

[1]Measured twice.

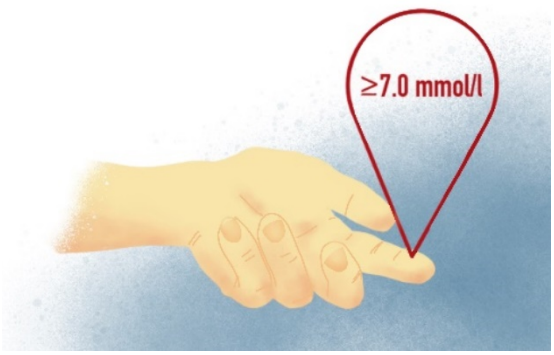



*Figure 1. Diabetes is defined as a state of recurring hyperglycemia*

### 1.1.2 SUBTYPES OF DIABETES

The heterogeneity of the diabetes disease was known before the discovery of insulin (2). Today, we often stratify diabetes into two main subtypes, type 1 and type 2 diabetes, where type 1 is characterized by an impaired insulin production, while type 2 is characterized by an impaired insulin response (8). Other less common subtypes are the maturity onset diabetes in young (MODY), and the latent autoimmune diabetes in adults (LADA) which affect younger individuals (9, 10). Both are associated with low insulin secretion, similar to type 1 diabetes, but with distinctly different underlying pathophysiology (9, 10). Recently, there have been attempts to stratify the diabetes disease into additional subtypes (11). This is important, as it can easily be argued that the underlying pathophysiology behind an individual first diagnosed with type 2 diabetes as an obese 40-year old, or a normal weight 80-year old individual is not necessarily the same. Separation into different subcategories with simple clinical measures could improve risk-stratification and help guide between treatment alternatives. This could improve clinical practice, as there are currently several treatment regimens supported by similar evidence.

These newer proposed subtypes are not yet accepted in clinical practice, nor defined by specific cut-offs applicable to the general clinician. However, they have been replicated in several studies (12, 13), and present with different risks of adverse outcomes (11, 13). In this new classification, the diabetes disease is divided into 5 subcategories, where 4 categories could be considered stratifications of the traditional insulin resistant type 2 diabetes, and the final represents individuals with autoimmune diabetes. The five categories are labelled severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild age-related diabetes (MARD) and severe autoimmune diabetes (SAID). While individuals with SIRD have a higher risk of diabetes kidney disease as well as liver fibrosis (11, 13), individuals with SAID present a higher risk of retinopathy (11). Hereon, this thesis will only focus on type 2 diabetes unless otherwise stated.

### 1.1.3 CONSEQUENCES OF DIABETES

Diabetes is a lethal condition, and elevated blood glucose can lead to premature death both in the acute phase and, if maintained, over time. The treatment of diabetes can also lead to other serious adverse events. Clinically, hypoglycaemia caused by too aggressive treatment can be a significant risk associated with premature death, especially in older individuals with cardiovascular comorbidities (14).

Diabetes is a systemic disease, affecting all organs in the body. Over time, manifest symptoms will occur throughout the body, with associated increased suffering, disabilities, costs and mortality. Ischaemic heart disease and cerebral stroke are the most, and third most common global causes of estimated years of life lost (15). This highlights the significance of reports demonstrating that individuals with diabetes are twice as likely to develop coronary heart disease, and 50% more likely to suffer an ischaemic stroke compared to individuals without diabetes (16).

Kidney disease is also a common consequence of type 2 diabetes, and will occur in at least half of all diabetes patients over time (17). Although the number of individuals progressing to end-stage renal disease has decreased, diabetes is still the leading cause for this outcome (18). Half of all individuals with diabetes will eventually also develop neuropathy (19), with consequences including a loss of sensory function in limbs, erectile dysfunction, gastroparesis and autonomic dysregulation. For some, neuropathy is manifest at the time of diabetes diagnosis, and progression is seen even in patients with good metabolic control (19).

Increased risks of peripheral artery disease, neuropathy and foot ulcers in diabetes together add up to a severely increased risk of lower limb amputation. It is estimated that half of all amputations in the United States are attributed to diabetes, with some studies even reporting numbers as high as 90% of all amputations (20). One third of all individuals with diabetes will also present with diabetic retinopathy. Although screening programs in many countries have improved early detection and intervention, diabetic retinopathy is still the leading cause of blindness in individuals of working-age (21). While these statistics include both type 1 and type 2 diabetes cases combined, the majority of all amputations and retinopathies occur in type 2 diabetes due to the higher prevalence of the disease (21, 22). Diabetes also increases the risk of several cancer forms, including liver cancers and pancreatic cancers (23), and presents close associations with Alzheimer's disease and other forms of dementia (24, 25).

The broad consequences of diabetes throughout the body, and the sometimes very early manifestations of complications highlight the importance of preventive action, routines for early detection, and active treatment of the disease. In light of these very serious outcomes, it is important to know that adequate treatment of diabetes will also mitigate the risk of complications significantly (26).

*"Statistics for the last thirty years show so great an increase in the number (of diabetes cases) that, unless this were in part explained by a better recognition of the disease, the outlook for the future would be startling."*

*-Elliott P. Joslin, 1921 (3)*

### 1.1.4 THE PHYSIOLOGICAL BACKGROUND TO TYPE 2 DIABETES

Type 2 diabetes is caused by a combination of genetic and environmental factors, where environment is the dominating factor for most (27). As previously mentioned, the importance of life-style in type 2 diabetes has been known for more than 100 years (2, 3). Several studies with remarkable results have reversed the condition through different approaches of caloric restriction, both through dietary interventions (28, 29), general life-style interventions (30) and obesity-surgery (31). While both life-style interventions and pharmacological interventions have been equally successful in reducing the incidence of type 2 diabetes, life-style changes were shown to be the most sustainable (32, 33).

One predominant model of disease currently advocated is that diabetes is the consequence of a sustained positive energy-balance, passing the threshold of the individual's maximum energy storage capacity (34, 35). Following this hypothesis, all individuals have a genetically predisposed maximum storage capacity of excess energy in their body. This can also be described as a maximum kilogram of body-fat mass for that person. During overfeeding, excess energy will be stored in the adipose tissue as triglycerides as a reserve for a later time point resulting in an increased bodyweight. If the positive energy-balance is maintained, the body will eventually reach its maximum capacity. In line with the first law of thermodynamics and the continuity equation, any additional energy introduced to the body of the individual must either be transformed into heat or momentum and leave the body, or be stored elsewhere. The storage of triglycerides in other organs than adipose tissue is termed ectopic fat deposition. Increased levels of lipids in the liver, pancreas, skeletal muscle, and blood is well-described (36-39), and is closely associated with both prevalent and incident type 2 diabetes. During type 2 diabetes development, an insufficient insulin secretion is also seen in the pancreas, as well as increases in endogenous glucose production in the liver (34, 40). Together, these changes result in increased blood glucose levels in the fed and fasted state.

Further supporting the hypothesis of type 2 diabetes as a consequence of a passed maximum energy storage, are studies in individuals with congenital generalized lipodystrophy (CGL) (41). Individuals with CGL have a severely impaired capacity to store triglycerides in the adipose tissue, and consequently present with a higher degree of ectopic fat deposition and deranged metabolic control (42, 43).

### 1.1.5 THE ENVIRONMENTAL BACKGROUND TO TYPE 2 DIABETES

Modifiable life-style factors are determinants for incident type 2 diabetes. Interventions with simple recommendations on diet and physical activity for individuals at high risk to develop type 2 diabetes have been successful at stopping the disease (30, 44). Several dietary components present independent risk factors for incident type 2 diabetes, including a low intake of dietary fibres (45), high intake of alcohol (46-48), saturated fats (49) and total energy (50). Perhaps not surprisingly, the single largest dietary risk factor has been shown to be intake of glucose itself (51). Over the last decades, there has been a significant increase in daily caloric intake globally, leading to what has been referred to as a pandemic of obesity and type 2 diabetes (52). A low level of physical activity is also a risk factor for type 2 diabetes, demonstrating that it is not only energy intake, but also energy expenditure which is important in the disease (53).

### 1.1.6 THE TYPE 2 DIABETES PATIENT

There are over 400 million individuals with diabetes in the world, and 90% of these have type 2 diabetes (54). A large proportion of people who meet the diagnostic criteria of type 2 diabetes remain undiagnosed (55). Commonly seen traits in individuals with type 2 diabetes are outlined by examining the characteristics of approximately 270 000 individuals participating in a study from the Swedish diabetes registry (56). This particular study included individuals with type 2 diabetes and no advanced diabetes complications, such as a medical history of leg amputations, cerebral stroke or acute myocardial infarction. The individuals presented with an average age around 60 years, a body mass index (BMI) of 30 $kg/m^2$, a balanced representation between men and women and a large proportion (around half of all participants) with concurrent medication for hypertension and statins (56). In addition, sex differences are well known in type 2 diabetes (57), prevalence varies globally (55) and between different age groups (15). However, a general image can sometimes be helpful for an overall understanding of a disease.

*"The individual overweight is at least twice, and at some ages forty times, as liable to the disease. For the prevention of more than half of the cases of diabetes in this country, no radical undernutrition is necessary…"*

*-Elliott P. Joslin, 1921 (3)*

*"The preparation of insulin finally removed the major objection to the concept of a pancreatic internal secretion and its important functional significance in carbohydrate turnover. Thus, the possible existence of a pancreatic diabetes could be affirmed in a positive sense. However, as is evident in other areas of the natural sciences, each new solution of a puzzle suggests more questions and presents more puzzles."*

*- Oscar Minkowski, 1929 (1)*

### 1.1.7 MEASURING INSULIN RESISTANCE

It is generally agreed that type 2 diabetes is preceded by a time of insulin-resistance (34, 58). However, while type 2 diabetes is a well-defined disease, this is not the case for what constitutes a state of insulin resistance (59). Insulin resistance can be discussed on population level (60, 61), individual level (62-65), organ level (66) and on a cellular level (67), unfortunately challenging the usefulness of the term.

Several methods have been proposed for the scientific measurement of insulin resistance in living humans, some more invasive than others (62-65). The euglycemic insulin clamp technique assesses insulin resistance through a continuous insulin infusion while controlling the systemic glucose levels via a dynamic infusion rate of glucose (62). Several composite measures based on clinical variables in fasting and during an oral glucose tolerance test (63-65) have also successfully been developed over the years. However, there is currently no consensus of which method, or what cut-offs to use within the field, and comparative studies regarding the predictive value of these methods for incident type 2 diabetes are not easily found.

Mechanistic studies propose manifestations of insulin resistance on the organ level (66). These manifestations occur in several organs and historically, the liver and muscle were among the first to be studied (68). The adipose tissue, gut, kidney and brain are other highly metabolic organs often studied in this context (34). These latter discoveries have been significant, as we now have disease modifying drugs specifically related to several of these organs, including PPAR-γ agonists, GLP-1 agonists, SGLT2-inhibitors and for selected individuals also leptin injections. On a cellular level, insulin resistance can occur at three levels, semantically separated into pre-receptor defects, receptor defects and post-receptor defects (67, 69).

Taken together, it is imperative to consider the heterogeneity of the term insulin resistance in any study of type 2 diabetes. While there is evidence of some form of insulin resistance in all these aspects in the evolution of type 2 diabetes, specific reports should be considered contextual, and may not always be generalizable.

## 1.2 ADIPOSE TISSUE PHYSIOLOGY

The adipose tissue is a central organ in the pathophysiology of type 2 diabetes. In obesity, and type 2 diabetes, changes in adipose tissue, function and histology are evident. Initially the size of adipocytes increases with obesity, although this increase is not infinite. Changes to other cells in the tissue are also clear, including a progressive fibrosis (70) and infiltration of macrophages, a phenomenon often referred to as a low-grade chronic inflammation (71).

The regulation of adipose tissue metabolism is complex as it depends on a multitude of factors. Adipose tissue blood flow (72), insulin levels (73), adrenergic effects (74), fasted or fed state (75), and substrate availability (76) are only a few of the key regulators in this process. Adipose tissue is the source of circulating free fatty acids, with the subcutaneous adipose tissue making the largest contribution (77). Adipocytes store excess energy in the form of triglycerides, and degrade these to release glycerol and free fatty acids through the process of lipolysis in the fasted state and after adrenergic stimulation (74). While obesity in itself does not appear to alter the levels of free fatty acids (78), the association between free fatty acids and metabolic dysregulation in the insulin resistant state is well described (79, 80). Elevated levels of free fatty acids in type 2 diabetes are considered to contribute to adverse effects through lipotoxicity (34), and directly influence the metabolism of the liver where the fatty acids are used as energy and substrate in hepatic triglyceride production (81, 82).

The significance of adipose tissue localization is also considered relevant in type 2 diabetes. It has been proposed that visceral adipose tissue is more detrimental to metabolic control, compared to subcutaneous adipose tissue (83, 84). As men have a proportionally higher amount of fat stored viscerally, this is suggested to contribute to the increased cardiovascular risk in middle-aged men compared to women (85). In studies with surgical interventions, removing visceral fat appears to improve metabolic control, compared to studies removing large amounts of subcutaneous fat without any metabolic alterations (86, 87). However, the strong collinearity between these depots and the larger absolute mass of subcutaneous tissue must be considered from a clinical perspective in this discussion.

Several adipose tissue genes important in type 2 diabetes have also been identified in genetic studies of individuals with familiar mutations. Genes related to insulin signalling (insulin receptor, *AKT2*), adipose tissue maturation

(*PPARG*), and lipid handling (*PLIN1*) are associated with adverse metabolic outcomes for carriers of the mutations (88-91).

