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„Effects of different interventions on adipokine levels in diabetes: systematic reviews and meta-analyses“

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“What you get by achieving your goals is not as important as what you become by achieving your goals.”
– Henry David Thoreau

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Output papers

Paper 1: Effects of Omega-3 Supplementation on Adipocytokines in Prediabetes and Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Becic T., Studenik C. Diabetes Metab J. 2018;42(2):101-116.

Paper 2: Effects of Vitamin D Supplementation on Adipocytokine Levels in Patients with Abnormal Glucose Metabolism: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Becic T., Studenik C. J Pharmol Clin Res. 2018;5(5):555674

Paper 3: Exercise Increases Adiponectin and Reduces Leptin Levels in Prediabetic and Diabetic Individuals: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Becic T., Studenik C., Hoffmann G. Med Sci 2018;6(4). pii: E97

Abstract

Background: Diabetes mellitus is one of the most common metabolic disorders today. In 2017, there were around 450 million individuals suffering with diabetes and this number is expected to rise to more than 690 million in 2045. The disease is characterized by blood glucose levels which are well above the normal values. Such high blood glucose levels are damaging to several organs in the body, so that a range of complications develops, including cardiovascular disease, kidney disease and nerve and eye damage. Type 2 diabetes, which is strongly associated with overweight and obesity, represents the most prevalent type of disease and is associated with significant morbidity and mortality. Most individuals with type 2 diabetes have prediabetes before they develop full-blown disease. This state represents a high-risk condition as around 70% of prediabetic individuals show a constant deterioration of blood glucose levels and go on to develop over disease. Adipose tissue has long been considered an inert energy storage system. However, in the 1990s, a paradigm shift was brought about with the dicovery of numerous signalling molecules from the adipose tissue, collectively known as adipokines. In 1994, leptin was discovered, followed by numerous other molecules, so that today, more than 600 adipokines are known. However, the function of most of these proteins is not well-understood. Some of them have been functionally well-characterized, such as the „adipostat“ leptin, anti-inflammatory adiponectin and pro-inflammatory tumor necrosis factor-alpha and interleukins. These adipokines have a range of important physiological functions, many of which are highly relevant in diabetes. Dearrangements in levels of adipokines have been observed in prediabetes and diabetes, which led to suggest that these molecules are implicated in the development and progression of diabetes. As current interventions are not effective at halting the

ever-growing global burden of diabetes, complementary interventions which are feasible, affordable and easy to implement are highly needed.

Given the relevance of adipokines, elucidating the impact of such interventions on their levels is of high importance.

Methods: In order to characterize the quantative effect of different interventions on levels of adipokines in prediabetic and diabetic individuals, systematic reviews and meta-analyses were conducted on omega-3/fish oil supplementation, vitamin D supplementation and exercise. The studies were planned, conducted and reported in accordance with current standards (PRISMA, Cochrane Handbook). Multiple databases (e.g. PubMed, Medline, EMBASE) were searched as sources of potential randomized controlled trials for inclusion. Strict inclusion and exclusion criteria were applied to studies. Pooled effects of interventions were assessed as mean difference using the random effects model. In order to test for robustness of results, several statistical analyses were conducted, including a sensitivity, subgroup and publication bias analysis. Results were published in peer-review journals.

Results and Conclusion: With regards to omega-3/fish oil supplementation, an increase in adiponectin levels and a reduction in TNF-alpha levels was found, whereas no changes were found in levels of leptin, plasminogen-activator inhibitor 1, resistin and interleukin-6. Vitamin D suplementation reduces levels of the pro-inflammatory levels of interleukin-6, but does not affect levels of leptin, adiponectin and tumor necrosis factor-alpha. Exercise, especially aerobic exercise, increases adiponectin and reduces leptin in this population. The results demonstrate that these interventions have a significant effect on circulating levels of adipokines in prediabetic and type 2 diabetic

individuals. Introducing these interventions into the management of these conditions could have a positive clinical impact and improve treatment outcomes.

Zusammenfassung

Hintergrund: Diabetes mellitus ist eine der häufigsten metabolischen Erkrankungen. In 2017 gab es mehr als 450 Millionen Erkrankte weltweit und die projizierte Ziffer für 2045 ist mehr als 690 Millionen. Die Erkrankung ist characterisiert durch zu hohe Blutzuckerspiegel. Chronisch erhöhter Blutzucker fügt mehreren Organen erheblichen Schaden zu, so dass sich eine Reihe von diabetischen Komplikationen entwickeln, z.B. kardiovaskuläre Erkrankungen, Nierenversagen sowie Nerven- und Augenschäden. Diabetes mellitus Typ 2 ist die häufigste Form der Erkrankung. Sie ist stark assoziiert mit Übergewicht und führt zu erheblicher Morbidität und Mortalität. Die meisten Typ 2 Diabetiker leiden an Prediabetes, einer Vorstufe der Erkrankung. Prediabetes ist ein Hochrisikozustand, denn 70% der Betroffenenen zeigen eine konstante Verschlechterung der Blutzuckerspiegel und entwickeln letztendlich Diabetes. Fettgewebe wurde sehr lang als ein inertes Energiespeichersystem betrachtet. Dies hat sich in den 1990er mit der Entdeckung zahlreicher Signalmoleükule geändert, die als Adipokine bezeichnet werden. In 1994 wurde zuerst Leptin entdeckt, gefolgt von zahlreichen anderen Proteinen, so dass heute mehr als 600 Adipokine bekannt sind. Die genaue Funktion der meisten Adipokine ist allerdings noch immer nicht bekannt. Manche wurden gut charackterisiert, z.B. Leptin, Adiponektin, Tumor Nekrose Faktor-alpha und Interleukine. Diese Adipokine haben eine Reihe sehr wichtiger physiologischer Funtionen, welche auch in Diabetes relevant sind. Pathologisch veränderte Konzentrationen dieser Moleküle wurden in Prediabetes und Diabetes entdeckt und es wurde gezeigt, dass sie in der Entstehung und Fortschreitung der Erkrankung eine wesentliche Rolle spielen. Derzeitige Interventionen sind oft nicht effektiv in der Behandlung der Erkrankung, so dass komplentäre Interventionen, die

leistbar und leicht umsetzbar sind, notwendig sind, um eine erfolgreiche Krankheitskontrolle herbeizuführen. Die Untersuchung des Einflusses solcher Interventionen auf Adipokine ist sehr wichtig aufgrund der hohen Relevanz dieser Signalmoleküle bei Diabetes.

Methodologie: Systematiches Review und Meta-analyse von Omega-3 Fettsäuren/Fischöl Supplementierung, Vitamin D Supplementierung und körperlicher Aktivität wurde durchgeführt, um den Effekt dieser Interventionen auf diverse Adipokine quantitativ zu ermitteln. Die Studien wurden im Einklang mit derzeitigen Standards durchführt (PRISMA, Cochrane Handbook). Mehrere Datenbanken (z.B. PubMed, Medline, EMBASE) wurden systematic durchgesucht, um potentielle randomisierte kontrollierte Studien zu identifizieren, die nach Prüfung von Ein- und Ausschlusskriterien in die Analyse aufgenommen wurden. Anhand des Models der randomisierten Effekte wurden die gepoolten Effekte der Interventionen als Mitteldifferenz errechnet. Mithilfe von Sensitivitäts-, Subgruppen- und Publikationsbiasanalysen wurde die Robustheit der Ergebnisse immer getestet. Die Ergebnisse wurden in peer-review Journals publiziert.

Ergebnisse: Supplementierung mit Omega-3 Fettsäuren/Fischöl erhöht die Konzentration von Adiponektin und vermindert die Konzentration von Tumor Nekrose Faktor-alpha bei der untersuchten Population, hat aber keine Auswirkungen auf die Konzentration von Leptin, Plasminogen Aktivator Inhibitor-1, Resistin und Interleukin-6. Vitamin D führt zu einer Reduktion der Blutspiegel des pro-inflammatorischen Interleukin-6, verändert aber nicht die Konzentration von Leptin, Adiponektin und

Tumor Nekrose Faktor-alpha. Körperliche Aktivität, insbesondere aerobisches Training, erhöht Adiponektinspiegel und reduziert Leptinspiegel. Die Ergebnisse zeigen, dass die untersuchten Interventionen einen deutlichen Einfluss auf die Blutspiegel von Adipokinen bei Prediabetikern und Typ 2 Diabetikern haben. Somit können diese Interventionen positive Auswirkungen in der Behandlung der Erkrankten haben.

1. Classification and diagnosis of diabetes mellitus

Diabetes mellitus encompasses a group of metabolic disorders generally characterized by hyperglycemia due to defects in insulin secretion from the pancreatic beta-cells, insulin action in target tissues or their combination (1).