*"It is not necessary to know all the answers concerning the forces of nature for them to become useful to human needs. It suffices to understand the laws by which the forces act to master them."*

*- Oscar Minkowski, 1929 (1)*

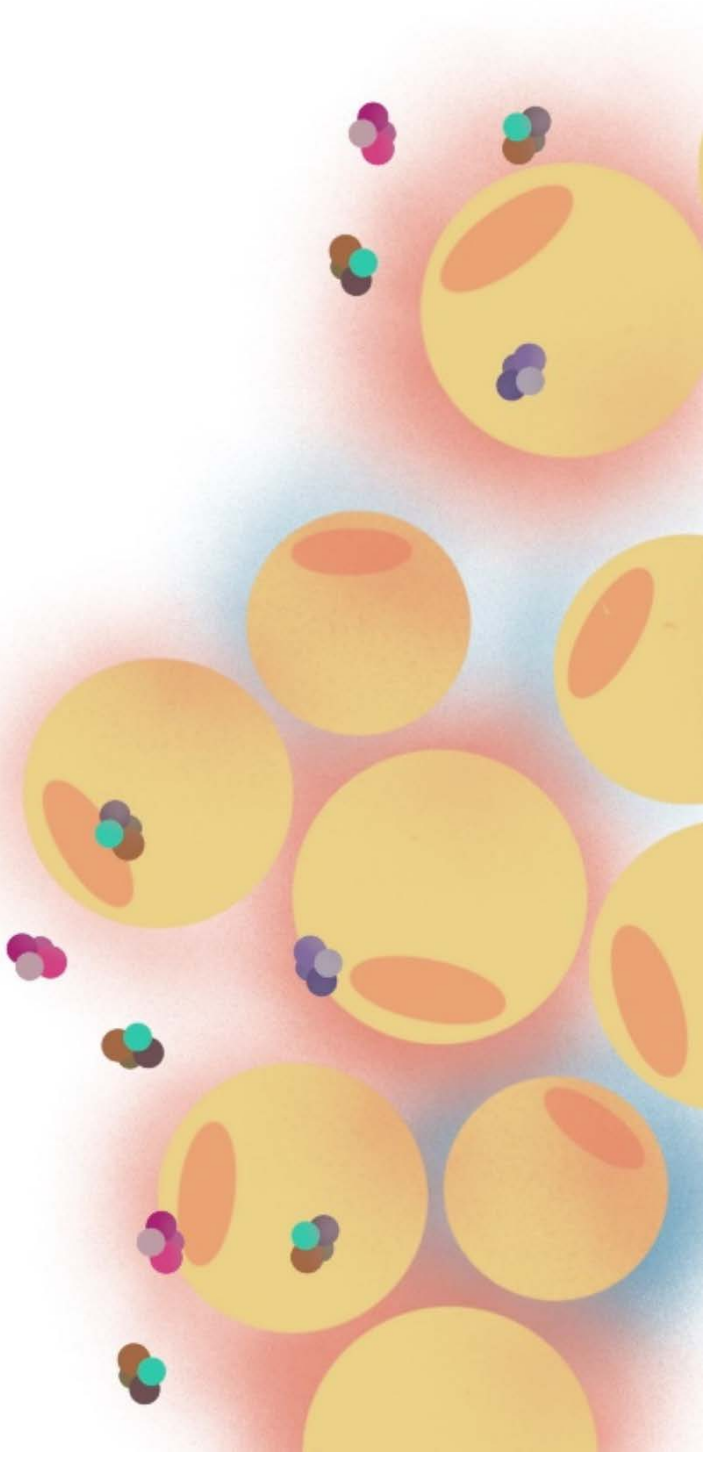
### 1.2.1 ADIPOKINES

The adipose tissue is currently often described as an endocrine organ, due to the active secretion of different factors to surrounding cells and into the circulation. These factors are generally described as adipokines, cytokines released from adipocytes (92). During the progression to manifest type 2 diabetes, the secretion of factors from the adipose tissue changes. One of the most important adipokines is leptin (93), a hormone regulating satiety and energy intake when the signal is intact. Individuals with obesity and type 2 diabetes have high leptin levels but do not experience the appropriate satiety response (94). This has led to a proposed model of leptin resistance in type 2 diabetes. The discovery of leptin has yet to have any direct impact on the majority of individuals with type 2 diabetes. However, it has shown drastic results in children with Berardinelli-Seip syndrome, a rare congenital lipoatrophy disorder with metabolic aberrations as well as in congenital leptin deficiency (42, 95). The most commonly used animal models for genetically induced obesity related diabetes, the ob/ob and db/db animals, are both obese due to a dysfunctional leptin signal (94).

Adiponectin is another adipokine often studied, which appears to be secreted in higher levels in adipocytes from lean and insulin sensitive individuals (96). The protein is specific for adipocytes, and therefore used biochemically as an adipocyte marker (97, 98). Although often related to obesity, insulin sensitivity and type 2 diabetes, the specific function, direct metabolic effect, and clinical relevance has yet to be demonstrated (99-101).

Tumour necrosis factor alpha (TNF-α) is a potent pro-inflammatory factor secreted from macrophages in the adipose tissue (102). It is therefore not appropriate to define it as an adipokine specifically. However, it is well established that adipose tissue containing hypertrophic adipocytes produces elevated levels of TNF-α (103, 104), and it is also believed that this release in itself has an important role in the development of type 2 diabetes (71). Today, TNF-α suppression is a well-established treatment in several conditions including rheumatoid arthritis and inflammatory bowel disease. Although smaller studies intervening on TNF-α in type 2 diabetes exist, the scientific evidence of any clinically relevant effects are still missing.

*Figure 2. Adipocytes secrete different factors depending on their size.*



# 1.3 GALECTIN-1

Another factor secreted from the adipose tissue is galectin-1. This protein is complex, from a biochemical standpoint, a cellular standpoint, a physiological standpoint, and as a natural consequence, from a clinical standpoint. A brief overview of these aspects will therefore be presented. It should be stressed that currently available data are highly fragmented, and based on studies with very specific aims, possibly influencing the bigger picture substantially.

## 1.3.1 FUNDAMENTALS OF GALECTINS WITH FOCUS ON GALECTIN-1

Lectins are defined biochemically as proteins with the ability to bind to carbohydrates. Galectins are a family of lectins, with similar structures and amino-acid sequences, that all are able to bind the carbohydrate galactose (105). In total, 15 galectins are currently known in human, and these are highly conserved between different mammals. Over the years, several galectins have carried different names, but these are now called galectin-1 to -15 in order of discovery (106).

Galectin-1 is a small protein of 135 amino-acids, secreted through an atypical pathway to the extracellular space (107). Galectin-1 is expressed in a variety of tissues, and the interest in adipose tissue galectin-1 has only emerged recently. Several studies have now demonstrated an altered regulation of galectin-1 on gene or protein level in the adipose tissue during a variety of metabolic states including PPAR-γ activation (108), dietary interventions (109), and in experimental animal models (110-113). Galectin-1 is reportedly altered in child-obesity (114), gestational diabetes (115) and in type 2 diabetes (116). Several studies in type 1 diabetes and diabetic retinopathy have also found a deviation in galectin-1 regulation, and some studies have explored a protective role in type 1 diabetes (117-121).

Although several studies on galectin-1 in adipose tissue function and metabolic regulation have emerged in recent years, the majority of the scientific literature has examined the role of galectin-1 in relation to cancer, inflammation, T-cell functionality and neovascularization (107, 122-125).

*Figure 3. Galectin-1 (white) dimerises at physiological concentrations and is constituted of beta-sheets, forming a concave surface with a carbohydrate-binding site (red) in the groove.*

### 1.3.2 GALECTIN-1 MOLECULAR STRUCTURE

Galectin-1 consists of a series of beta-sheets, together forming a flat and slightly concave surface, with the cavity constituting the carbohydrate binding domain which can hold up to a tetrasaccharide large molecule (106). In line with the highly conserved structure of galectins, and the common trait of galactoside binding, a large overlap in carbohydrate binding is seen between different galectins, each binding the disaccharide *N*-acetyllactosamine found on many cellular glycoproteins (107).

Galectin-1 can take three different forms in human physiology, as a monomer, a homo dimer, and an oxidised protein. Monomeric or dimeric formation is believed to be concentration dependent, and both have similar ligand binding capabilities (126). The oxidised form does not have carbohydrate binding ability, and is therefore not believed to have any functional role extracellularly (107). Galectins have both autocrine and paracrine functions (106), and it is believed that extracellular functions of galectin-1 largely depend on the carbohydrate-recognising domain (CHR), in contrast to its intracellular functions.

Galectin-1 gene knockout in mice results in viable animals, indicating that galectin-1 is not essential for survival (127). This is intuitively contradictive with the high degree of galectin-1 genetic structure conservation between mammals. This is otherwise typically seen in genes regulating essential physiological functions. These observations have therefore resulted in the hypothesis that galectins may interchange in physiological function, and that the knock-out of one galectin may provoke a counter-regulatory response in other galectins (128). This concept is further endorsed by the similarities in binding affinity between different galectins in their CHR-domain.

### 1.3.3 GALECTIN-1 SIGNALLING

Many studies over the years have proposed ligands or receptors for galectin-1. A common trait for several of these ligands is the common carbohydrate structures presented in the proteins. It has therefore been suggested that it is not a specific protein, but rather a carbohydrate sequence that is the ligand of galectin-1. Identified ligands include fibronectin, laminin, neuropilin-1, VEGFR2, and CD 146, although several others have also been proposed (107, 129-131). One of the most studied ligands is neuropilin-1, with several independent reports demonstrating a direct interaction between the two proteins (130, 132-134). A potential galectin-1 to neuropilin-1 interaction is particularly interesting, as neuropilin-1 has a functional role in the lipid uptake of endothelial cells (135). However, the wide variety of ligands for galectin-1

has raised questions regarding the way galectin-1 mediates its effects (105). It could be through a distinct signalling pathway or more convoluted protein-protein or protein-glycan interactions (106, 136).

To complicate things further, galectin-1 binds to glycolipids in addition to its capability to bind to glycosylated proteins. It is believed that galectin-1 secretion is mediated through the binding to glycolipids, allowing for a Golgi-independent secretion from the cell. This pathway would also guarantee a correct folding of the protein as it would be dependent on the carbohydrate recognizing domain to pass the cell wall (107). The binding of galectins on glycolipids is known to occur on the cellular surface, and is believed to have a functional role in protein sorting and structuring of lipid rafts (106). Taken together, there are currently several proposed molecular mechanisms through which galectin-1 can evoke an effect, both distinct protein ligand-signalling pathways and through cell-surface protein complex formations.

*"...the discovery of insulin demonstrates that research, even though not directly guided by purely practical aims, will sooner or later result in findings that become useful in medical practice."*

*- Oscar Minkowski, 1929 (1)*

### 1.3.4 THE CLINICAL POTENTIAL OF GALECTIN-1 INTERVENTION

Several recent reviews have pointed to a variety of medical conditions where galectin-1 is altered, including autoimmune disease, cancer, cardiovascular disease and metabolic disease (122, 137-139). Tumours expressing galectin-1 are associated with increased malignancy and mortality (123-125), and high levels of galectin-1 are found in the vitreous fluid of individuals with retinopathy, possibly associated with the VEGF-signal (120, 140).

High levels of galectin-1 have also been reported in the ischaemic lesions after a myocardial infarction in mice, in the brain of gerbils after an ischaemic stroke and associations have been reported in several cardiovascular conditions (137, 141-143). The wide variety of tissues and different medical conditions associated with galectin-1 raises questions around the potential specificity in targeting galectin-1 pharmaceutically.

### 1.3.5 CLINICAL INTERVENTIONS ON GALECTIN-1

Galectin-1 inhibitors have been in development for many years, and are currently used in *in vitro* research (144). Clinically, there are no available drugs for this purpose, although early clinical trials are ongoing. Furthermore, clinical trials have progressed to phase 2 and 3 for galectin-3 inhibitors in non-alcoholic steatohepatitis and pulmonary fibrosis (145). Several approaches to galectin-1 have been evaluated for the use in patients, including antibody therapy, peptide-based blockers, and most commonly, carbohydrate-based inhibitors of the CHR-domain (144, 145). Development of pharmacological interventions are complicated, and challenged by the impracticalities if administration cannot be administered orally, is not tolerated or has unfavourable pharmacokinetic properties (145). Agents interfering with the CHR-domain also need to be assessed for cross-reactivity for other galectins if they are designed to target a specific galectin. The highly conserved nature of the galectin family presents challenges in the development of inhibitors specific for a given galectin, and cross-reactivity with other galectins is often a matter of dose (128, 145). Furthermore, as it is not known if galectins can replace one another in physiological functions, it is not obvious whether specificity should be strived for. In line with this reasoning, some agents are only referred to as “galectin-inhibitors”.

*Figure 4. Galectin-1 (green, bottom right) is believed to play a role in several tissues, including the abdominal adipose tissue and the pancreas. It is also altered in medical conditions including diabetic retinopathy, during a myocardial infarction or a cerebral stroke, and in several cancer forms.*

# 2 AIM

The overall aim of this thesis project was to screen for a new protein relevant in type 2 diabetes using the adipose tissue secretome.

Specific aim of Study I: To identify new proteins in human subcutaneous adipose tissue possibly related to type 2 diabetes development, and perform an initial assessment on one candidate protein.

Specific aim of Study II: To validate the potential metabolic role of the newly identified protein galectin-1 in a large community-based sample of middle-aged individuals.

Specific aim of Study III: To evaluate the potential causal role of circulating galectin-1 on incident type 2 diabetes and related conditions in a large longitudinal community-based cohort. As a secondary objective, assessment of causality through a Mendelian randomization study would be examined on the primary outcome and secondary outcomes of significance.

Specific aim of Study IV: To compare the metabolic association profile of circulating galectin-1 with galectin-3, the most widely studied galectin.