In general, there are four main aetiological categories of diabetes based on criteria set out by the World Health Organization (WHO) and the American Diabetes Association (ADA) (1-3):

1. Type 1 diabetes, which results from absolute insulin deficiency mostly due to an autoimmune disorder
2. Type 2 diabetes, which results from insulin resistance in target tissues with progressive loss of beta-cell secretory function
3. Gestational diabetes mellitus, which occurs in the second or third trimenon of pregancy without prior overt diabetes
4. Other specific forms of diabetes, such as genetic abnormalities in insulin secretion (e.g.; maturity-onset diabetes of the young – MODY) or action (mutations in the insulin receptor), endocrinopathies (e.g., acromegaly), injuries to the exocrine pancreas (e.g., due to cystic fibrosis), diabetes induced by medication (e.g., with glucocorticoid therapy), and infection-associated diabetes (e.g., congenital rubella virus).

Type 1 and type 2 diabetes mellitus account for the absolute majority of diabetes cases. Type 1 diabetes, also called insulin-dependent diabetes and juvenile diabetes, accounts for approximately 5-10% of diabetes cases and is due to an autoimmune reaction leading to destruction of pancreatic beta cells and absolute insulin deficiency. Type 2

diabetes, also known as non-insulin dependent diabetes or adult-onset diabetes, affects about 90-95% of diseased individuals and its main hallmark is insulin resistance mostly associated with obesity and sendentary lifestyle (1-3).

The vast majority of patients with type 2 diabetes have impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) before they develop full-blown disease (4). The terms juvenile diabetes and adult diabetes should no longer be used, as both types of diabetes can be diagnosed at any age (5,6), which particularly becomes apparent as with global obesity epidemic the incidence of type 2 diabetes shifts more towards the younger age (6).

The diagnosis of diabetes mellitus is made based on plasma glucose levels after a 2 hours 75 g oral glucose tolerance test (OGTT) already for decades or under fasting conditions. The cut-off values used today were set out by the Expert Committe on the Diagnosis and Classification of Diabetes in 1997 (7), which reviewed results of large epidemiological studies which correlated retinopathy with plasma glucose levels. Plasma glucose cut-off following OGTT was confirmed to be at 200 mg/dl (11.1 mmol/L), but the long-used fasting plasma glucose cut-off value of 140 mg/dl (7.8 mmol/l) was revised and reduced to 126 mg/dl (7.0 mmol/L). Besides acute markers of hyperlycemia, HbA_{1c} is used as a marker of chronic hyperglycemia, as it reflects blood glucose levels over the past 2-3 months. The use of HbA_{1c} for diagnostic purposes with a threshold of $\geq$6.5% was recommended by an International Expert Committee in 2003 (8) and is endorsed by the ADA (1) and WHO (9). However, WHO acknowledges that only levels above the established threshold of 6.5% are diagnostically indicative for diabetes but lower levels do not exclude diabetes otherwise diagnosed by plasma glucose levels (9). Accordingly, WHO recommends the use of FPG and 2h plasma

glucose after an OGTT, while the ADA recommends testing the HbA_{1c} first, followed by FPG and 2-PG. Another important discordance between WHO and ADA criteria is the threshold for IFG, which is set at 110 mg/dl and 100 mg/dl, respectively, as shown in Table 1.

Table 1. Diagnostic criteria for diabetes mellitus, IGT and IFG.

Condition	WHO criteria[3,9]	ADA criteria[1]
Diabetes mellitus	**HbA_{1c}:** Can be used as diagnostic parameter only if ≥6.5% (≥48 mmol/mol); lower value does not exclude diabetes *Recommended:* **FPG** ≥126 mg/dl (≥7 mmol/L) or **2h-PG** ≥200 mg/dl (≥11.1 mmol/L)	*Recommended:* **HbA_{1c}** ≥6.5% (≥48 mmol/mol) **FPG** ≥126 mg/dl (≥7 mmol/L) or **2h-PG** ≥200 mg/dl (≥11.1 mmol/L)
IGT	**2h-PG** 140 – 199 mg/dl (7.8 – 11.1 mmol/L)	**2h-PG** 140 – 199 mg/dl (7.8 – 11.1 mmol/L)
IFG	**FPG** 110-125 mg/dl (6.1 – 6.9 mmol/L)	**FPG** 100-125 mg/dl (5.6 – 6.9 mmol/L)

IGT – impaired glucose tolerance, IFG – impaired fasting glucose, 2h-PG – 2-hours post-load plasma glucose, FPG – fasting plasma glucose

2. Global burden of diabetes

Diabetes mellitus represents a serious public health problem worldwide as prevalence rates continute to rise. A pooled population-analysis of 751 population-based studies with from WHO showed that in 1980, the number of individuals suffering from disease was estimated to be 108 million. This number quadrupled until 2014, reaching 422 million. The increase in prevalence rates has been stronger in men (4,3% to 9%) than in women (5.0% to 7.9%) (10). According to the most recent data from the International Diabetes Federation, there were 451 million diseases individuals in 2017 and the number is projected to increase to 693 million by 2045.

Importantly, half of all cases worldwide in 2017 were undiagnosed, particularly in low-income countries, underlying the importance of strengthening screening measures in order to meet timely diagnosis and reduce the global diabetes burden. In terms of regional prevalence, it is projected that the highest increase will be seen in Africa, where the number of affected individuals will rise from 16 million in 2017 to 41 million in 2045, followed by Middle East and Africa (39 million in 2017 to 67 million in 20145) and South and Central America (26 million in 2017 to 42 million in 2045), representing relative increases of 156%, 72% and 62%, respectively (11).

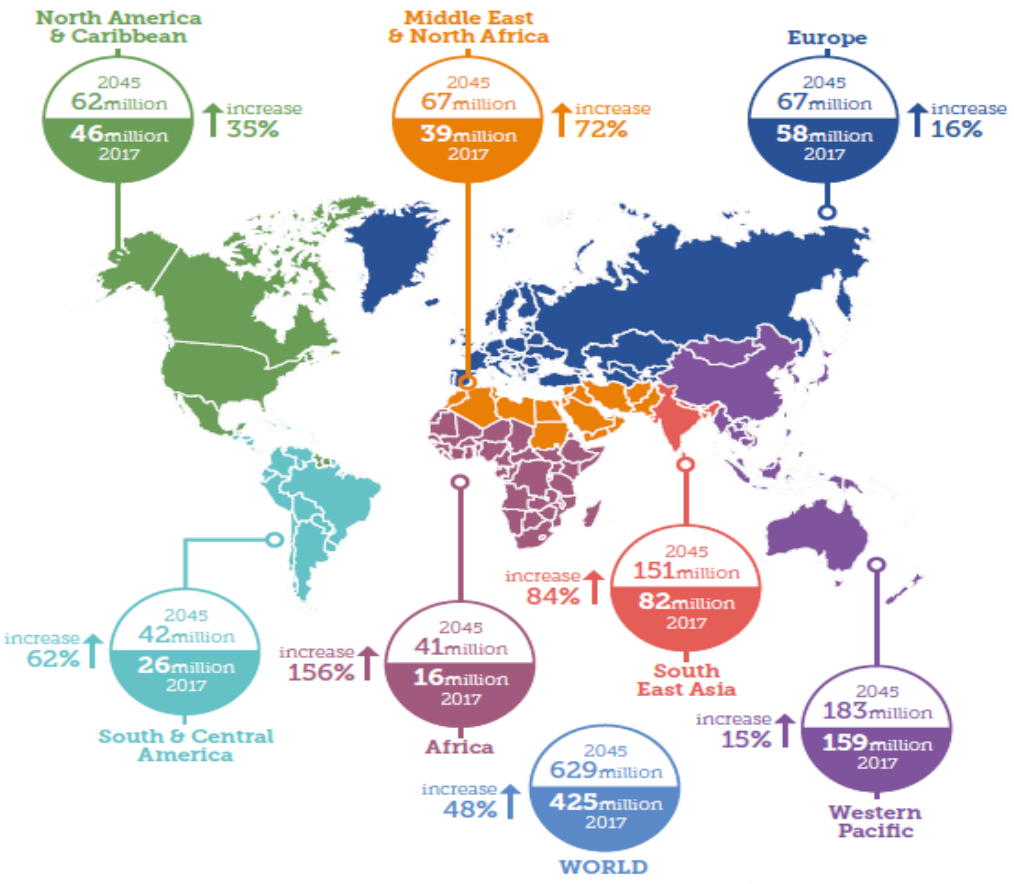

Figure 1. Global prevalence rates of diabetes in 2017 and projections for 2045 (Source: IDF Diabetes Atlas 2017)

Similarly, the number of individuals with IGT was estimated to be 374 million in 2017, and is projected to reach 587 million in 2045, with a clear trend of increasing prevalence with higher age (11). With regards to mortality, diabetes was responsible for 5.0 million deaths in 2017, accounting for 9.9% of the global all-cause mortality. About one third of these deaths occurred in individuals below 60 years of age, i.e. premature deaths.

3. Normal glucose metabolism

Under basal conditions glucose is utilized by insulin-dependent tissues at a rate of about 2.0 mg/kg/min (13). Approximately half of glucose is taken up and used by the brain (14). The rest is utilized by the liver and gastrointestinal tissues (15) and muscle (16) at equal proportions. Glucose stems from endogenous production to greatest part by the liver, while a smaller part is produced by the kidney (13, 17), which produce glucose at an amount to match the utilization rate (13). The production of glucose originates from two biochemical pathways – glycogenolysis (the breakdown of stored glycogen) and gluconeogenesis (de novo synthesis from other substrates, e.g. certain aminoacides) – to an equal amount (17, 18).

After a carbohydrate-containing meal, there is an increase in plasma glucose concentration, which leads to insulin secretion from the pancreatic beta-cells. The increaed glucose and insulin concentration lead to increased glucose uptake by the liver, gastrointestinal system and muscles, and at the same time inhibit endogenous production of glucose (13, 15, 19). The fat tissue takes up only a small fraction of the post-prandial glucose (5%), but its role in normal glucose homeostasis goes beyond direct glucose metabolization. Hyperinsulinemia exerts a strong antilipolytic effect, i.e. there is a decreased release of free fatty acids (FFA) from the adipose tissue and consequently lower FFA plasma circulation.

This further stimulates glucose utilization by the muscle tissue and supressess endogenous production of glucose. This way, plasma FFA levels play a crucial role in glucose metabolism (20-25).

4. Pathophysiology of type 2 diabetes mellitus

The pathopyhsiological characterization of diabetes mellitus stems from one of its main symptoms – polyuria. The name of the disease originates from the Greek word „diabainen“, which stands for „go through“, i.e. the increased urine output seen in diseased individuals. Later, the term „mellitus“ (honey) was added in the 17th century in order to differentiate this disease from the non-glycosuric diabetes insipidus (26).

First evidence pointing towards two different types of diabetes emerged in 1930s (27), and the introduction of radioimmunassay techniques in the 1950s definitely brought about the distinction between insulin-dependent and non-insulin dependent diabetes (28).

The central role of insulin resistance in type 2 diabetes was confirmed experimentally in 1979 in a seminal paper by DeFronzo et al., where they state that „tissue sensitivity to physiologic hyperinsulinemia is reduced in most maturity-onset diabetics” (29). Multiple studies in the 1980s and 1990s shed light on the interplay between obesity, insulin resistance and insulin secretion defects and clearly establish insulin resistance as a precursor state of type 2 diabetes (30-38).

Obesity is the single most important risk factor for developing type 2 diabetes. In the seminal Nurses Health Study, women who had a body mass index (BMI) above 35 had a 45-fold higher risk of eventually developing type 2 diabetes then those with a BMI below 22. In men, a BMI ≥ 35 was associated with a 42-times higher risk of diabetes compared to a BMI < 23.0 in a large US cohort study (39).

The association between obesity and increased insulin production has been known for more than five decades (40). The progression from normoglycemia over prediabetes to

overt type 2 diabetes follows a relatively long period of progressive loss of beta-cell function (41).

Obese individuals show significantly lower insulin sensitivity compared to lean individuals, a state which is initially compensated by an overproduction of insulin in an attempt to keep glucose levels in physiological range; this initial state is called euglycemic hyperinsulinemia (38). The onset of this state can lie back up to 15 years before overt disease (42). However, with progressive insulin resistance, the compensatory capacity of beta-cells becomes exhausted, leading to decreased insulin secretion – hyperglycemic hypoinsulinemia (38). Glucose levels at this stage are elevated following glucose load, which can be detected with an OGTT test. This impaired glucose tolerance is then soon followed by impaired fasting glucose, as the endogenous hepatic production of glucose does not get suppressed. These two conditions, collectivelly known as prediabetes, are accompanied by further reduction in beta-cell function with up to 80% reduced insulin secretion (43, 44), ultimately leading to beta-cell failure and development of overt type 2 diabetes (42). Prediabetes is a high-risk condition and up to 70% of prediabetic individuals will eventually progress to develop full-blown disease (45). However, this condition represents a window of opportunity to introduce interventions to reverse the condition and stop further progression to frank diabetes (46).

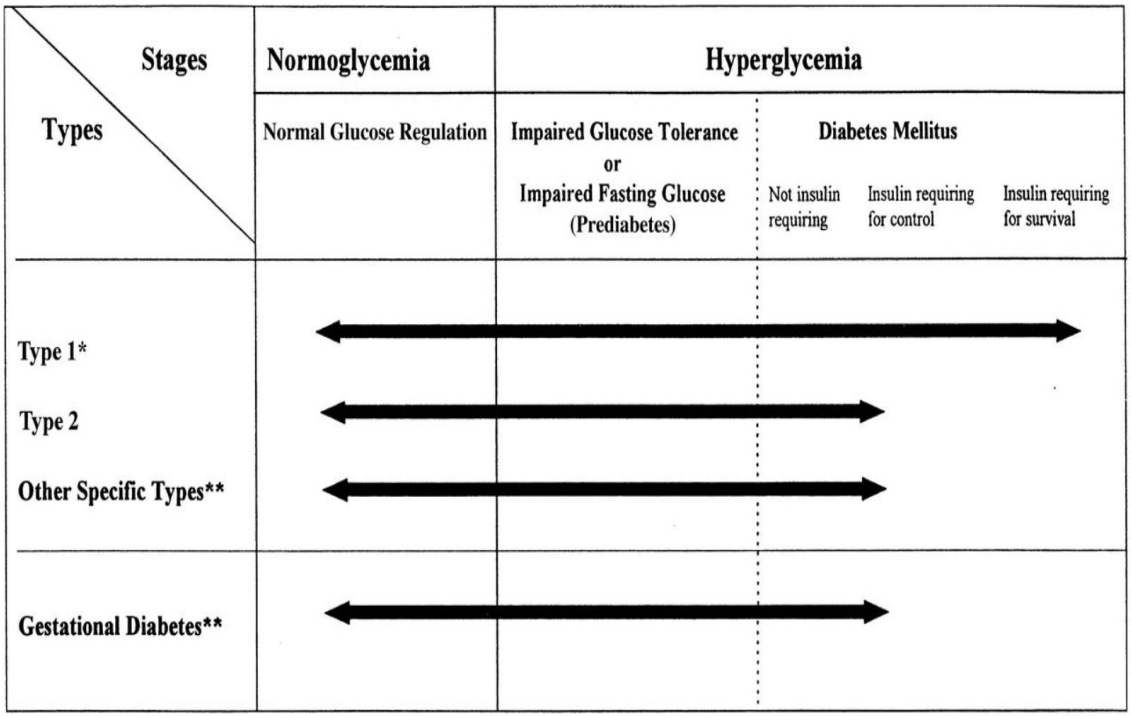

Figure 2. Stages and etiology of glycemic disorders (1)

5. Diabetic complications

Diabetes mellitus is associated with multiple life-limiting and life-threatening complications. Cardiovascular disease (CVD) is a highly prevalent complication seen in diabetes. The association between CVD and diabetes is long known from seminal studies. The DECODE study showed that both undiagnosed and diagnosed diabetes are associated with CVD-related mortality and that even within the normoglycemic range, CVD-mortality increases with rising plasma glucose levels (47-49). In the Framingham study, diabetic individuals had a two- to fourfold higher risk of developing coronary artery disease (CAD) or myocardial infarction (MI) (50). CVD mortality in non-diabetics is higher in men than in women, but co-occurrence of diabetes reduces the

gender difference (51). Therefore, diabetes is an independent risk factor for CVD mortality, including from stroke and heart disease (52).

The increase in risk is so dramatic that diabetic individuals with no prior history of CAD have the same mortality risk as patients with a prior MI (53). Not surprisingly, about 75% of all diabetics die from CVD (50). A plethora of factors are associated with the development of CVD in diabetes. Insulin resistance is not only the hallmark of type 2 diabetes, but is also central to CVD development and is implicated in a dissadvantageous atherogenic profile long before the onset of overt type 2 diabetes (54). Chronic subclinical inflammation is also associated with insulin resistance and is strongly correlated with atherosclerosis (55, 56). Another important factor is oxidative stress, which is chronically increased in states of insulin resistance, CVD and type 2 diabetes (57). Hypercoaguability due to plateles hyperaggregability and overactivation of coagulation factors also plays a major role (58, 59). Hypertension is approximately twice as frequent in diabetic individuals as compared to healthy individuals, and is often considered to be the single most important contributing factor to CVD in diabetics (60).