Specific aim of Study V: To explore the functional role of galectin-1 in human subcutaneous adipose tissue *in vivo* and *in vitro* in a cross-sectional study of individuals undergoing an oral glucose tolerance test, and through the modulation of galectin-1 activity in a system of cultured preadipocytes during differentiation to adipocytes.

# 3 METHODOLOGICAL CONSIDERATIONS

## 3.1 STUDY DESIGN

All studies include compromises, and it is important to consider the study design when setting up a new study, or evaluating one already published.

Small experimental studies are highly dependent on the sample selection. Inclusion- and exclusion-criteria can be used to select a sample with less influence of confounding factors, and a smaller variability between individuals. This design increases the statistical power of the sample, allowing for statistical comparisons between different groups with a smaller number of study participants. Using heavily resource-demanding techniques that may be expensive or require the presence of a full team of study personnel for a full day can sometimes demand this sort of design. However, the price of the high selectivity at inclusion is a loss of representability of the study results. For this reason, smaller exploratory studies should later be validated in larger studies with a less strict selection process to mitigate this limitation and improve generalizability.

Larger observational studies have the benefit of large numbers of participants and a consequent increase in statistical power. This allows for the detection of smaller but statistically significant differences in a population. Furthermore, the higher statistical power also allows for the statistical adjustment of confounding factors in mathematical models. This is the background to the workflow implemented in this thesis. Study I was conducted on a strictly selected sample, and validated in Study II and Study III, population-based studies with large sample sizes.

## 3.2 CONFOUNDING AND CAUSALITY

The identification of new factors related to a disease process generally raises the question of confounding and causality. In Study I, the close correlation between galectin-1 and BMI, and the difference in BMI between the two study groups immediately raised the question of a potential confounding effect of BMI on our observation. Statistical adjustment of confounding variables can be performed in several ways. In Study I, adjustment for BMI was performed through ANCOVA, and suggested a difference in interstitial galectin-1 levels independent of BMI. The following epidemiological studies, Study II, III and IV also adjusted for BMI in all linear models to assess the BMI-independent influence of other variables.

It should be noted that adjustments for confounding variables comes at a price. A loss in statistical power of the given analysis is a natural consequence as the degrees of freedom decrease for each additional variable in a regression model. For this reason, general rules of thumb have been proposed regarding how many confounding factors are appropriate to include, based on a given sample size (146, 147). Furthermore, if causality is unknown, relevant associations may disappear when adjusting for variables down-stream the exposure of interest. Directed acyclic graphs can provide support when constructing statistical models of exposures and outcomes. However, information regarding the causal relationship between variables included in the model is central for this process.

Challenges can occur when discussing causality as it not in itself absolute. Mendelian randomization analysis can demonstrate a causal direction between an exposure and an outcome, but this does not eliminate the existence of other confounding factors in additional analysis. This is illustrated in Study III, where both a Mendelian randomization analysis and Cox-regression models are utilised to examine the association between galectin-1 and type 2 diabetes.

*Figure 5. Single-nucleotide polymorphism (SNPs) can be used in genome-wide association studies to identify a genetic predictor of circulating protein levels. This information can then be used in Mendelian randomization analysis to examine the causal relationship between the protein and an outcome, based on the assumption that SNPs occur randomly in large populations.*

## 3.3 MENDELIAN RANDOMIZATION STUDIES

The availability of large data sets of individuals with whole genome sequencing data, as well as a clear characterization of key clinical outcomes has opened the door for genetically based studies on causality for different outcomes. The analysis is built on three assumptions, and for readability, these will be discussed in the context of circulating galectin-1 set as the exposure, and type 2 diabetes as the outcome (148). The first assumption is that the single-nucleotide polymorphism (SNP) included in the analysis to represent galectin-1 levels is indeed associated with circulating galectin-1 levels. The second assumption is that the SNP does not share a common cause with type 2 diabetes. That is, the cause behind the mutation in the SNP is not also the cause of type 2 diabetes. Finally, the third assumption is that the SNP does not increase the risk of type 2 diabetes in any other way than through galectin-1.

An inherent limitation in the analysis is the statistical power. A low frequency of the genetic variant, a small effect size or a limited sample can all undermine the reliability of the final results of the analysis (149). We examine the causal role of galectin-1 in Study III with two outcomes, type 2 diabetes and chronic kidney disease. This was possible to do thanks to large consortium datasets available to the scientific community, with hundreds of thousands of individuals contributing with their genetic data and information on medical outcome in the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) (150), and Chronic Kidney Disease Genetics (CKDgen) (151) collaborations.

## 3.4 CHALLENGES IN DEFINING DIABETES

The clarity of the ADA-definition of diabetes makes it very useful in clinical practice. However, in studies of type 2 diabetes it is not always sufficient for several reasons. Firstly, as the clinical manifestations of the disease change over time, with loss of endogenous insulin production and the development of other systemic comorbidities, studies may require a specification of disease progression in order to be useful. Secondly, the definition is not sufficient to stratify between different subtypes, including type 1 diabetes, LADA, and MODY. As additional stratifications of diabetes have now been proposed, yet additional considerations may be necessary.

Furthermore, the specific values specified for glucose and HbA1c levels to define diabetes could be considered arbitrary on a continuous scale. Individuals with very similar metabolic phenotype may be classified differently depending on the first decimal value of the plasma glucose concentration the days the

individuals were in contact with their physician. Consequently, similarities between phenotypes will make it difficult to identify differences between individuals with and without the disease.

Cohort studies are also highly dependent on the data available to them for classification of presence or absence of a diagnosis. These studies have a high throughput of participants and several types of bias can influence the disease classification for the individual participant. Participants may not be aware of a diagnosis or may not consider it relevant for the study to know. Mistakes on reporting a specific condition to the clinical research form can occur. As an example, participants can arrive to the study without being fasted, resulting in high plasma glucose levels, which are physiological. Cross-validation of medical diagnoses can be conducted through comparisons of the patient's stated medical history, with information on patient medication and data on prescriptions and diagnoses from national registries.

In these studies, different definitions have been used to define type 2 diabetes. Study I and Study V only include newly diagnosed individuals with type 2 diabetes, although with differences in heredity for diabetes and BMI. Study II pooled all type 2 diabetes cases together, although some were diagnosed upon inclusion and other had lived with the disease for many years. In Study III, registries were also used, and it was assumed that individuals developing diabetes without specification all had type 2 diabetes due to the age upon inclusion to the study.

## 3.5 MICRODIALYSIS

The possibility to continuously monitor physiological processes directly in a tissue, with the opportunity to perform simultaneous interventions, makes the microdialysis technique such an interesting technique. Technically, the method is performed by suturing a thin catheter through the skin and the tissue of interest. The catheter is constructed from a semi-permeable dialysis membrane, where the pore-size determines the size of the molecules allowed to equilibrate between the lumen and the surrounding tissue. The catheter is then perfused at a very slow rate, in the magnitude of microliters per minute. The solution used for perfusion of the catheters, the perfusate, can be adapted in accordance with what molecules should be sampled, and to fit any down-stream analysis (80). Osmolarity, pH, and the presence of necessary binding proteins are central considerations in the final composition of the perfusate. The perfusate can also be spiked with compounds used as external references in sample analysis, or drugs interfering locally with the tissue (152). Endogenous metabolites can

also be used as internal references to adjust for differences in recovery rate between catheters (153).

Microdialysis was used in Study I to identify galectin-1 and other candidate proteins (154). In Study V, interstitial glycerol levels were measured to estimate the adipocyte glycerol release in vivo (80). Interstitial protein sampling by microdialysis is in principle a straightforward technique through the perfusion of inserted semi-permeable catheters. However, differences in chemical composition between the perfusing solution and the interstitial fluid can potentially affect the mass-transport through the membrane wall. Some proteins bind to carrier proteins in the interstitial fluid, and may therefore not be correctly identified in downstream analysis. Other proteins may bind to the catheter wall resulting in lower levels in the dialysate fluid leaving the catheter. In order to get a higher sensitivity for less abundant proteins in Study I, we filtered out 14 highly abundant plasma proteins from the dialysate fluid. This procedure could potentially result in losses of proteins bound to the removed proteins, as well as other proteins binding unspecifically to the filter cartridge.

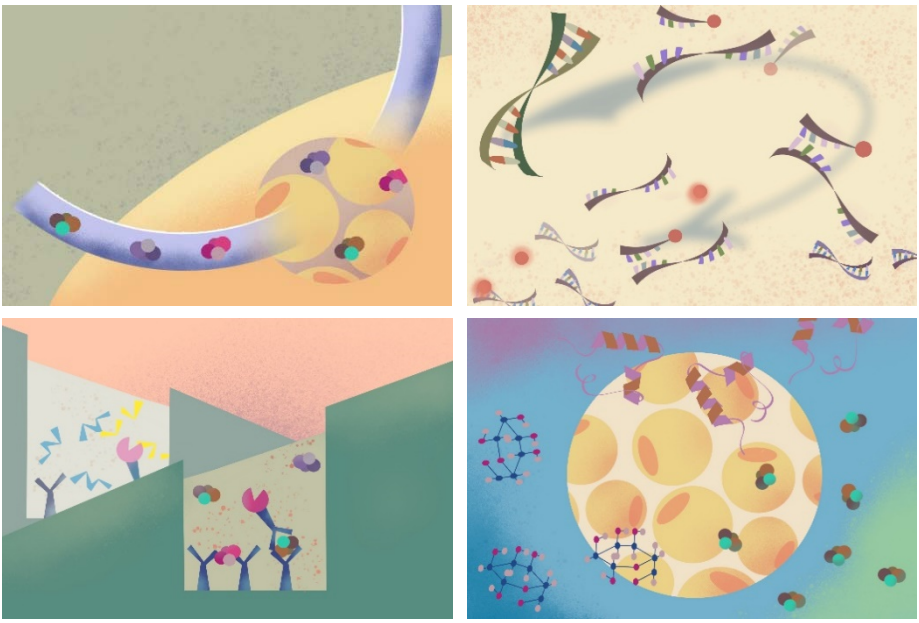


*Figure 6. Infographical summaries of some biochemical procedures used in the project. Microdialysis is a method enabling in vivo sampling of interstitial fluid from the subcutaneous adipose tissue (top left). Quantitative PCR is a method for precise gene-expression quantification of a given gene in a genetic sample (top right). Enzyme-linked immunosorbent assay (ELISA) is a precise method of absolute quantification of a given protein in a fluid-based sample using enzyme-linked antibodies (bottom left). In vitro culture of cells allows for specific modulation of the extracellular environmental factors including glucose, insulin concentrations and other specific compounds.*

# 3.6 MEASUREMENTS

The quantitative focus of this thesis rests heavily on the absolute measurements of all variables within the studies. It is therefore important to highlight aspects of the science of measuring. As previously discussed, the definition of type 2 diabetes is clearly defined by distinct values of glucose or HbA1c. However, the utilization of the glucose criteria requires the quantification of how many glucose molecules exist in a given volume of human plasma. Quantifying glucose can be done through several methods and based on different principles including electrochemical, photometric, colourimetric and biosensoring (155). However all measurements are associated with bias, and will present differently regarding precision, sensitivity, specificity, repeatability and reproducibility, which must be considered (156).

Measurement uncertainty can also be separated in three categories, preanalytical-, analytical-, and postanalytical uncertainty (156). While it is important to strive for a minimal influence of uncertainty through all steps of any study, it is also important to acknowledge their existence. This opens up to the possibility of quantifying the magnitude of uncertainty in order to assess the suitability of the method, and to verify the overall quality of the analysis (157). These uncertainties have had a significant influence in diabetes research, as glucose presents great variability both on a daily and hourly basis (158). Furthermore, HbA1c has also presented challenges throughout modern history, and is currently reported in two different units in scientific publications (159). Standardization and optimization procedures in accredited hospital laboratories are important and cumbersome, as the measurements reported often directly influence the care and treatment of a specific individual (160). However, the time, resources and technical qualifications required to meet this standard may not always be realistic in an experimental laboratory environment. Therefore, it is important to always keep measurement uncertainties in mind when chemical analyses have been conducted *in house*.

## 3.6.1 SINGLE VARIABLE MEASUREMENTS

Several biochemical methods have been used in this project; routine analysis of established clinical markers including fasting plasma glucose and HbA1c at the hospital laboratory, experimental measurements of proteins including galectin-1 and gene expression analysis of specific genes, and unconditional omics' analysis. Experimental procedures such as microdialysis, xenon-based adipose tissue blood flow measurement, as well as functional studies of glucose uptake have also been conducted. The theoretical background behind each and all of these techniques is so comprehensive that it will not be

discussed in detail in this thesis. Instead, only key methodological considerations are briefly discussed.

In Study I, galectin-1 was identified through Liquid chromatography - tandem mass spectrometry (LC-MS/MS). As the sample-size was small, and no adjustment for multiplicity was done in the statistical analysis of the proteomic data, we validated our observations through a reanalysis of the dialysate fluid with enzyme-linked immunosorbent assay (ELISA), and quantified the galectin-1 gene expression, *LGALS1*, in isolated adipocytes from the same individuals. As these observations aligned, the risk of a false positive analysis was minimized.

All fluid-based analyses of galectin-1 were performed with the same brand of ELISA (Study I, II, III, IV and V). For each study, all assays were also ordered from the same manufacturing batch to minimize variability. The absolute levels of galectin-1 were also compared between the studies to ascertain assay consistency. The assay has also been evaluated with recombinant galectin-1 from independent sources to ascertain that it is indeed galectin-1 that is measured. In line with these procedures, *LGALS1* expression has also been measured through independent measurements, with quantitative PCR (qPCR), and RNA-sequencing (RNAseq) presenting similar results in Study V.

### 3.6.2 MULTI-VARIABLE MEASUREMENTS, OMICS DATA

The unbiased study of any scientific problem can lead to new insights outside the current paradigm. A comprehensive assessment of a set of molecules, such as proteins or metabolites is one way to identify new markers in a given disease. The methods utilised for such assessment are often summarized with the suffix -omics, further specified by the type of molecules analysed such as genomics, proteomics and metabolomics (161). Combining several omics techniques can allow for a comprehensive characterization, on several layers of cellular activity (162). However, the large number of different outcomes analysed, in combination with large variability within individuals and between individuals, introduces a large risk of false discoveries (161). Furthermore, the discrepancy between different omic modalities is also notorious (108). Taken together, the utilization of omics can provide new insights, and allow for a detailed characterization of cellular processes. However, should also be interpreted with due caution.

In this project, we use several different omics techniques, to identify galectin-1, and to gain more knowledge about the protein itself. Proteomics through LC-MS/MS was used in Study I to identify galectin-1 as a potential adipokine related to type 2 diabetes (154, 163). In Study III and Study IV, galectin-1 and

galectin-3 were quantified through proteomics, using an antibody based method in the All New Diabetics In Scania (ANDIS) and Prospective investigation of Obesity, Energy and Metabolism (POEM) cohorts respectively (164). Study III also employs whole genome sequencing in order to allow for a genome wide association study of circulating galectin-1 (165). Study IV also utilised imiomic data (166) and metabolomic data (167) to examine the association profile of galectin levels to imaging data and metabolomics data. In addition, genomics data acquired through RNAseq was used in Study V to examine the correlations between *LGALS1* expression and the gene expression of genes related to adipose tissue function.

## 3.7 STATISTICS

Due to the wide scope of the different study designs included in this thesis, the statistical methods will be discussed on a principal level. It is well established that all statistical methods have their own inherent limitations (168), and that the decision of selecting a given method before another can bias the outcome to a false-positive or false-negative result (169), often referred to a type I or a type II error. Discussion of the risk for false positive associations, especially in studies with many parallel outcomes have resulted in statistical adjustments for multiplicity (170, 171), as well as proposals of using *p*-value thresholds lower than the commonly used $p<0.05$ (172).

In general, there are specific assumptions behind each statistical method that should be considered before implementing them into a project plan. The sample selection, variable distribution and, inherent variability of any measurement are some things to consider (173). However, although often used as criteria, they are not absolute, and there are often several independent methods used within the field to test if these assumptions are fulfilled. As an example, several different methods can be used to test whether a variable has a normal distribution (173). Furthermore, what truly constitutes a random sample can be discussed, and if it is reasonable to only conduct scientific studies on samples meeting these assumptions perfectly. It can be argued that the strict mathematical assumptions required for the correct utilization of a given statistical test may not always be possible, leaving the scientist with the alternatives of no statistics or bad statistics (174). In line with such reasoning, it can be argued that a clear result may not even need any p-value to stand on its own (78, 175), or that the interpretation of the results is more important than the statistics themselves (176). Replication is another method available to complement the inherent weaknesses of both statistical and experimental methods utilised. Through repeated observations of the same phenomena in

different experimental settings, and through different statistical and biochemical procedures, a true causal relationship can be assumed with more certainty (177).

In this project, we used simple and robust statistical methods without any adjustments for multiplicity in Study I in order to maximize the probability of identifying any candidate protein. In order to validate our identified target, we performed a validating cross-sectional study with a larger sample size while adjusting for confounding factors in Study II. Furthermore, we proceeded with a second cross-sectional validation in an independent cohort through our baseline data in Study III, all with similar results and further adjustments for additional confounding factors. Measurements of galectin-1 were also performed through independent methods in Study I (LC-MS/MS, ELISA and qPCR), Study II (ELISA) and Study III (ELISA and proximity extension assay/PEA). We also examined the association between galectin-1 not only with the outcomes of body mass index and type 2 diabetes, but with functional outcomes related to metabolism and adipose tissue function measured through a wide range of different methods in Study I, Study IV and Study V.

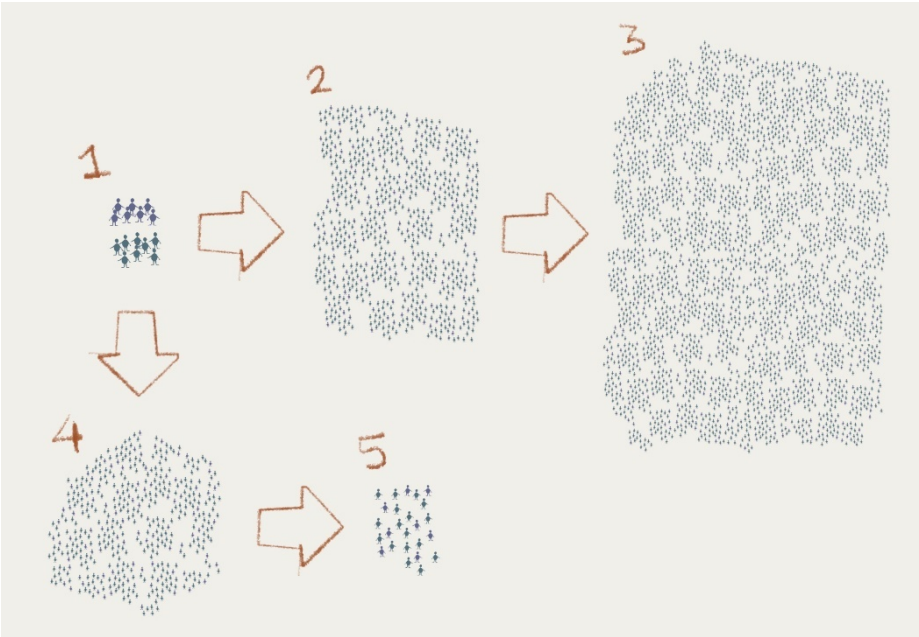



*Figure 7. Galectin-1 was identified as a candidate protein related to adipose tissue and type 2 diabetes in Study I. In Study II and Study III, the association with type 2 diabetes was validated in two independent cohorts, one cross-sectional and one longitudinal. The functional associations of galectin-1 as an agent involved in adipose tissue metabolism was further explored in Study IV and Study V.*

## 3.8 ETHICAL ASPECTS

All studies have been approved by the regional ethical committees, and all participants have given written and informed consent to the participation in the studies (ethical approval numbers: 2009-035, 2016-1009, 2017-T-109, 2018-T-163, LU 51-90, LU 204-00, 469/2006, 2009/633, 2006-584, 2009-359, 2011-367, 2012-676, 2014-198, 2009-057, 844-12, T844-13, T474-15, T967-15 and 2019-04911). Studies have also been conducted in accordance with the principles outlined in the Declaration of Helsinki (178). All studies in this thesis have been conducted in humans, often invasive, and participants have had very little to gain for themselves when participating. Therefore, the outmost respect must be warranted to anyone who contributes in a scientific study. Through collaborative efforts, much knowledge has been acquired surrounding the research questions without unnecessary additional experiments in the participants. Instead, existing studies with overlapping interests have all contributed with unique data, together forming a comprehensive and more robust final image, while limiting the discomfort of volunteering participants.

*"Medical progress is based on research that ultimately must include studies involving human subjects."*

*-World Medical Association, 2013(178)*

# 4 RESULTS AND DISCUSSION

## 4.1 STUDY I

It is well established that both obesity as a condition, and the adipose tissue as an organ, have central roles in the development of type 2 diabetes. Several biochemical processes and signalling pathways discovered in the adipose tissue already have an established central role in clinical treatment, including PPAR-γ agonists in type 2 diabetes, and leptin in congenital leptin deficiency syndrome (43, 179). This knowledge led to the hypothesis that there were still undiscovered proteins in the human adipose tissue, central for the type 2 diabetes disease.

*Figure 8. Galectin-1 was measured in serum, adipose tissue interstitial fluid and on gene level in 8 lean individuals without, and 7 individuals with type 2 diabetes in the exploratory Secretome-study.*

### 4.1.1 RESULTS IN STUDY I

Combining microdialysis, a minimally invasive technique used for interstitial-fluid protein sampling, with proteomics provided a possibility to characterize the human subcutaneous adipose tissue secretome *in vivo*. The technique was developed and used to compare the secretome in 8 lean individuals without type 2 diabetes, and 7 individuals with newly diagnosed type 2 diabetes, and a family history of type 2 diabetes. We identified 36 proteins with different protein expression-levels by comparing the secretome of individuals with and without a type 2 diabetes diagnosis. One of the secreted proteins upregulated in individuals with type 2 diabetes was galectin-1, which we characterized further. Validation analysis of galectin-1 in subcutaneous interstitial fluid verified increased levels of galectin-1 in the adipose tissue, and gene expression analysis further indicated increased mRNA levels of *LGALS1* in isolated adipocytes from the same individuals. There was also a significant correlation between dialysate galectin-1, *LGALS1* expression, and HbA1c. While there was no difference in circulating levels of galectin-1 between the two groups, there were significant correlations with waist-hip ratio and adipocyte cell size. Furthermore, *LGALS1* expression increased in the subcutaneous adipose tissue of individuals subjected to high caloric intake, and was reduced in individuals with a reduced caloric intake. Treatment of

adipocytes with recombinant galectin-1 also reduced glucose uptake independently of insulin stimulation.

### 4.1.2 DISCUSSION OF STUDY I

The combination of microdialysis and proteomics in subcutaneous adipose tissue had not previously been conducted. However, the overall results demonstrate that the method is feasible and several candidate proteins were identified. This study focused on galectin-1 as a protein with higher adipose tissue protein expression in individuals with type 2 diabetes, and validation experiments in the same population were consistent with the initial analysis. Dietary experiments also indicated that galectin-1, at least in part was affected by caloric intake, and experiments on primary adipocytes suggested a functional role on glucose uptake.

Together the results strengthen each other, as results from independent experiments all fit with the established pathophysiological model behind type 2 diabetes. While the correlations between galectin-1, HbA1c and adipocyte size were facilitated by the absolute differences in all these variables by selection of the two groups, the nonparametric Spearman correlation analysis was chosen to mitigate this bias (168). Furthermore, the observations on changes of adipose tissue *LGALS1* expression were consistent with the knowledge that high-calorie diet is a risk factor of type 2 diabetes (50) and observed in two independent populations (180, 181).

However, it should be considered that the small sample included in the study, and the lack of statistical adjustment for multiplicity increased the risk of identifying proteins which were not relevant to the general population of individuals with type 2 diabetes. Individuals in the study were all white, and aged between 40 and 65 years old. Furthermore, only men were included in this study. These limitations were deliberate, in order to reduce within-sample variability, but with consequent compromise to the generalizability of the results. As an example, it is well known that type 2 diabetes has significantly different trajectories and implications depending on ethnicity and sex (57, 182).

In conclusion, the proteomic analysis of subcutaneous adipose tissue dialysate fluid identified galectin-1 as a candidate protein altered in type 2 diabetes, with a possible dietary regulation and effects on adipocyte glucose uptake.

## 4.2 STUDY II

To validate our previous observation, proposing galectin-1 as a protein from the adipose tisse and altered in type 2 diabetes, we analysed the serum levels of galectin-1 in 989 participants aged 50-65 years, from the community based Swedish CArdioPulmonary bioImage Study (SCAPIS) pilot.

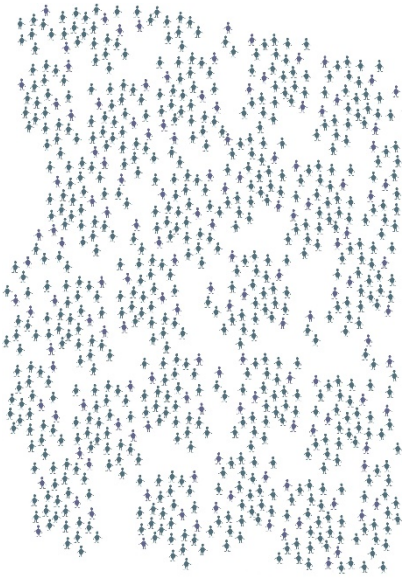


*Figure 9. Galectin-1 was measured in 989 individuals in the cross-sectional SCAPIS-pilot study.*

### 4.2.1 RESULTS IN STUDY II

We found that galectin-1 correlated to several markers of metabolic disease, with the strongest correlation to BMI, C-reactive protein (CRP), fasting insulin, the insulin resistance estimate Homeostatic model assessment-index (HOMA) (64), and TNF-α.

In linear models, we found that galectin-1 was inversely associated with type 2 diabetes, and also associated with BMI, age and sex independently. In a second model, type 2 diabetes was replaced with fasting glucose, fasting insulin and CRP. Interestingly, galectin-1 was independently associated with fasting insulin and CRP, and presented an inverse association with glucose.

### 4.2.2 DISCUSSION OF STUDY II

This study validates our observations of galectin-1 as a protein associated with type 2 diabetes, proposed in Study I. However, after adjustments for the strong association with BMI, the association between galectin-1 and type 2 diabetes was inverse. This observation was unexpected, while aligning with the inverse association with glucose observed in model 2.

The strong association with BMI was consistent between men and women, although slightly higher levels of galectin-1 were observed in women. This could be explained by differences in adipose tissue distribution, and is an interesting observation for several reasons. Firstly, galectin-1 was identified in a study consisting only of men, and this was the first evaluation in women. Although the sex difference was statistically significant in the linear models, the absolute values of galectin-1 for men and women were of similar

magnitude for both lean and obese. Nonetheless, as it is well known that type 2 diabetes presents different risks of complications between men and women, a potential factor mediating these differences could be of scientific relevance.