Diabetic dyslipidemia characterized by fasting and postprandial hypertriglyceridemia, low high-density lipoprotein (HDL) levels, elevated low-density lipoprotein (LDL) levels and predominance of small-dense LDL particles is a major risk factor for CVD as well (61). Obesity, which is common to the absolute majority of type 2 diabetic patients, elevates the risk for CVD in itself significantly, and combined with diabetes, the risk increases further (62). Other important factors include endothelial dysfunction with the formation of advanced glyosylation end products (AGEs) and macrophage cellular abnormalites which aggrevate insulin resistance and promote atherosclerotic plaque formation and rupture. Diabetic cardiomyopathy with ventricular dysfunction due to

structural and functional cardiac changes is also frequently observed and is associated with insulin resistance and hyperglycemia. The complex interplay between all these factors leads to damage to smaller and bigger blood vessels, so that diabetic CVD is characterized by concomitant micro- and macrovascular damage (63). Micro- and macrovascular complications in diabetes develop simultaneously (64).

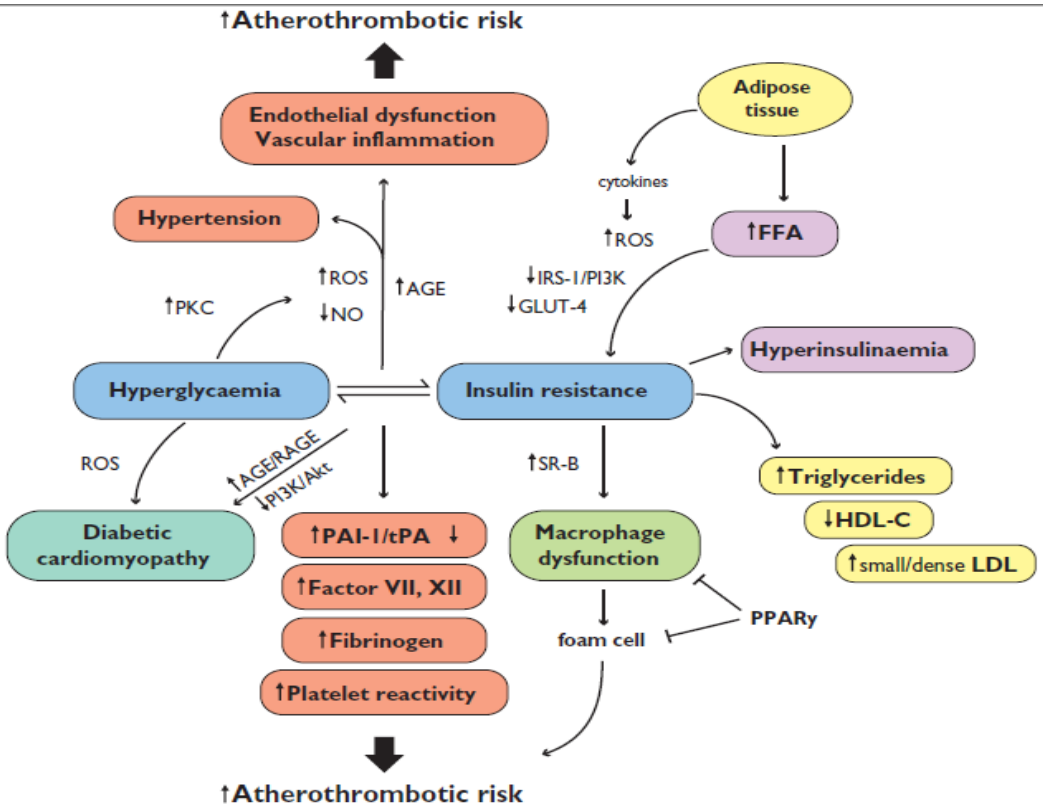

Figure 3. Pathophysiology of CVD in type 2 diabetes (63).

Microvascular complications frequently affect other organs as well, including the eyes, kidneys and nerves.

Diabetic retinopathy is the most commonly occurring microvascular complication of diabetes (65). It is associated with significant morbidity, as it increases the risk for

significant visual impairmant and blindness. In the United States, about 10.000 cases of blindness are caused by diabetic retinopathy every year (66). Globally, diabetic retinopathy affects more than 100 million people and the prevalence of related vision impairment and blindness rose by 64% annd 27% between 1990 and 2000, respectively. Remarkable overlap has been identified between diabetic retinopathy and incidence of CVD in several studies (68-71).

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) worldwide. Uncontrolled hyperglycemia, hypertension and dyslipidemia are suggested as the most important clinical determinants for the onset and progression of diabetic nepropathy. The natural course of nephropathy is characterized by the amount of albumin secreted in the urine. Microalbuminuria represents a condition of low, but abnormal levels of albumin present in the urine (30-299 mg/24 hours). Macroalbuminuria (≥ 300 mg/24 hours) represents overt nephropathy and is associated with an increased risk of ESRD (72). The majority of diabetic patients have microalbuminuria before the development of full-blown disease, and 20-40% of those will progress to overt nephropathy; another 20% will progress to develop ESRD (73, 74).

Diabetes causes a range of neuropathic disorders, including both acute and chronic syndromes. Polyneuropathy is characterized as a "symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvessel alterations as a result of chronic hyperglycemia exposure and cardiovascular risk covariates" (75). It is the most common neuropathic complication and affects up to 50% of diabetic individuals.

It is associated with foot ulceration and amputation, gait disturbances, neuropathic pain and a wide range of sensory symptoms (76, 77). Other neuropathic syndromes include diabetic autonomic neuropathy, cranial neuropathy, mononeuropathy and neuropathy-associated cachexia (78). Although hyperglycemia undoubtedly plays a role in the development of diabetic neuropathy, other factors are important, as good glucose control only minimally reduces the risk of its development (79). These include excess accumulation of adipose tissue, oxidative stress, mitochondrial dysfunction, AGEs development and inflammatory cytokines (80, 81).

6. Importance of adipose tissue in type 2 diabetes mellitus

The absolute majority of type 2 diabetic individuals are overweight or obese (82). The classical definition of overweight and obesity is based on the body mass indey (BMI), which categorizes individuals with a BMI >18.5 but <25.0 as normal weight, ≥25.0 - <30.0 as overweight and ≥30.0 as obese. However, BMI is based purely on body weight and fails to account for body composition or specifically for the amount of body fat mass. BMI has therefore been called into critic as a marker of metabolic health (83). Within the normal BMI range, individuals with higher body fat percentage have almost a 3-fold higher risk for developing diabetes (84). Body fat percentage is also a better predictor of disorders typically associated with diabetes, such as cardiovascular disease (85). However, not only excess adiposity predisposes to insulin resistance and diabetes, but also adipose tissue loss, such as in cases of lipodystrophy, pointing to a critical role of adipose tissue in maintaining glucose homeostasis and preserving insulin sensitivity (86, 87).

Adipose tissue has an important fat storage functions – it stores energy in the form of triglycerides for later use, such as in periods of extensive fasting or famine. However, the discovery of its secretory function led to a paradigm shift and the perception of adipose tissue from an inert storage tissue changed to an endocrine organ. Comprised of adipocytes, connective tissue, nerve tissue, stromovascular cells and different immune cells, adipose tissue forms a highly active metabolic site which not only responds to signals from endocrine hormones and central nervous system, but also secretes a plethora of signalling molecules that act locally (autocrine/paracrine) or systematically (endrocrine), which are collectivelly known as adipocytokines.

The diversity of functions of these molecules makes adipose tissue a key physiological component which acts on a variety of bodily functions, including energy metabolism, neuroendocrine function and immune function and an interesting intervention target (88).

Research in the area of adipocytokines was pioneered by Cook and Spiegelman with the discovery of adipsin in 1987 (89). In 1994, the most prominent adipocytokine, leptin, was discovered (90), which set the stage for a host of molecules to be discovered afterwards.

7. Adipose tissue structure and function

7.1 Types and distribution of adipose tissue

There are two main types of adipose tissue – white adipose tissue (WAT) and brown adipose tissue (BAT).

WAT is the predominant type and is responsible for energy storage and represents the largest energy reservoir in the body. In cases of excess incoming energy in form of caloric surplus or decreased energy output through reduced energy expenditure, WAT stores energy for later usage. Besides that, WAT is responsible for adipocytokine production and secretion, and it also serves as mechanical protection for vital organs (91). Metabolically, WAT can synthesize triglycerides (lipogenesis) and break them down in order to release fatty acids into the blood stream (lipolysis) as well as uptake glucose (92). WAT is generally located under the skin (subcatenous fat), primarily in the area of abdomen, thighs and buttocks; or intra-abdominally (visceral fat), where it sits primarily around the omentum, bowel and perirenal area. In addition, there are minor WAT depots located in other body areas, including the muscles, heart, face and bone marrow.