Correlations between galectin-1 and BMI, insulin, triglycerides and the inflammatory markers CRP and TNF-α together indicate that galectin-1 is not only a marker of obesity, but associated with physiological manifestations seen in pathologic obesity. Insulin and triglycerides are often high in early phases of diabetes and in states of insulin resistance, and low-grade inflammation, specifically measured as TNF-α is a well-known trait of metabolically unhealthy obesity. Together, these observations suggest a functional role of galectin-1 in the progression from obesity to type 2 diabetes. However, the cross-sectional design of the study does not allow for any conclusions regarding causality.

In conclusion, galectin-1 correlates with BMI and is independently associated with type 2 diabetes, confirming results from our previous study with some caveat as this association was inverse in serum after adjustment for BMI. Furthermore, correlations with other metabolic markers point to a possible functional role of galectin-1 in obesity and type 2 diabetes.

## 4.3 STUDY III

After the discovery that galectin-1 was altered in the circulation in type 2 diabetes, also after adjustment for BMI, the question remained if galectin-1 could have a predictive or causal role in the development of the disease. For this reason, we analysed serum galectin-1 in the circulation of 4022 individuals from the cardiovascular cohort in the Malmö Diet and Cancer Study.

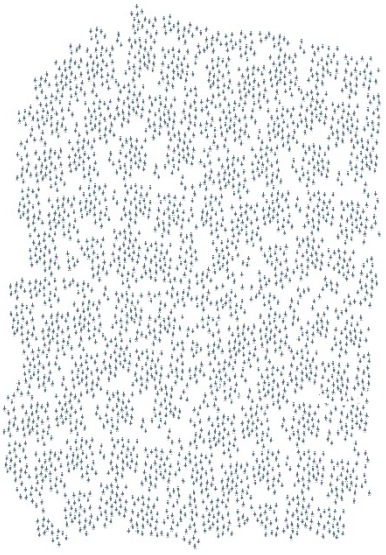


*Figure 10. Galectin-1 was measured in 4022 individuals in the longitudinal Malmö Diet and Cancer Study.*

### 4.3.1 RESULTS IN STUDY III

In addition to our previously observed strong associations with BMI, BMI-adjusted positive associations with insulin and CRP, and inverse association with fasting glucose, we also discovered an even stronger association with estimated glomerular filtration rate (eGFR). Moreover, high serum galectin-1 was associated with incident type 2 diabetes in a Cox proportional hazards regression model adjusted for other established risk factors. In a secondary analysis, the influence of galectin-1 on other diabetes related diseases was examined, but did not reveal any statistically significant results. While there was an association between baseline galectin-1 and incident CKD in models adjusted for established risk factors, it did not persist after adjustment for baseline eGFR. Furthermore, information on galectin-1 levels did not improve model discrimination of type 2 diabetes or CKD in any significant way.

The influence of galectin-1 on incident type 2 diabetes and CKD studied through Mendelian randomization analysis did not provide evidence of any causal effect. However, the inclusion of several SNPs in the analysis decreased the *p*-value to $p$=0.08, and $p$=0.05 for the outcomes type 2 diabetes and CKD respectively, while presenting similar effect sizes. It has been shown that type 2 diabetes can be further stratified into 4 subgroups, with different inherent risks of complication between these groups. Therefore, the causal effects of genetically predicted high galectin-1 on eGFR was assessed separately in these 4 groups, in a stratified Mendelian randomization analysis. While there was no evidence of any causal effects of galectin-1 on eGFR levels in the large sample of individuals from CKDgen or in individuals with type 2 diabetes in general,

galectin-1 suggested a kidney protective effect in participants presenting with a severely insulin resistant diabetes (SIRD).

### 4.3.2 DISCUSSION OF STUDY III

Our observation that increased galectin-1 was associated with incident type 2 diabetes was an important piece of information regarding the relevance of galectin-1 in the pathophysiology of type 2 diabetes. The longitudinal design of the study also allowed for an assessment of galectin-1 as a biomarker of disease. However, there was no apparent value of galectin-1 in addition to established biomarkers. The observed associations with BMI, insulin, triglycerides and the inverse association with glucose further support our previous observations from Study II in an independent cohort.

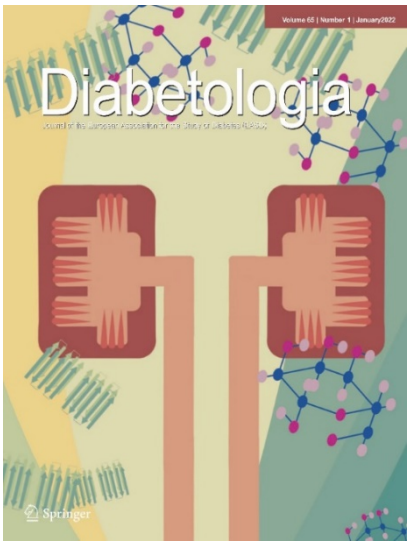



*Figure 11. The cover of the 2022 January issue of Diabetologia featured an illustration of Study III, with galectin-1 in green, glucose in magenta and two kidneys in the center.*

The Mendelian randomization study did not provide ample evidence of any causal effect of galectin-1 on diabetes. However, it should be noted that the absolute *p*-value was low, which should raise caution to the interpretation of the results. While no causality can be concluded from this analysis, it is probably not wise to completely exclude it based on this study alone. There have previously been examples of conflicting Mendelian randomization studies, which together highlight the importance of cautious interpretations of individual studies (99, 100, 183). The observations of a predictive role of serum galectin-1 on type 2 diabetes incidence, together with the lack of evidence for a casual effect, could also suggest that galectin-1 is a mediator of environmental factors. This proposal is also in line with the diet induced changes in *LGALS1* expression in the subcutaneous adipose tissue observed in Study I.

The close association between galectin-1 and markers of kidney function, was not expected, but several reports have previously demonstrated associations between galectin-1 and kidney disease in different settings (139, 184-187). While galectin-1 levels did not associate with incident CKD after adjustment for baseline eGFR, arguments can be made both for and against such

adjustment. As CKD is defined by eGFR, adjustment for eGFR may introduce bias to the outcome. Furthermore, while the Mendelian randomization analysis did not demonstrate a causal association between galectin-1 and CKD, the opposite would have been true if we had not conducted Bonferroni adjustments for multiplicity. As the Mendelian randomization was performed to confirm observations from the longitudinal analysis, a conservative approach was determined to be most appropriate. However, others have argued that adjustment for multiplicity in hypothesis-driven studies may be counterproductive (170). Taken together the contradictory results from these statistical analyses, resulting in outcomes completely dependent on the decisions made during the statistical analysis calls for caution in the final interpretation of these outcomes. As valid arguments for the benefits of both conclusions can be made, additional studies in independent samples appear to be the best way forward to resolve these uncertainties.

The discovery of a potential kidney protective effect of galectin-1 in individuals with SIRD is very interesting and could eventually provide new insights in the fields of diabetes kidney disease if validating studies emerge. Kidney protective effects of galectin-1 have previously been explored with promise in an experimental setting, supporting a functional role in this context (64). However, considering the many studies on galectin-1 as a promoting factor in cancer disease, it may be challenging to develop directed therapies with recombinant galectin-1 in humans.

In conclusion, galectin-1 is not only associated with obesity and diabetes in cross-sectional studies, but high galectin-1 is also a risk factor in incident diabetes. While there is no evidence of causality between galectin-1 and type 2 diabetes, this study points to a functional role in the disease, possibly as a mediator of environmental factors.

# 4.4 STUDY IV

Through our previous studies, it has become apparent that galectin-1 is closely associated with metabolically unfavourable adiposity, as associations with BMI, insulin and triglycerides were consistent between Study II and Study III. However, galectin-1 is not the only galectin with a proposed role in type 2 diabetes. In fact, there are many more studies on galectin-3 and type 2 diabetes, currently registered on https://pubmed.ncbi.nlm.nih.gov/ today than there are for galectin-1 and type 2 diabetes. Furthermore, it has been proposed that galectins could have overlapping functionality, although clinical evidence is currently missing. For this reason, we decided to examine the association pattern of circulating galectin-1 and -3 with other markers of adiposity and metabolic disease in the well characterized POEM-cohort.

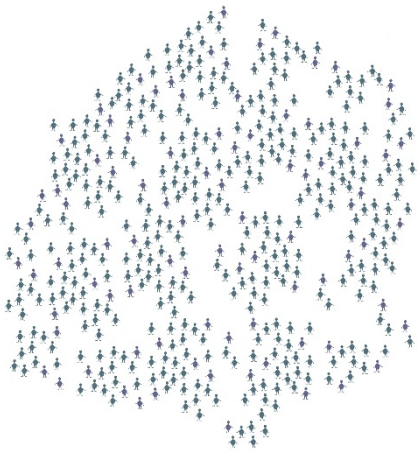


*Figure 12. Galectin-1 was measured in 502 individuals in the cross-sectional POEM-study.*

## 4.4.1 RESULTS STUDY IV

We measured galectin-1 and galectin-3 in the 502 participants in the cross-sectional community-based POEM-study. Initial association analysis revealed distinctly different association patterns between the two galectins after adjustment for age, sex, smoking and physical activity as galectin-1 was significantly associated with almost all metabolic variables analysed unlike galectin-3. However, as there is a strong association between galectin-1 and BMI, BMI-adjustments were also included in a second analysis. After this adjustment, galectin-1 was still associated with fat mass, visceral and subcutaneous adipose tissue, C-peptide, Matsuda index and fasting triglycerides. There was also an inverse association with fasting glucose. Galectin-3 did not associate with any marker of weight or adiposity, and adjustments for this variable did not change the outcomes of the analysis. Similar to galectin-1 there was an inverse association with fasting glucose, and a positive association with fasting triglycerides. Similar to the association between galectin-1 and C-peptide, galectin-3 was associated with fasting insulin and a *p*-value of 0.087 for C-peptide. Galectin-3 also associated with total cholesterol and LDL-cholesterol.

In line with the association profiles of clinical variables, the whole-body imiomic analysis revealed distinct differences between galectin-1 and galectin-3. The strong association between galectin-1 and all adipose tissue depots, specifically subcutis, shines in stark contrast to the lack of associations with galectin-3. Interestingly, the clarity in the different associations with body fat was matched by an equally clear similarity in the BMI-adjusted associations with almost all metabolites analysed in the metabolomic analysis. Positive associations were found with all fatty-acid groups except when stratified to subgroups of different polyunsaturated fats. Similarly, triglyceride levels were also associated with both galectins in a similar magnitude, with few exceptions.

While associations were seen between both measures of body fat and lipid metabolism, there was no clear indication of any causal or genetic effect of galectin-1 on the outcomes of BMI or any fat depot for either galectin.

### 4.4.2 DISCUSSION STUDY IV

This study supports our previous observations of a close association between galectin-1 and the adipose tissue and provides new information as the strongest association is with general fat mass, not specified to any one locale. While some associations with fasting glucose, insulin and Matsuda index were significant after adjustment for BMI, the strength of the associations were drastically reduced. This could indicate that the main link between galectin-1 and insulin resistance is mediated through obesity, or body mass. Interestingly, while galectin-3 did not reveal any associations with body mass or fat distribution associations with glucose and insulin aligned with the associations seen for galectin-1, glucose and C-peptide.

The almost identical associations seen with the metabolomic profiling for both galectins strongly indicate some joining factor between them. If this similarity is due to regulatory, functional or confounding factors remains to determine. However, it is known that galectin-1 and galectin-3 share affinity to the *N*-acetyllactosamine structure found in many glycoproteins (118) and also share some specific ligands (129, 188) which could indicate an overlapping functionality. Analysing SNPs associated with circulating galectin-1 and -3 did not reveal any clear association with BMI or adipose tissue distribution. This could have several explanations including a limited statistical power in the analysis, or a non-genetic association between galectin-1 and markers of adiposity. This observation aligns with the lack of association between galectin-1 and type 2 diabetes in the Mendelian randomization analysis in Study III, and the associations with galectin-1 and changes in caloric intake in Study I.

Taken together, our findings indicate that galectin-1, but not galectin-3 is closely associated with the adipose tissue, with no clear distinction regarding adipose tissue locale. Furthermore, the similar association patterns shared between galectin-1 and galectin-3 could indicate overlapping functionality.

## 4.5 STUDY V

Galectin-1 is consistently associated with all markers of adiposity, in all populations examined and through all techniques used for these assessments. Furthermore, animal experiments have indicated a functional role of galectin-1 in adipose tissue physiology (110-112, 189, 190). However, evidence of a similar role of galectin-1 in human adipose tissue physiology is currently missing. For this reason, we analysed serum galectin-1 before an oral glucose tolerance test (OGTT), together with *LGALS1* expression in human subcutaneous adipose tissue, and performed correlations with markers of metabolism in blood and on the gene level. We also modulated the galectin-1 protein effect through *in vitro* experiments on Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes, and during differentiation to cultured adipocytes.

*Figure 13. Galectin-1 was measured in 25 individuals in the experimental MD-Lipolysis study*

### 4.5.1 RESULTS STUDY V

Serum galectin-1 revealed the strongest correlations with kilograms of body fat, glycerol release rate per $10^4$ adipocytes and a positive correlation with insulin resistance, measured as Matsuda index. Galectin-1 also correlated with insulin, glycerol and free fatty acid levels during the OGTT. RNA extracted from subcutaneous adipose tissue in the same participants was analysed with RNAseq, and correlation analysis was conducted between *LGALS1* expression and other genes related to adipose tissue functionality. *LGALS1* was consistently correlated with all genetic markers of lipid uptake and adipogenesis, and also correlated with the lipogenesis marker *DGAT2*, the adipocyte marker *LEP* and the lipolysis marker *PLIN1*.