With varying WAT location in the body, its endocrine and metabolic function changes as well. Visceral fat secretes more molecules involved in inflammatory processes than subcutaneous fat and excess visceral fat accummulation generally predisposes to development of cardio-metabolic disorders, including diabetes. The distribution of WAT depends on several factors, including age (with increasing age there is a shift towards accummulation of visceral fat), sex (men tend to store more fat in the upper

body area – android distribution, while women store fat predominantly in the lower body area – gynoid distribution), and genetics.

BAT, on the other hand, is responsible for heat production through fatty acid oxidation. This is made possible through relatively high levels of expression of uncoupling protein-1 (UCP) in mitochrondria of BAT cells, which enables the uncoupling of respiratory chain to ATP synthetase enzyme. This unique property of metabolic heat production makes BAT an important component of thermoregulation through non-shivering thermogenesis. BAT is unique to mammals, and in humans, it is found at highest levels in fetuses and newborns, with levels rapidly dropping afterwards (93). In adults, higher than usual BAT levels are observed in individuals residing in cold climates, primarily in the cervical, supraclavicular and paravertebral regions (94, 95, 96).

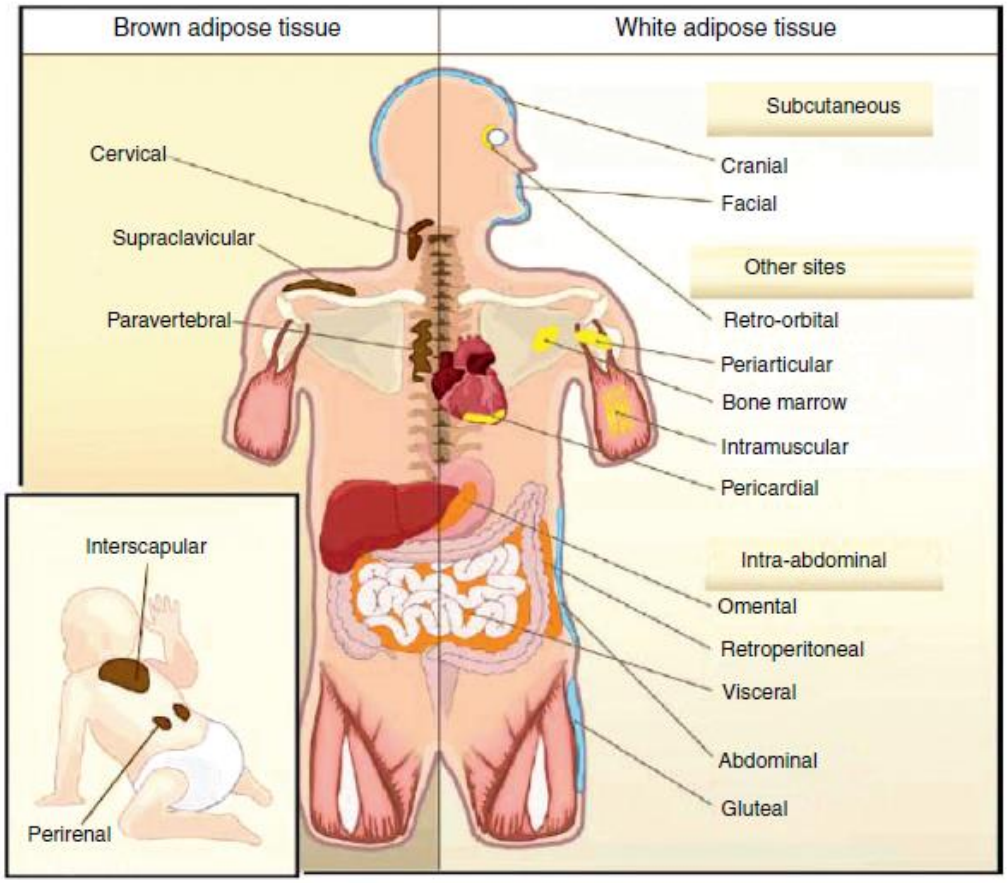

Figure 4. Distribution of WAT and BAT in the human body (91).

7.2. Cells of adipose tissue

7.2.1 Adipocytes

7.2.1.1 Development

Adipocytes are the main cell type of adipose tissue. White adipocytes are round-shape cells with a single lipid storage droplet, thin cytoplasm, low concentration of mitochondria and a flattened nucleus in the cell periphery. Their size depends on the amount of stored lipids so that their diameter ranges from 20 to 200 μm (97).

Adipocytes arise from mesenchymal stem cells, which are located in the perivascular fraction of the stromal compartment in adipose tissue. Apart from adipocytes, different cell lines are derived from these stem cells, including the chondroblasts, osteocytes and skeletar striated muscle cells (98). During gastrulation, the mesenchymal stem cells can express the myogenic transcription factor Myf5. Mesenchymal stem cells of the paraxial mesoderm express Myf5 and develop into brown adipocytes or striated muscle cells. On the other hand, stem cells of the lateral mesoderm do not express Myf5 and differentiate into white adipocytes and vascular-associated pericytes (99). A unique type of adipocytes was recently discovered, termed brown-like or beige adipocytes. These cells develop from vascular cells residing in the adipose tissue and express low UCP-1 levels under basal conditions. Following beta-adrenergic stimulation associated with cold exposure or exercise, beige adipocytes can significantly increase UCP-1 levels and are involved in thermogenesis (100, 101).

Adipogenesis starts through activation of the *CCAT/enhancer-binding protein β* (C/EBPβ) gene expression. The resulting protein binds on the promoters of *peroxisome proliferator-activated receptor γ* (PPARγ) and C/EBPα and activates the expression of a protein complex which further activates a large number of genes typical for adipocyte phenotype (102, 103).

Through the expression of *steroid response element-binding protein 1c* (SREBP1c) in precursor cells that do not express CD137 antigen on the cell surface (CDd137-negative), a set of genes is activated which leads to final differentiation into white adipocytes (104, 105). On the other hand, in CD137-positive precursor cells, there is an

expression of UCP-1, leading to the proliferation of beige adipocytes (106, 107). In Myf5 expressing stem cells, PPARγ activates *PRD1-BF-1-RIZ1 homologous domain containing protein-16* (PRDM16), which also results in the expression of UCP-1 and leads to differentiation into brown adipocytes (108).

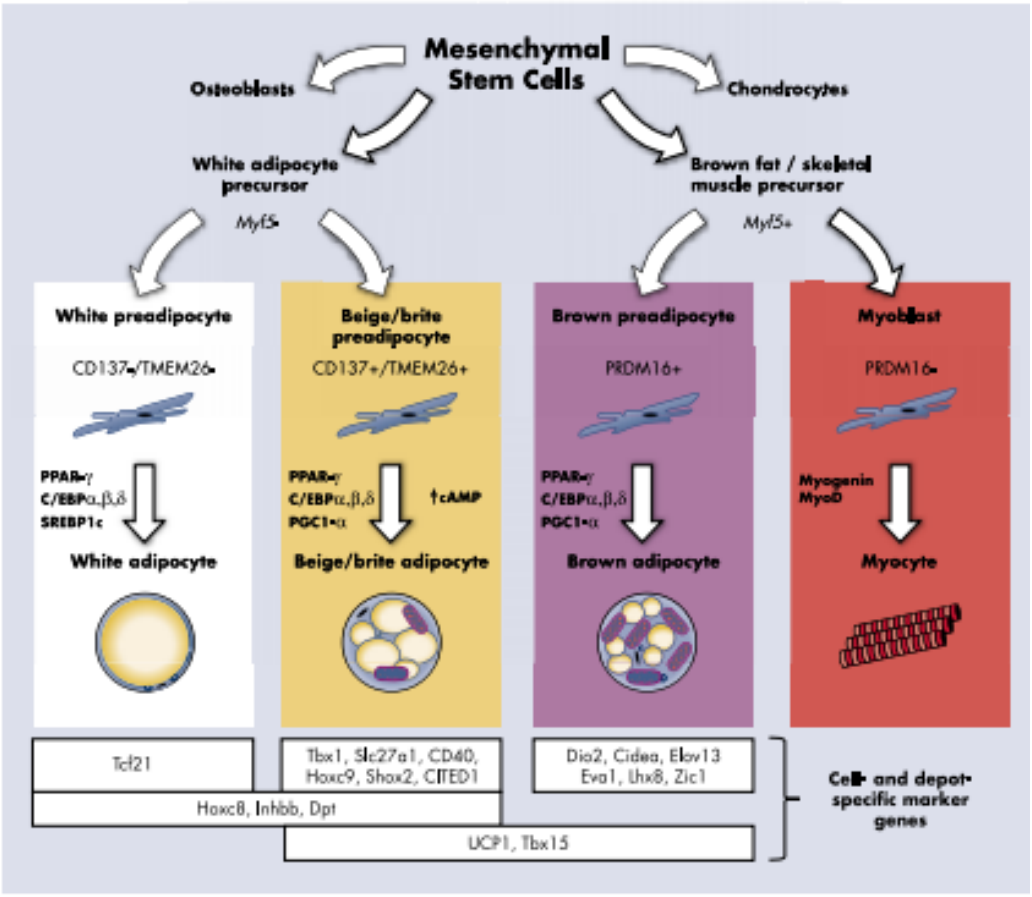

Figure 5. Development of adipocytes from mesenchymal stem cells (109).

7.2.1.2 Size and number

Adipose tissue can expand by increasing the size of adipocytes (hypertrophy) or by increasing cell number (hyperplasia). Hyperplasia is the primary growth mechanism in childhood. With increasing age, hypertrophy becomes the primary growth mechanism – adipocytes can grow substantially by storing lipids to compensate for the storage need with excess energy, such as seen in obesity (110).

There is significant variance in adipocyte size, even in samples taken from the same body location. The propensity of some adipocytes to accummulate more lipids is due to differences in expression of genes which regulate lipogenesis. Predominance of larger adipocytes is associated with proinflammtory profile and metabolic syndrome, while a higher number of smaller adipocytes is associated with better insulin sensitivity (111).

There is an upper limit of how much lipids can be stored intracellulary in adipocytes; once the storage need exceeds the growth capacity, there is ectopic fat accummulation, as seen in obesity (112).

While adipocyte volume is determined through lipogenesis and lipolysis, adipocyte number is determined by the proliferation, differentiation and apoptosis of preadipocytes and apoptosis of adipocytes. Preadipocytes proliferation, differentation and apoptosis vary according to location of fat tissue, affecting fat distribution patterns and adipose tissue plasticity in the body. While subcutaneous adipose tissue contains

approximately an equal proportion of slowly and fast proliferating preadipocytes, visceral adipose tissue contains predominantly the slowly proliferating cell type (113).

Remarkably, in one given individual, the number of adipoyctes stays the same regardless of age, body weight and leanness status. This ows to the fact that the actual number of adipocytes is set early in life, during childhood and early adolescence, and it stays constant throughout life. The seminal study by Spalding et al. (114) demonstrated that the anual turnover of adipocytes is about 10% and the renewal rate does not change even in obesity.

7.2.1.3 Transdifferentation

Brown and beige adipocytes can develop through differentation from precursor cells, including Myf5-positive precursor cells and Myf5-negative white adipocyte precursor cells or through differentation from white adipocytes directly (115). In humans, there is generally a shift towards more white and less brown adipocytes with increasing age. However, at any given age, the transdifferentiation between white and brown adipocytes is possible in a bidirectional manner (116). The direction of the process is regulated by the energy status (117), acute food intake (118) and thermogenesis (119). Energy excess, cold exposure and beta-adrenergic stimulation promote the differentiation of white adipocytes into brown adipocytes, while hunger and caloric deficit promote the creation of white adipocytes (120, 121). The process of transdifferentation is regulated primarily through PPARγ and ist downstream signaling, includijng PGC-1α, PRDM16 i UCP-1 (122, 123). The activation of PPARγ signalling pathway is also crucial in several

other process, including the supression of inflammation through decreased production of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα), increased metabolic efficiency (increased uptake of glucose in skeletal muscle and reduced hepatic gluconeogenesis as well as decreased plasma concentration of free fatty acids), improved LDL/HDL ratio, increased production of adiponectin and reduced leptin production (124). Several pharmacologic agents, such as thiazolidinediones, activate the PPARγ pathway, promoting the development of brown adipocytes and leading to positive metabolic effects (125, 126).

7.2.2 Immune cells in the adipose tissue

First studies pointing towards the role of immune cells in the adipose tissue were conducted in mice and showed that obesity is associated with infiltration of immune cells, such as macrophages, in WAT (127). Cells of both the innate and adaptive immune systems accummulate in WAT (127-130). The most abundant immune cells are macrophages, as they represent more than half of leukocytes in lean and obese individuals (127).

In lean humans, macrophages make up about 5% of all WAT cells. However, in obesity, this number can go as high as 505 (131). Accummulation of macrophages goes hand in hand with the expansion of adipose tissue, but the relationship also holds in reverse as well, as with weight loss, there is substantial reduction in the numbers of macrophages in subcutaenous as well as visceral fat (132-134).

Obesity alters not only the number of macrophages, but also their morphology and localization within the adipose tissue. In lean humans, macrophages are small in size and

interspersed between adipocytes. In obesity, they form aggregates around lipid droplets resulting from dead adipocytes (135-137).

The accummulation of macrophages in WAT results from increased synthesis of chemokines by adipocytes in obesity, mainly the monocyte chemoattractant protein-1 (MCP-1), which leads to infiltration of monocytes from blood into the tissue (138). Leptin from adipocytes stimulate the production of adhesion molecules, such as interecellular adhesion molecule-1 (ICAM-1) on endothelial cells, which enables the adhesion of monocytes on the endothelium (139). Adipocytes futher secrete colony stimulating factor-1 (CSF-1), which promotes the differentation of monocytes into mature macrophages (140).

The phenotype of macrophages may be pro-inflammatory or anti-inflammatory and is dependent on the activation pathway. Macrophages activated through cytokines secreted by T-helper cells, such as interferon-gamma (INFγ), develop into the pro-inflammatory M1 phenotype. On the other side, macrophages activated by IL-4 or IL-13 develop into the anti-inflammatory M2 phenotype (141). In type 2 diabetics, the dysbalance between the two phenotypes is also observed in peripheral blood and bone marrow and has clinical relevance in diabetic microvessel disease (142). M1 macrophages are the main cell type responsible for the secretion of pro-inflammatory cytokines, including TNF-alpha, IL-1 beta and IL-6 (143).

Although macrophages represent the largest and the best studied myeloid cell type in the adipose tissue, obesity and diabetes are associated with changes in other cells as

well. Neutrophils make up less than 1% of immune cells in the adipose tissue of lean animals, but in obesity, their number can double. In rat models of obesity and metabolic syndrome, neutrophil infilitration sets on very early and there is altered cell production, function and phenotype (144). While some evidence shows that neutrophil infiltration is transient (145), other show that it is a chronic state (146). The role of accummulation and activation of neutrophils in adipose tissue in obesity-associated pathogenesis has also been demonstrated in humans (147).

Dendritic cells are closely related to macrophages, but their role in adipose tissue is poorly understood. Recent evidence points towards an accummulation of dendritic cells in the adipose tissue of obese individuals (148) and an independent role of dendritic cells in obesity-associated infammation (149).

Mast cells, which are normally involved in allergic reactions, are also increased in adipose tissue of obese as compared to lean individuals and their occurence is in close association with blood vessels (150).

Their function has been implicated in chronic inflammation and fibrosis of WAT, and their number and phenotype correlates with diabetic parameters (151).

Of the cells of the inate immune system, eosinophiles are the only type of cells whose number is reduced in the adipose tissue in obesity and diabetes. As a consequence, the reduction in their number leads to decreased activation of M2 macrophages and hyperactivation of M1 macrophages, resulting in inflammation (152).

Obesity and associated metabolic disorders are also associated with increased number of T-cells (153) and B-cells in the adipose tissue (154, 155). CD3+ T-cells are the most common occuring immune cells after macrophages in adipose tissue in cases of obesity (152). CD4+ and CD8+ T-cells both infilitrate visceral and subcutaneous adipose tissue. However, pro-inflammatory T-helper cells (Th1) reside primarily in the visceral fat, contributing to the general pro-inflammatory nature of visceral adipose tissue, and their abundance there is associated with C-reactive protein as marker of inflammation. To the contrary, occurence of anti-inflammatory T-helper cells (Th2) in visceral adipose tissue is inversely associated with systemic inflammation and insulin resistance (156).