To further examine a functional role of galectin-1 in adipocytes, the association between galectin-1 and the genetic markers of adipocyte function was further explored in an *in vitro* system of SGBS preadipocytes. During differentiation to mature cultured adipocytes, galectin-1 protein levels were increased in media and in the cells. Furthermore, inhibition of galectin-1 in SGBS preadipocytes treated with a pharmacological inhibitor, or small inhibitory RNA (siRNA) for *LGALS1* significantly reduced the genetic markers of lipid uptake, *FABP4* as well as *CD36 (191)*.

Treating SGBS cells with a low dose of a pharmacological inhibitor of galectin-1 during differentiation to mature adipocytes resulted in cells with a lower triglyceride content, as well as a reduced gene expression of the lipid uptake marker *CD36,* the lipogenesis marker *DGAT2*, the glucose uptake marker *GLUT4* and the differentiation marker *PGC1A*. In addition, high dose treatment with the same inhibitor almost completely blocked the differentiation process resulting in a supressed expression of genes related to lipid uptake, lipogenesis, differentiation, lipolysis, thermogenesis and glucose uptake.

### 4.5.2 DISCUSSION STUDY V

Measurements of galectin-1 in the MD-Lipolysis study revealed several very interesting correlations between galectin-1 and markers of adiposity, insulin resistance and lipid metabolism. Nevertheless, there are some important considerations associated with these observations. The fact that the study is constituted of a pooled sample from three distinctly different phenotypes which differ in these very variables could bias the study outcomes. However, the serum levels of galectin-1 were not significantly different from a statistical perspective, meaning that these correlations are to a large degree explained by within-group variability and not group differences. Furthermore, the observations on adiposity and insulin align with results in the population-based cohorts in Study II and Study III. The correlation seen with adipocyte size was also previously observed in Study I, providing support also to the new correlations observed with glycerol, and free fatty acids.

Although it may appear surprising that there were no differences in galetin-1 levels in serum or on genetic level between lean, obese and obese with type 2 diabetes, this was not completely unexpected. Firstly, there was no difference in circulating levels of galectin-1 between the groups in Study I either, and it is possible that the lack of difference between lean and obese is constituted by a type 2 error as the sample sizes in both Study I and Study V are very small. Furthermore, the gene expression analysis in Study I was performed on isolated adipocytes, while the analysis in this study was performed on whole adipose tissue. As previously discussed, the selection criteria for the diabetes patients were also different between the two studies. The individuals in Study I were all male, had an average BMI of 25.9 $kg/m^2$, and all had a family history of type 2 diabetes. In contrast, participants in the MD-Lipolysis study were all obese, and included both males and females. These discrepancies illustrate the contextual nature of functional studies. However, the similar observations in these studies point to a functional role of galectin-1 in the adipose tissue in type 2 diabetes as independent designs reach the same conclusion (192).

The genetic associations between *LGALS1* and markers of adipocyte functionality align with the clinical markers of lipid metabolism, and suggest a direct functional role in the adipocyte. Inhibition of galectin-1 supressed markers of lipid uptake both through pharmacological and genetic methods in preadipocytes. Furthermore, pharmacological inhibition also reduced triglyceride content in mature adipocytes. As the inhibition of galectin-1 during differentiation also reduced the markers of glucose uptake and lipogenesis, it is not certain if galectin-1 directly influences a specific metabolic process such as lipid uptake, or is involved in a general anabolic program in the cell. Several reports have previously found high levels of galectin-1 in anabolic tissues with high proliferation such as in differentiating cells, during wound healing, or in aggressive tumours. This could indicate a metabolic role of galectin-1 in these settings (125, 134, 193). Similar associations are also known for other metabolic hormones such as insulin (194-196), although evidence of any direct interaction between galectin-1 and insulin is currently missing.

Taken together, our observations between galectin-1 on both protein and genetic level in vivo and in vitro point to a functional role in the human adipose tissue, specifically in the adipocyte. The clinical implications of this association, the influence of intercellular cross-talk, and the potential influence of other galectins should be further explored in new studies.

# 5 CONCLUSIONS

Taken together, this thesis shows that the galectin-1 protein is highly expressed in the adipose tissue and is related to obesity, insulin resistance and type 2 diabetes, suggesting a possible functional role in this setting.

Specifically, this thesis demonstrates that:

- Proteomic analysis of subcutaneous adipose tissue microdialysate fluid is a feasible method for identification of novel disease-related proteins.

- Galectin-1 is altered in both the subcutaneous interstitial fluid and circulation in individuals with type 2 diabetes.

- Dietary interventions appear to alter *LGALS1* expression in the subcutaneous adipose tissue.

- There is a robust association between circulating galectin-1 and markers of adiposity. These associations are seen with both adipocyte size, as well as total adipose tissue mass.

- Circulating galectin-1 is associated with cross-sectional measurements of markers of insulin resistance, as well as an increased risk of incident type 2 diabetes over time.

- Galectin-1 appears to have a functional role in adipose tissue physiology related to lipid metabolism.

# 6 FUTURE PERSPECTIVES

The identification of galectin-1 as an adipose tissue protein related to lipid metabolism, insulin resistance and type 2 diabetes provides a solid ground for further advances. However, several crucial questions remain.

While galectin-1 is increased in the subcutaneous adipose tissue of newly diagnosed individuals with type 2 diabetes, the population-based studies show that circulating galectin-1 is lower in individuals with type 2 diabetes compared to BMI-matched individuals without the disease. Longitudinal studies with repeated galectin-1 measurements over time could provide new insights on how galectin-1 changes during disease development.

Furthermore, the studies presented in this thesis focus strongly on galectin-1 in adipocytes. However, as indicated in Study III, galectin-1 could provide kidney protective effects in some individuals at risk of diabetic kidney disease. This is an important observation, also aligning with earlier reports on galectin-1 and kidney disease. Future studies on the role of galectin-1 in diabetic kidney disease would be of interest to investigate the implications of this observation.

The experimental work conducted in Study I and Study V suggest a functional role of galectin-1 in adipocyte metabolism. Human metabolism is complex, convoluted, and rarely in steady state during daily life. Experiments are therefore contextual, reflecting the fasted or fed state, and highly dependent on nutrient composition and availability. Furthermore, *in vivo* cross talk, between endothelial cells, adipocytes, macrophages and other cells in the adipose tissue are challenging to reflect in cell-culture experiments. Nonetheless, a deeper understanding of the metabolic implications of galectin-1 on a cellular level should be further explored going forward.

Study IV also reveals striking similarities in the metabolic association profiles of galectin-1 and galectin-3. These similarities should be considered in future functional experiments of galectin-1. While overlapping functionality between galectins has been previously suggested, this concept is not reflected in most mechanistic studies on galectins today. The significance of individual galectin effects, and the relative influence of galectin-family effects are important to conclude, especially when considering the ongoing development of galectin inhibitors for pharmacological intervention. Should future inhibitors be specific to one isoform, or should they strive for inhibition of the whole galectin-family?

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# REFERENCES


1. Minkowski O. Historical development of the theory of pancreatic diabetes by Oscar Minkowski, 1929: introduction and translation by Rachmiel Levine. *Diabetes.* 1989;38(1):1-6.
2. Joslin EP. The Treatment of Diabetes Mellitus. *Can Med Assoc J.* 1916;6(8):673-84.
3. Joslin EP. The Prevention of Diabetes Mellitus. *JAMA.* 1921, republished 2021;325(2):190-.
4. Alberti KGMM, Zimmet PZ, and Consultation W. Definition, diagnosis and classification of diabetes mellitus and its complications part 1: Diagnosis and classification of diabetes mellitus - Provisional report of a WHO consultation. *Diabetic Medicine.* 1998;15(7):539-53.
5. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva; 2011.
6. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2011;34 Suppl 1:S62-9.
7. American Diabetes Association Professional Practice C, American Diabetes Association Professional Practice C, Draznin B, Aroda VR, Bakris G, Benson G, et al. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2022. *Diabetes Care.* 2022;45(Suppl 1):S17-S38.
8. American Diabetes A. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care.* 2021;44(Suppl 1):S15-S33.
9. Broome DT, Pantalone KM, Kashyap SR, and Philipson LH. Approach to the Patient with MODY-Monogenic Diabetes. *J Clin Endocrinol Metab.* 2021;106(1):237-50.
10. Buzzetti R, Tuomi T, Mauricio D, Pietropaolo M, Zhou Z, Pozzilli P, et al. Management of Latent Autoimmune Diabetes in Adults: A Consensus Statement From an International Expert Panel. *Diabetes.* 2020;69(10):2037-47.
11. Ahlqvist E, Storm P, Karajamaki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol.* 2018;6(5):361-9.
12. Slieker RC, Donnelly LA, Fitipaldi H, Bouland GA, Giordano GN, Akerlund M, et al. Replication and cross-validation of type 2 diabetes subtypes based on clinical variables: an IMI-RHAPSODY study. *Diabetologia.* 2021;64(9):1982-9.
13. Zaharia OP, Strassburger K, Strom A, Bonhof GJ, Karusheva Y, Antoniou S, et al. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. *Lancet Diabetes Endocrinol.* 2019;7(9):684-94.

14. International Hypoglycaemia Study G. Hypoglycaemia, cardiovascular disease, and mortality in diabetes: epidemiology, pathogenesis, and management. *Lancet Diabetes Endocrinol.* 2019;7(5):385-96.
15. Collaborators GBDCoD. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2018;392(10159):1736-88.
16. Emerging Risk Factors C, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* 2010;375(9733):2215-22.
17. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primers.* 2015;1:15018.
18. Burrows NR, Li Y, and Geiss LS. Incidence of treatment for end-stage renal disease among individuals with diabetes in the U.S. continues to decline. *Diabetes Care.* 2010;33(1):73-7.
19. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. *Nat Rev Dis Primers.* 2019;5(1):41.
20. Barnes JA, Eid MA, Creager MA, and Goodney PP. Epidemiology and Risk of Amputation in Patients With Diabetes Mellitus and Peripheral Artery Disease. *Arterioscler Thromb Vasc Biol.* 2020;40(8):1808-17.
21. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care.* 2012;35(3):556-64.
22. Malik RA, Tesfaye S, and Ziegler D. Medical strategies to reduce amputation in patients with type 2 diabetes. *Diabet Med.* 2013;30(8):893-900.
23. Ling S, Brown K, Miksza JK, Howells L, Morrison A, Issa E, et al. Association of Type 2 Diabetes With Cancer: A Meta-analysis With Bias Analysis for Unmeasured Confounding in 151 Cohorts Comprising 32 Million People. *Diabetes Care.* 2020;43(9):2313-22.
24. Michailidis M, Moraitou D, Tata DA, Kalinderi K, Papamitsou T, and Papaliagkas V. Alzheimer's Disease as Type 3 Diabetes: Common Pathophysiological Mechanisms between Alzheimer's Disease and Type 2 Diabetes. *Int J Mol Sci.* 2022;23(5).
25. Biessels GJ, and Despa F. Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. *Nat Rev Endocrinol.* 2018;14(10):591-604.
26. Diabetes C, Complications Trial Research G, Nathan DM, Genuth S, Lachin J, Cleary P, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in

insulin-dependent diabetes mellitus. *N Engl J Med.* 1993;329(14):977-86.
27. Unnikrishnan R, Pradeepa R, Joshi SR, and Mohan V. Type 2 Diabetes: Demystifying the Global Epidemic. *Diabetes.* 2017;66(6):1432-42.
28. Lean ME, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet.* 2018;391(10120):541-51.
29. Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, et al. Very Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiological Changes in Responders and Nonresponders. *Diabetes Care.* 2016;39(5):808-15.
30. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med.* 2001;344(18):1343-50.
31. Sjostrom L, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med.* 2004;351(26):2683-93.
32. Haw JS, Galaviz KI, Straus AN, Kowalski AJ, Magee MJ, Weber MB, et al. Long-term Sustainability of Diabetes Prevention Approaches: A Systematic Review and Meta-analysis of Randomized Clinical Trials. *JAMA Intern Med.* 2017;177(12):1808-17.
33. Gillies CL, Abrams KR, Lambert PC, Cooper NJ, Sutton AJ, Hsu RT, et al. Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes in people with impaired glucose tolerance: systematic review and meta-analysis. *BMJ.* 2007;334(7588):299.
34. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes.* 2009;58(4):773-95.
35. Taylor R, and Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond).* 2015;128(7):405-10.
36. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care.* 2006;29(6):1337-44.
37. van der Zijl NJ, Goossens GH, Moors CC, van Raalte DH, Muskiet MH, Pouwels PJ, et al. Ectopic fat storage in the pancreas, liver, and abdominal fat depots: impact on beta-cell function in individuals with impaired glucose metabolism. *J Clin Endocrinol Metab.* 2011;96(2):459-67.

38. Reaven GM. The role of insulin resistance and hyperinsulinemia in coronary heart disease. *Metabolism.* 1992;41(5 Suppl 1):16-9.
39. Muoio DM. Revisiting the connection between intramyocellular lipids and insulin resistance: a long and winding road. *Diabetologia.* 2012;55(10):2551-4.
40. Tripathy D, Eriksson KF, Orho-Melander M, Fredriksson J, Ahlqvist G, and Groop L. Parallel manifestation of insulin resistance and beta cell decompensation is compatible with a common defect in Type 2 diabetes. *Diabetologia.* 2004;47(5):782-93.
41. Garg A. Clinical review#: Lipodystrophies: genetic and acquired body fat disorders. *J Clin Endocrinol Metab.* 2011;96(11):3313-25.
42. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med.* 1999;341(12):879-84.
43. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature.* 1997;387(6636):903-8.
44. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care.* 1997;20(4):537-44.
45. Schwingshackl L, Hoffmann G, Lampousi AM, Knuppel S, Iqbal K, Schwedhelm C, et al. Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. *Eur J Epidemiol.* 2017;32(5):363-75.
46. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, et al. Alcohol as a risk factor for type 2 diabetes: A systematic review and meta-analysis. *Diabetes Care.* 2009;32(11):2123-32.
47. Yuan S, and Larsson SC. An atlas on risk factors for type 2 diabetes: a wide-angled Mendelian randomisation study. *Diabetologia.* 2020;63(11):2359-71.
48. Knott C, Bell S, and Britton A. Alcohol Consumption and the Risk of Type 2 Diabetes: A Systematic Review and Dose-Response Meta-analysis of More Than 1.9 Million Individuals From 38 Observational Studies. *Diabetes Care.* 2015;38(9):1804-12.
49. Luukkonen PK, Sadevirta S, Zhou Y, Kayser B, Ali A, Ahonen L, et al. Saturated Fat Is More Metabolically Harmful for the Human Liver Than Unsaturated Fat or Simple Sugars. *Diabetes Care.* 2018;41(8):1732-9.
50. Ley SH, Hamdy O, Mohan V, and Hu FB. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. *Lancet.* 2014;383(9933):1999-2007.