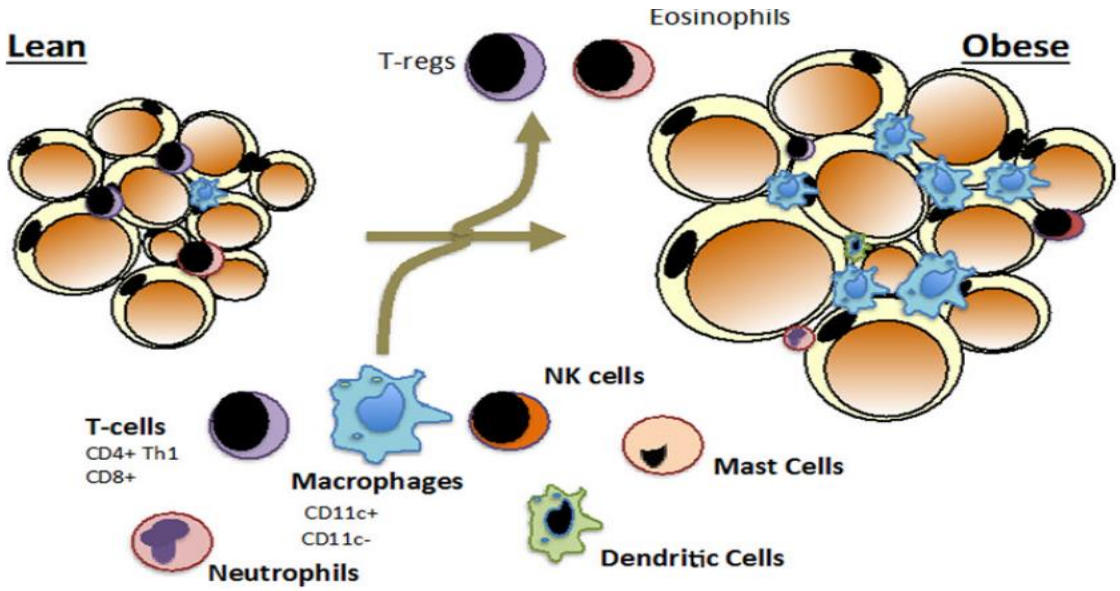

Figure 6. Immune cells of adipose tissue in lean and obese individuals (157)

8. Adipokines

Adipocytes, preadipocytes, immune and endothelial cells and fibroblasts all secrete bioactive peptides which can exert both local and systemic effects, collectively known as adipokines. There have been more than 600 adipokines discovered until today, while most of them still have to be functionally characterized (158). Adipokines affect a plethora of physiological proccesses. Within the adipose tissue, they modulate adipogenesis, migration of immune cells into the adipose tissue and metabolic function of adipocytes. Their systemic effects are exerted in a variety of target organs, e.g. the brain, heart, vessels, liver and muscles, where they affect appetite and satiety, energy expenditure, inflammation, insulin secretion and sensitivity and metabolic homeostasis and many other bodily processes, all of which are clinically relevant for diabetes and related comorbidities (159, 160). Interestingly, the function of majority of adipokines is still not known. With a growing need to develop effective interventions to tackle the global burden of diabetes, elucidating the function, molecular targets and clinical relevance of these adipokines is important.

Adipokines have recetnly been put at the spotlight of obesity research as their secretion pattern from the adipose tissue is associated with individual risk of developing cardio-metabolic disorders typically associated with obesity. With the onset of dysfunction in adipose tissue there is a remarkable shift in adipokine secretion towards a diabetogenic, pro-inflammatory and atherogenic profile (161-63). The clinical relevance of adipokines in diabetes is not restricted to obese individuals – in non-obese diabetic individuals, there is signficnt change in circulating levels of adipokines (164).

Well-characterized adipokines have already been introduced into the clinical practice. They can serve as biomarkers of individual treatment success both for pharmacotherapy and lifestyle interventions (165). Others, such as dipeptidyl-peptidase 4 (DPP-4) have been introduced as drug targets in diabetes (166). Having been recognized as a central integrator of glucose homeostasis and other physiological processes relevant in diabetes, manipulation of adipokine profile so as to decrease the circulation of pro-glycemic and increase the circulation of anti-glycemic adipokines in diseased individuals and those at risk has been acknowledged as a promising strategy in developing tailored therapies (167).

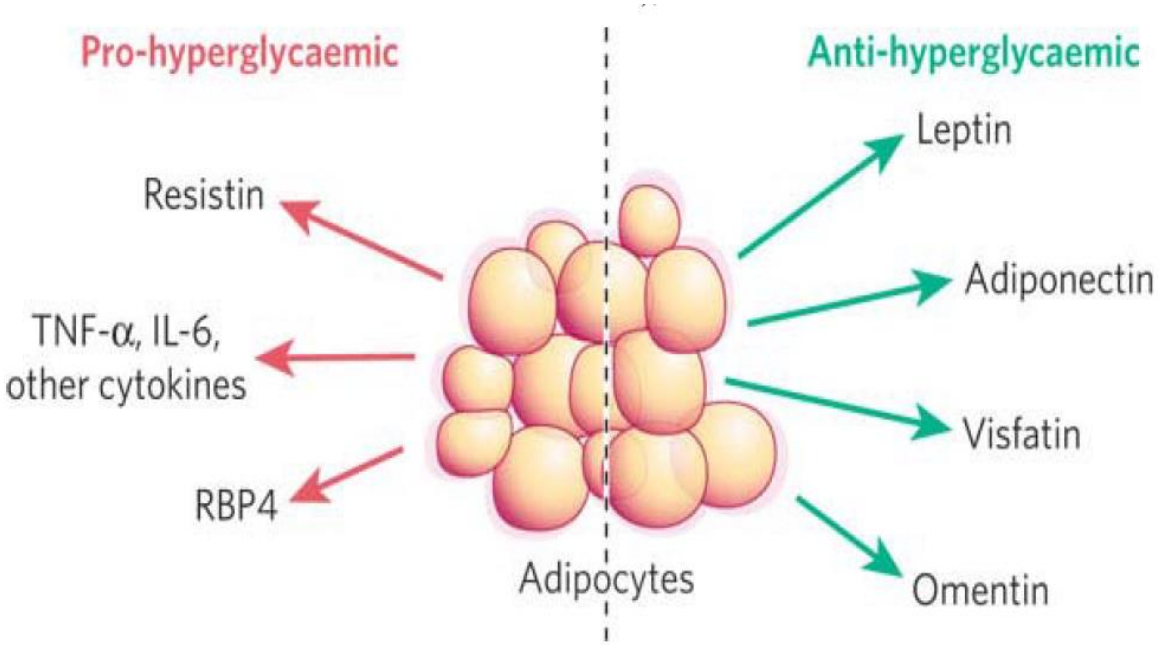

Figure 7. Influence of adipokines on glucose homeostasis (167).

8.1 Adiponectin

Adiponectin is an adipokine which is secreted from mature adipocytes. Its effects are multifold, including an increase in insulin sensitivity, regulation of lipid metabolism, cardioprotective and anti-atherogen, anti-inflammatory1 and tumor suppressing effects (168-172).

8.1.1 Production and metabolism

Adiponectin is encoded by a gene located on 3q27, which also hosts a set of other genes coding for proteins involved in the pathogenesis of cardio-metabolic disorders (173). Research indicates that the secretion and circulating levels of adiponectin are genetically determined (174).

The primary production site of adiponectin are mature adipocytes, while preadipocytes secrete significantly less adiponectin (175). Besides adipose tissue, minimal amounts of adiponectin are secreted from the epithelium of the colon, liver and striated muscles (176). Females have higher circulating levels of adiponectin as compared to males (177, 178), as male sex hormones supress the production (179). Adiponectin concentration in the serum ranges between 2-20 µg/ml (179) and changes in a circadian manner, as highest concentrations are observed early in the day and lowest at night (180).

Overweight and obese individuals have paradoxically lower adiponectin levels than normal-weight individuals despite higher amount of adipose tissue, its main production site. This is due to decreased secretion of adiponectin in adipocytes in obesity, so that generally, an inverse association exists between circulating adiponectin levels and BMI (181-183).

Decreased production of adiponectin in adipocytes of obese individuals is also evident through lower concentrations of intracellular mRNA in adipose tissue samples of such individuals (175, 184).

There seems to be a difference in the secretion levels of adiponectin in different sites of SAT (184). Some studies suggest higher secretion from the SAT than VAT (185), but contrary evidence also exists (186, 187). Nevertheless, visceral type of obesity and ectopic fat accummulation in the liver are independent predictors of circulating adiponectin levels (188). Weight loss through caloric restriction (168) or through bariatric surgery (186) leads to increased levels of adiponectin, while there is inconsistent evidence with regards to effects of exercise (189, 190).

8.1.2 Isoforms and receptors

The structure of adiponectin is heterogenous. In blood plasma, adiponectin circulates in globular or longitudinal form. The globular form enhances fat oxidation in the liver and striated muscles while the longitudinal form decreases the hepatic production of glucose (190, 191). Furthermore, there are three different molecular forms of adiponectin: low molecular weight (LMW), which is a trimer; medium molecular weight (MMW), a hexamer, and the 12-18 monomeric units containing high molecular weight (HMW) adiponectin (192). The HMW form ist he perdominant form intracellulary in adipocytes, while circulating adiponectin is mostly LMW (193).