51. Basu S, Yoffe P, Hills N, and Lustig RH. The relationship of sugar to population-level diabetes prevalence: an econometric analysis of repeated cross-sectional data. *PLoS One.* 2013;8(2):e57873.
52. Seidell JC. Obesity, insulin resistance and diabetes--a worldwide epidemic. *Br J Nutr.* 2000;83 Suppl 1:S5-8.
53. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet.* 2012;380(9838):219-29.
54. Chatterjee S, Khunti K, and Davies MJ. Type 2 diabetes. *Lancet.* 2017;389(10085):2239-51.
55. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018;138:271-81.
56. Rawshani A, Rawshani A, Franzen S, Sattar N, Eliasson B, Svensson AM, et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med.* 2018;379(7):633-44.
57. Huebschmann AG, Huxley RR, Kohrt WM, Zeitler P, Regensteiner JG, and Reusch JEB. Sex differences in the burden of type 2 diabetes and cardiovascular risk across the life course. *Diabetologia.* 2019;62(10):1761-72.
58. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes.* 1988;37(12):1595-607.
59. van der Aa MP, Knibbe CA, Boer A, and van der Vorst MM. Definition of insulin resistance affects prevalence rate in pediatric patients: a systematic review and call for consensus. *J Pediatr Endocrinol Metab.* 2017;30(2):123-31.
60. Tan VM, Lee YS, Venkataraman K, Khoo EY, Tai ES, Chong YS, et al. Ethnic differences in insulin sensitivity and beta-cell function among Asian men. *Nutr Diabetes.* 2015;5:e173.
61. Li L, Zou X, Huang Q, Han X, Zhou X, and Ji L. Do East Asians With Normal Glucose Tolerance Have Worse beta-Cell Function? A Meta-Analysis of Epidemiological Studies. *Front Endocrinol (Lausanne).* 2021;12:780557.
62. DeFronzo RA, Tobin JD, and Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237(3):E214-23.
63. Matsuda M, and DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* 1999;22(9):1462-70.
64. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, and Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-9.

65. Simental-Mendia LE, Rodriguez-Moran M, and Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord.* 2008;6(4):299-304.
66. Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, and Yki-Jarvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. *Gastroenterology.* 2008;135(1):122-30.
67. Kolterman OG, Gray RS, Griffin J, Burstein P, Insel J, Scarlett JA, et al. Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. *J Clin Invest.* 1981;68(4):957-69.
68. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes.* 1988;37(6):667-87.
69. Golden MP, Charles MA, Arquilla ER, Myers GL, Lippe BM, Duckworth WC, et al. Insulin resistance in total lipodystrophy: evidence for a pre-receptor defect in insulin action. *Metabolism.* 1985;34(4):330-5.
70. Grunberg JR, Elvin J, Paul A, Hedjazifar S, Hammarstedt A, and Smith U. CCN5/WISP2 and metabolic diseases. *J Cell Commun Signal.* 2018;12(1):309-18.
71. Hotamisligil GS. Inflammation and metabolic disorders. *Nature.* 2006;444(7121):860-7.
72. Frayn KN, and Karpe F. Regulation of human subcutaneous adipose tissue blood flow. *Int J Obes (Lond).* 2014;38(8):1019-26.
73. Shafrir E, and Raz I. Diabetes: mellitus or lipidus? *Diabetologia.* 2003;46(3):433-40.
74. Young SG, and Zechner R. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. *Genes Dev.* 2013;27(5):459-84.
75. Bickerton AS, Roberts R, Fielding BA, Hodson L, Blaak EE, Wagenmakers AJ, et al. Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period. *Diabetes.* 2007;56(1):168-76.
76. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, and Sul HS. Regulation of lipolysis in adipocytes. *Annu Rev Nutr.* 2007;27:79-101.
77. Tan GD, Fielding BA, Currie JM, Humphreys SM, Desage M, Frayn KN, et al. The effects of rosiglitazone on fatty acid and triglyceride metabolism in type 2 diabetes. *Diabetologia.* 2005;48(1):83-95.
78. Karpe F, Dickmann JR, and Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes.* 2011;60(10):2441-9.
79. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science.* 1992;258(5083):766-70.

80. Fryk E, Olausson J, Mossberg K, Strindberg L, Schmelz M, Brogren H, et al. Hyperinsulinemia and insulin resistance in the obese may develop as part of a homeostatic response to elevated free fatty acids: A mechanistic case-control and a population-based cohort study. *EBioMedicine.* 2021;65:103264.
81. Nielsen S, and Karpe F. Determinants of VLDL-triglycerides production. *Curr Opin Lipidol.* 2012;23(4):321-6.
82. Reddy JK, and Rao MS. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol.* 2006;290(5):G852-8.
83. Birkenfeld AL, and Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. *Hepatology.* 2014;59(2):713-23.
84. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol.* 2020;8(7):616-27.
85. Palmer BF, and Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol.* 2015;402:113-9.
86. Klein S, Fontana L, Young VL, Coggan AR, Kilo C, Patterson BW, et al. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med.* 2004;350(25):2549-57.
87. Thorne A, Lonnqvist F, Apelman J, Hellers G, and Arner P. A pilot study of long-term effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. *Int J Obes Relat Metab Disord.* 2002;26(2):193-9.
88. Hattori Y, Hirama N, Suzuki K, Hattori S, and Kasai K. Elevated plasma adiponectin and leptin levels in sisters with genetically defective insulin receptors. *Diabetes Care.* 2007;30(11):e109.
89. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, et al. A family with severe insulin resistance and diabetes due to a mutation in AKT2. *Science.* 2004;304(5675):1325-8.
90. Hegele RA, Cao H, Frankowski C, Mathews ST, and Leff T. PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes.* 2002;51(12):3586-90.
91. Gandotra S, Le Dour C, Bottomley W, Cervera P, Giral P, Reznik Y, et al. Perilipin deficiency and autosomal dominant partial lipodystrophy. *N Engl J Med.* 2011;364(8):740-8.
92. Hammarstedt A, Gogg S, Hedjazifar S, Nerstedt A, and Smith U. Impaired Adipogenesis and Dysfunctional Adipose Tissue in Human Hypertrophic Obesity. *Physiol Rev.* 2018;98(4):1911-41.
93. Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, et al. Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. *Proc Natl Acad Sci U S A.* 1995;92(15):6957-60.

94. McGarry JD. Appetite control: Does leptin lighten the problem of obesity? *Curr Biol.* 1995;5(12):1342-4.
95. Beltrand J, Beregszaszi M, Chevenne D, Sebag G, De Kerdanet M, Huet F, et al. Metabolic correction induced by leptin replacement treatment in young children with Berardinelli-Seip congenital lipoatrophy. *Pediatrics.* 2007;120(2):e291-6.
96. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol.* 2000;20(6):1595-9.
97. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257(1):79-83.
98. Hu E, Liang P, and Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem.* 1996;271(18):10697-703.
99. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert MF, Warren LL, et al. Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. *Diabetes.* 2013;62(10):3589-98.
100. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, et al. Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. *Diabetes.* 2013;62(4):1338-44.
101. Herder C, Peltonen M, Svensson PA, Carstensen M, Jacobson P, Roden M, et al. Adiponectin and bariatric surgery: associations with diabetes and cardiovascular disease in the Swedish Obese Subjects Study. *Diabetes Care.* 2014;37(5):1401-9.
102. Hotamisligil GS, Shargill NS, and Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259(5091):87-91.
103. Skurk T, Alberti-Huber C, Herder C, and Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab.* 2007;92(3):1023-33.
104. Sethi JK, and Hotamisligil GS. Metabolic Messengers: tumour necrosis factor. *Nat Metab.* 2021;3(10):1302-12.
105. Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, et al. Galectins: a family of animal beta-galactoside-binding lectins. *Cell.* 1994;76(4):597-8.
106. Johannes L, Jacob R, and Leffler H. Galectins at a glance. *J Cell Sci.* 2018;131(9).
107. Camby I, Le Mercier M, Lefranc F, and Kiss R. Galectin-1: a small protein with major functions. *Glycobiology.* 2006;16(11):137R-57R.
108. Ahmed M, Neville MJ, Edelmann MJ, Kessler BM, and Karpe F. Proteomic analysis of human adipose tissue after rosiglitazone

treatment shows coordinated changes to promote glucose uptake. *Obesity (Silver Spring).* 2010;18(1):27-34.
109. Roumans NJT, Vink RG, Bouwman FG, Fazelzadeh P, van Baak MA, and Mariman ECM. Weight loss-induced cellular stress in subcutaneous adipose tissue and the risk for weight regain in overweight and obese adults. *Int J Obes (Lond).* 2017;41(6):894-901.
110. Mukherjee R, and Yun JW. Lactobionic acid reduces body weight gain in diet-induced obese rats by targeted inhibition of galectin-1. *Biochem Biophys Res Commun.* 2015;463(4):1311-6.
111. Mukherjee R, Kim SW, Park T, Choi MS, and Yun JW. Targeted inhibition of galectin 1 by thiodigalactoside dramatically reduces body weight gain in diet-induced obese rats. *Int J Obes (Lond).* 2015;39(9):1349-58.
112. Mukherjee R, and Yun JW. Pharmacological inhibition of galectin-1 by lactulose alleviates weight gain in diet-induced obese rats. *Life Sci.* 2016;148:112-7.
113. Rhodes DH, Pini M, Castellanos KJ, Montero-Melendez T, Cooper D, Perretti M, et al. Adipose Tissue-Specific Modulation of Galectin Expression in Lean and Obese Mice: Evidence for Regulatory Function. *Obesity.* 2013;21(2):310-9.
114. Acar S, Paketci A, Kume T, Tuhan H, Gursoy Calan O, Demir K, et al. Serum galectin-1 levels are positively correlated with body fat and negatively with fasting glucose in obese children. *Peptides.* 2017;95:51-6.
115. Blois SM, Gueuvoghlanian-Silva BY, Tirado-Gonzalez I, Torloni MR, Freitag N, Mattar R, et al. Getting too sweet: galectin-1 dysregulation in gestational diabetes mellitus. *Mol Hum Reprod.* 2014;20(7):644-9.
116. Liu X, Feng Q, Chen Y, Zuo J, Gupta N, Chang Y, et al. Proteomics-based identification of differentially-expressed proteins including galectin-1 in the blood plasma of type 2 diabetic patients. *J Proteome Res.* 2009;8(3):1255-62.
117. Jiang GX, Cui YF, Zhong XY, Tai S, Liu W, Wang ZD, et al. Use of a cocktail regimen consisting of soluble galectin-1, rapamycin and histone deacetylase inhibitor may effectively prevent type 1 diabetes. *Arch Med Res.* 2009;40(5):424-6.
118. Perone MJ, Bertera S, Shufesky WJ, Divito SJ, Montecalvo A, Mathers AR, et al. Suppression of autoimmune diabetes by soluble galectin-1. *J Immunol.* 2009;182(5):2641-53.
119. Gomez-Tourino I, Sanchez-Espinel C, Hernandez-Fernandez A, Gonzalez-Fernandez A, Pena-Gonzalez E, Rodriguez J, et al. Galectin-1 synthesis in type 1 diabetes by different immune cell types: reduced synthesis by monocytes and Th1 cells. *Cell Immunol.* 2011;271(2):319-28.

120. Kanda A, Noda K, Saito W, and Ishida S. Aflibercept Traps Galectin-1, an Angiogenic Factor Associated with Diabetic Retinopathy. *Sci Rep.* 2015;5:17946.
121. Kanda A, Dong Y, Noda K, Saito W, and Ishida S. Advanced glycation endproducts link inflammatory cues to upregulation of galectin-1 in diabetic retinopathy. *Sci Rep.* 2017;7(1):16168.
122. Salatino M, Croci DO, Bianco GA, Ilarregui JM, Toscano MA, and Rabinovich GA. Galectin-1 as a potential therapeutic target in autoimmune disorders and cancer. *Expert Opin Biol Ther.* 2008;8(1):45-57.
123. Thijssen VL, Barkan B, Shoji H, Aries IM, Mathieu V, Deltour L, et al. Tumor Cells Secrete Galectin-1 to Enhance Endothelial Cell Activity. *Cancer Research.* 2010;70(15):6216-24.
124. Banh A, Zhang J, Cao H, Bouley DM, Kwok S, Kong C, et al. Tumor galectin-1 mediates tumor growth and metastasis through regulation of T-cell apoptosis. *Cancer Res.* 2011;71(13):4423-31.
125. Wu H, Chen P, Liao R, Li YW, Yi Y, Wang JX, et al. Overexpression of galectin-1 is associated with poor prognosis in human hepatocellular carcinoma following resection. *J Gastroenterol Hepatol.* 2012;27(8):1312-9.
126. Salomonsson E, Larumbe A, Tejler J, Tullberg E, Rydberg H, Sundin A, et al. Monovalent interactions of galectin-1. *Biochemistry.* 2010;49(44):9518-32.
127. Rhodes DH, Pini M, Castellanos KJ, Montero-Melendez T, Cooper D, Perretti M, et al. Adipose tissue-specific modulation of galectin expression in lean and obese mice: evidence for regulatory function. *Obesity (Silver Spring).* 2013;21(2):310-9.
128. Barondes SH, Cooper DN, Gitt MA, and Leffler H. Galectins. Structure and function of a large family of animal lectins. *J Biol Chem.* 1994;269(33):20807-10.
129. Jouve N, Despoix N, Espeli M, Gauthier L, Cypowyj S, Fallague K, et al. The involvement of CD146 and its novel ligand Galectin-1 in apoptotic regulation of endothelial cells. *J Biol Chem.* 2013;288(4):2571-9.
130. Hsieh SH, Ying NW, Wu MH, Chiang WF, Hsu CL, Wong TY, et al. Galectin-1, a novel ligand of neuropilin-1, activates VEGFR-2 signaling and modulates the migration of vascular endothelial cells. *Oncogene.* 2008;27(26):3746-53.
131. Croci DO, Cerliani JP, Dalotto-Moreno T, Mendez-Huergo SP, Mascanfroni ID, Dergan-Dylon S, et al. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell.* 2014;156(4):744-58.
132. Thijssen VL. Galectins in Endothelial Cell Biology and Angiogenesis: The Basics. *Biomolecules.* 2021;11(9).

133. Wu MH, Chen YL, Lee KH, Chang CC, Cheng TM, Wu SY, et al. Glycosylation-dependent galectin-1/neuropilin-1 interactions promote liver fibrosis through activation of TGF-beta- and PDGF-like signals in hepatic stellate cells. *Sci Rep.* 2017;7(1):11006.
134. Lin YT, Chen JS, Wu MH, Hsieh IS, Liang CH, Hsu CL, et al. Galectin-1 accelerates wound healing by regulating the neuropilin-1/Smad3/NOX4 pathway and ROS production in myofibroblasts. *J Invest Dermatol.* 2015;135(1):258-68.
135. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, et al. Vascular endothelial growth factor B controls endothelial fatty acid uptake. *Nature.* 2010;464(7290):917-21.
136. Reily C, Stewart TJ, Renfrow MB, and Novak J. Glycosylation in health and disease. *Nat Rev Nephrol.* 2019;15(6):346-66.
137. Seropian IM, Gonzalez GE, Maller SM, Berrocal DH, Abbate A, and Rabinovich GA. Galectin-1 as an Emerging Mediator of Cardiovascular Inflammation: Mechanisms and Therapeutic Opportunities. *Mediators of Inflammation.* 2018.
138. Brinchmann MF, Patel DM, and Iversen MH. The Role of Galectins as Modulators of Metabolism and Inflammation. *Mediators Inflamm.* 2018;2018:9186940.
139. Al-Obaidi N, Mohan S, Liang S, Zhao Z, Nayak BK, Li B, et al. Galectin-1 is a new fibrosis protein in type 1 and type 2 diabetes. *FASEB J.* 2018:fj201800555RR.
140. Ridano ME, Subirada PV, Paz MC, Lorenc VE, Stupirski JC, Gramajo AL, et al. Galectin-1 expression imprints a neurovascular phenotype in proliferative retinopathies and delineates responses to anti-VEGF. *Oncotarget.* 2017;8(20):32505-22.
141. Tsai YL, Chou RH, Lu YW, Chang CC, Kuo CS, Huang PH, et al. Associations between galectin-1, left ventricular diastolic dysfunction, and heart failure with preserved ejection fraction. *J Cardiol.* 2022;79(3):371-5.
142. Ishibashi S, Kuroiwa T, Sakaguchi M, Sun L, Kadoya T, Okano H, et al. Galectin-1 regulates neurogenesis in the subventricular zone and promotes functional recovery after stroke. *Exp Neurol.* 2007;207(2):302-13.
143. Seropian IM, Cerliani JP, Toldo S, Van Tassell BW, Ilarregui JM, Gonzalez GE, et al. Galectin-1 controls cardiac inflammation and ventricular remodeling during acute myocardial infarction. *Am J Pathol.* 2013;182(1):29-40.
144. Blanchard H, Bum-Erdene K, Bohari MH, and Yu X. Galectin-1 inhibitors and their potential therapeutic applications: a patent review. *Expert Opin Ther Pat.* 2016;26(5):537-54.
145. Sethi A, Sanam S, Alvala R, and Alvala M. An updated patent review of galectin-1 and galectin-3 inhibitors and their potential therapeutic

applications (2016-present). *Expert Opin Ther Pat.* 2021;31(8):709-21.
146. Vittinghoff E, and McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol.* 2007;165(6):710-8.
147. Peduzzi P, Concato J, Kemper E, Holford TR, and Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol.* 1996;49(12):1373-9.
148. Davies NM, Holmes MV, and Smith GD. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *Bmj-British Medical Journal.* 2018;362.
149. Emdin CA, Khera AV, and Kathiresan S. Mendelian Randomization. *JAMA.* 2017;318(19):1925-6.
150. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat Genet.* 2018;50(11):1505-13.
151. Wuttke M, Li Y, Li M, Sieber KB, Feitosa MF, Gorski M, et al. A catalog of genetic loci associated with kidney function from analyses of a million individuals. *Nat Genet.* 2019;51(6):957-72.
152. Sjostrand M, Holmang A, and Lonnroth P. Measurement of interstitial insulin in human muscle. *Am J Physiol.* 1999;276(1):E151-4.
153. Strindberg L, and Lonnroth P. Validation of an endogenous reference technique for the calibration of microdialysis catheters. *Scand J Clin Lab Invest.* 2000;60(3):205-11.
154. Fryk E, Sundelin JP, Strindberg L, Pereira MJ, Federici M, Marx N, et al. Microdialysis and proteomics of subcutaneous interstitial fluid reveals increased galectin-1 in type 2 diabetes patients. *Metabolism.* 2016;65(7):998-1006.
155. Tirimacco R, Koumantakis G, Erasmus R, Mosca A, Sandberg S, Watson ID, et al. Glucose meters - fit for clinical purpose. *Clin Chem Lab Med.* 2013;51(5):943-52.
156. Beck SC, and Lock RJ. Uncertainty of measurement: an immunology laboratory perspective. *Ann Clin Biochem.* 2015;52(Pt 1):7-17.
157. Infusino I, and Panteghini M. Measurement uncertainty: Friend or foe? *Clin Biochem.* 2018;57:3-6.
158. Suh S, and Kim JH. Glycemic Variability: How Do We Measure It and Why Is It Important? *Diabetes Metab J.* 2015;39(4):273-82.
159. Little RR, and Sacks DB. HbA1c: how do we measure it and what does it mean? *Curr Opin Endocrinol Diabetes Obes.* 2009;16(2):113-8.
160. Vesper HW, Myers GL, and Miller WG. Current practices and challenges in the standardization and harmonization of clinical laboratory tests. *Am J Clin Nutr.* 2016;104 Suppl 3:907S-12S.
161. Hasin Y, Seldin M, and Lusis A. Multi-omics approaches to disease. *Genome Biol.* 2017;18(1):83.

162. Noble AJ, Purcell RV, Adams AT, Lam YK, Ring PM, Anderson JR, et al. A Final Frontier in Environment-Genome Interactions? Integrated, Multi-Omic Approaches to Predictions of Non-Communicable Disease Risk. *Front Genet.* 2022;13:831866.
163. Carlsohn E, Nystrom J, Karlsson H, Svennerholm AM, and Nilsson CL. Characterization of the outer membrane protein profile from disease-related Helicobacter pylori isolates by subcellular fractionation and nano-LC FT-ICR MS analysis. *J Proteome Res.* 2006;5(11):3197-204.
164. Lundberg M, Eriksson A, Tran B, Assarsson E, and Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res.* 2011;39(15):e102.
165. Drake I, Fryk E, Strindberg L, Lundqvist A, Rosengren AH, Groop L, et al. The role of circulating galectin-1 in type 2 diabetes and chronic kidney disease: evidence from cross-sectional, longitudinal and Mendelian randomisation analyses. *Diabetologia.* 2022;65(1):128-39.
166. Strand R, Malmberg F, Johansson L, Lind L, Sundbom M, Ahlstrom H, et al. A concept for holistic whole body MRI data analysis, Imiomics. *PLoS One.* 2017;12(2):e0169966.
167. Boone SC, van Smeden M, Rosendaal FR, le Cessie S, Groenwold RHH, Jukema JW, et al. Evaluation of the Value of Waist Circumference and Metabolomics in the Estimation of Visceral Adipose Tissue. *Am J Epidemiol.* 2022.
168. Bishara AJ, and Hittner JB. Reducing Bias and Error in the Correlation Coefficient Due to Nonnormality. *Educ Psychol Meas.* 2015;75(5):785-804.
169. Ioannidis JP. Why most published research findings are false. *PLoS Med.* 2005;2(8):e124.
170. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology.* 1990;1(1):43-6.
171. Benjamini Y, and Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Statistical Methodology.* 1995;57(1):289-300.
172. Vidgen B, and Yasseri T. P-Values: Misunderstood and Misused. *Frontiers in Physics.* 2016;4.
173. Vetter TR. Fundamentals of Research Data and Variables: The Devil Is in the Details. *Anesth Analg.* 2017;125(4):1375-80.
174. Stratton IM, and Neil A. How to ensure your paper is rejected by the statistical reviewer. *Diabet Med.* 2005;22(4):371-3.
175. Cohen J. The Earth Is Round (P-Less-Than.05). *American Psychologist.* 1994;49(12):997-1003.

176. Tsiamalou P, and Brotis A. Biostatistics as a Tool for Medical Research: What are we Doing Wrong? *Mediterr J Rheumatol.* 2019;30(4):196-200.
177. Hill AB. Environment and Disease - Association or Causation. *Proceedings of the Royal Society of Medicine-London.* 1965;58(5):295-+.
178. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA.* 2013;310(20):2191-4.
179. Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes.* 1998;47(4):507-14.
180. Svensson PA, Gabrielsson BG, Jernas M, Gummesson A, and Sjoholm K. Regulation of human aldoketoreductase 1C3 (AKR1C3) gene expression in the adipose tissue. *Cell Mol Biol Lett.* 2008;13(4):599-613.
181. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH, et al. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut.* 2008;57(5):649-54.
182. Rawshani A, Svensson AM, Rosengren A, Zethelius B, Eliasson B, and Gudbjornsdottir S. Impact of ethnicity on progress of glycaemic control in 131,935 newly diagnosed patients with type 2 diabetes: a nationwide observational study from the Swedish National Diabetes Register. *BMJ Open.* 2015;5(6):e007599.
183. Kizer JR. A tangled threesome: adiponectin, insulin sensitivity, and adiposity: can Mendelian randomization sort out causality? *Diabetes.* 2013;62(4):1007-9.
184. Okano K, Tsuruta Y, Yamashita T, Takano M, Echida Y, and Nitta K. Suppression of renal fibrosis by galectin-1 in high glucose-treated renal epithelial cells. *Exp Cell Res.* 2010;316(19):3282-91.
185. Liu Y, Long L, Yuan F, Liu F, Liu H, Peng Y, et al. High glucose-induced Galectin-1 in human podocytes implicates the involvement of Galectin-1 in diabetic nephropathy. *Cell Biol Int.* 2015;39(2):217-23.
186. Carlos CP, Silva AA, Gil CD, and Oliani SM. Pharmacological treatment with galectin-1 protects against renal ischaemia-reperfusion injury. *Sci Rep.* 2018;8(1):9568.
187. Kuo CS, Chou RH, Lu YW, Tsai YL, Huang PH, and Lin SJ. Increased circulating galectin-1 levels are associated with the progression of kidney function decline in patients undergoing coronary angiography. *Sci Rep.* 2020;10(1):1435.
188. Zhang Z, Zheng Y, Wang H, Zhou Y, and Tai G. CD146 interacts with galectin-3 to mediate endothelial cell migration. *FEBS Lett.* 2018;592(11):1817-28.
189. Baek JH, Kim DH, Lee J, Kim SJ, and Chun KH. Galectin-1 accelerates high-fat diet-induced obesity by activation of peroxisome

proliferator-activated receptor gamma (PPARgamma) in mice. *Cell Death Dis.* 2021;12(1):66.
190. Parray HA, and Yun JW. Combined inhibition of autophagy protein 5 and galectin-1 by thiodigalactoside reduces diet-induced obesity through induction of white fat browning. *IUBMB Life.* 2017;69(7):510-21.
191. Storch J, and Thumser AE. The fatty acid transport function of fatty acid-binding proteins. *Biochim Biophys Acta.* 2000;1486(1):28-44.
192. Hill AB. The Environment and Disease: Association or Causation? *Proc R Soc Med.* 1965;58:295-300.
193. Wang P, Mariman E, Keijer J, Bouwman F, Noben JP, Robben J, et al. Profiling of the secreted proteins during 3T3-L1 adipocyte differentiation leads to the identification of novel adipokines. *Cellular and Molecular Life Sciences.* 2004;61(18):2405-17.
194. Kaur S, Auger C, and Jeschke MG. Adipose Tissue Metabolic Function and Dysfunction: Impact of Burn Injury. *Front Cell Dev Biol.* 2020;8:599576.
195. Hopkins BD, Goncalves MD, and Cantley LC. Insulin-PI3K signalling: an evolutionarily insulated metabolic driver of cancer. *Nat Rev Endocrinol.* 2020;16(5):276-83.
196. Shahbazi M, Cundiff P, Zhou W, Lee P, Patel A, D'Souza SL, et al. The role of insulin as a key regulator of seeding, proliferation, and mRNA transcription of human pluripotent stem cells. *Stem Cell Res Ther.* 2019;10(1):228.