Once in the circulation, the different isoforms of adiponectin do not interconvert, so that the ratio between them depends on the secretion pattern from adipocytes. HMW representes the most active form of adiponectin and is typically reduced in obesity and associated disorders. Higher levels of circulating adiponectin in women is due to typically higher concentrations of HMW (194).

There are two types of adiponectin receptors: type 1 (AdipoR1) and type 2 (AdipoR2). Type 1 receptors are expressed in tissues throughout the body, but predominantly in striated muscles. Type 2 receptors are expressed primarily in the liver, while beta-cells of the pancreatic isle (195), macrophage and atherosclerotic plaque lesions (196) and the central nervous system (170) contain an equal proportion of both receptor types.

AdipoR1 receptors have a higher affinity towards the globular form of adiponectin, and through the activation of one of the key regulators of metabolism - the enzyme 5´-adenosine monophosphate activated protein kinase (AMPK) induce a higher uptake of glucose and beta-oxidation of free fatty acids in the muscle cells (170). Adiponectin effects in the liver which are mediated through AdipoR2 receptors also involve AMPK and lead to decreased hepatic glucose output and increased insulin sensitivity in the liver (197). It is suggested that HMW adiponectin acts primarily on the liver and that its primary effect is a hepatic increase in insulin sensitivity (198). Negative energy balance through decreased food intake or physical activity leads to increased expression of adiponectin receptors in the liver and muscles (199).

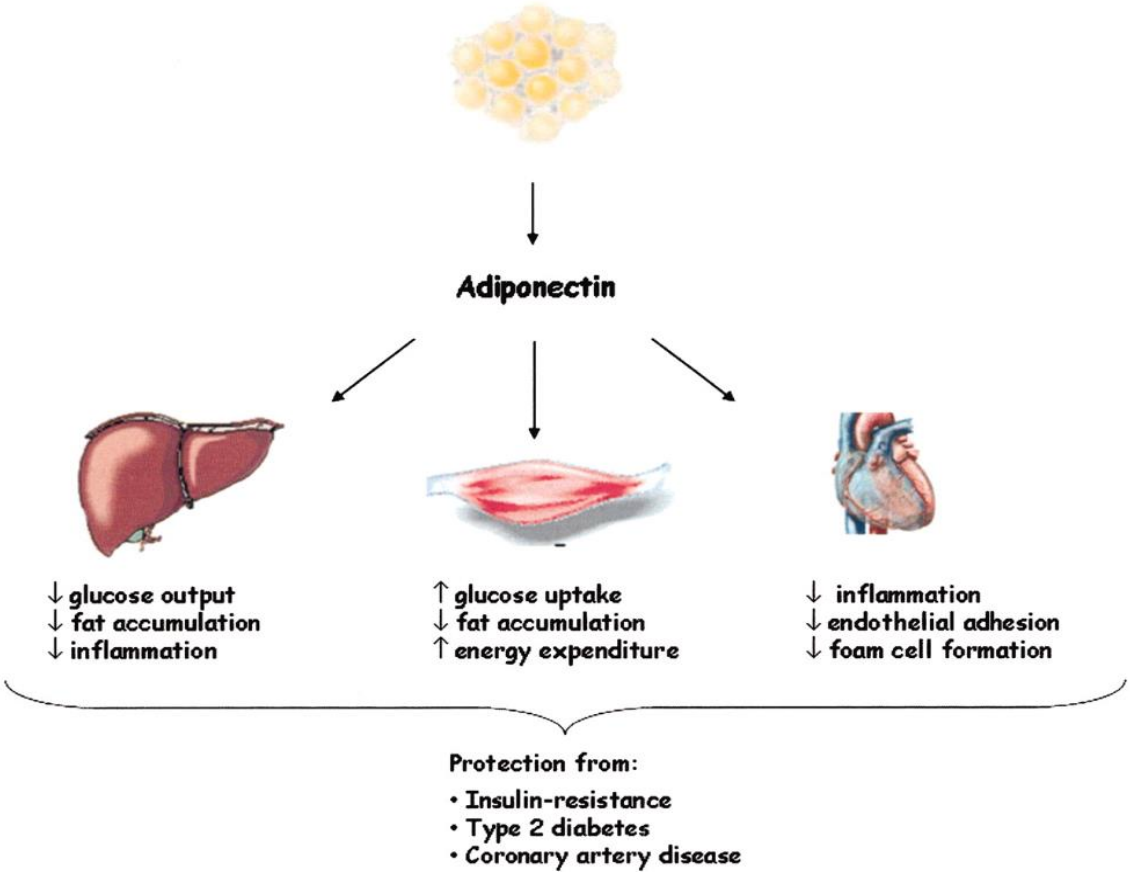

Figure 8. Physiological effects of adiponectin (200)

8.1.3 Role of adiponectin in prediabetes and diabetes

Hypoadiponectinaemia is positively associated with prediabetes and type 2 diabetes, independently of sex, BMI, measurement techniques of adiponectin, diagnostic criteria for diabetes and the length of follow-up (201). First-degree relatives of diabetic individuals have a lower secretion of adiponectin from adipose tissue and a higher degree of insulin resistance (202). An up-regulation of the adiponectin gene correlates with higher insulin sensitivity in healthy individuals but not in relatives of diabetics (203).

A decrease in circulatin adiponectin is an early predictor of insulin resistance (204). In reverse, improved insulin sensitivity induced by weight loss or pharmacotherapy goes together with higher adiponectin levels. Although the correlation between adiponectin levels and the insulin resistance are well-established, the exact mechanisms are still not elucidated (205). The metabolic effects of adiponectin, such as increased oxidation of free fatty acids in the liver, decreased hepatic glucose output and higher uptake of glucose and free fatty acids in the skeletal muscle all contribute to preserving insulin sensitivity in healthy individuals (206). Through its receptors in the skeletal muscles and liver, adiponectin modulates the activity of *adaptor protein containing PH domain, PTB domain and leucin zipper 1* (APPL1), which results in the phosphorylation of AMPK, p38 mitogen-activated protein kinase (p-38-MAPK) and Ras-related protein Rab5, thereby increasing beta-oxidation of free fatty acids, higher glucose uptake

through the translocation of glucose transporter 4 (GLUT4) in the muscles and decreased hepatic production of glucose through suppressed gluconeogenesis (207).

In obese individuals, inflammation mediated through TNF-alpha and other pro-inflammatory cytokines are negatively correlated with adiponectin levels and insulin sensitivity (208). The complex inter-play between adiponectin and glucose/insulin levels is subject of intensive research. Insulin leads to increased production of adiponectin in adipocytes (209), but higher levels of insulin suppress its production (210). The anti-glycemic effects of thiazolidinediones are partly through the stimulation of adiponectin secretion through PPAR, while other anti-diabetic drugs, such as metformin, exert their effects independently of adiponectin (211,212).
Adiponectin is associated with early dearrangements in glucose metabolism, as hypoadiponectinemia is a powerful risk marker of incident prediabetes in healthy individuals (213).

Likewise, low adiponectin levels correlate with IFG (214) and IGT (215). Furthermore, changes in adiponectin levels, together with changes in serum levels of 25(OH)D, the active form of vitamin D, are correlated with serum insulin levels. Low adiponectin is an independent risk factor for progression of prediabetes and type 2 diabetes (216).

In reverse, higher circulating levels of adiponectin are protective against glucose metabolism disorders and cardivascular disease (217). That adiponectin is at the intersection of the heart-metabolism axis has been confirmed in studies involving individuals who have suffered a myocardial infarction (218) and stroke (219), where

measurement of adiponectin levels adds significant prognostic value to glucose levels in predicting the risk of incident type 2 diabetes in this population.

8.1.4 Adiponectin and diabetic complications

Adiponectin is an anti-inflammatory adipokine and its levels are negatively associated with C-reactive protein (CRP), TNF-alpha and IL-6, and positively associated with other anti-iflammatory cytokines, such as IL-10 (220-222).

The anti-inflammatory effects are mediated through a number of mechanism and across different cells and tissues. Adiponectin suppresses CRP and TNF-α expression in adipose tissue, whereas TNF-α and IL-6, in return, suppress adiponectin expression. In cells of vascular endothelium, adiponectin inhibits the production of IL-8, vascular cell adhesion molecule-1 (VCAM-1) and reactive oxygen species (ROS) through cAMP-PKA-dependent signaling. AMPK-mediated stimulaton of endothelial nitrix oxide synthase (eNOS), which produces the vasodilatating agend NO is also an adiponectin effect.

In cells of the cardiac muscle, TNF-α production is suppressed through the stimulation of prostaglandin E2 (PGE2) production in the cyclooxygenase (COX) pathway. Furthermore, adiponectin decreases the production of TNF-α and IL-6 by suppressing the nuclear factor-kappa B (NF-κB), and increases the production of IL-10, which further leads to stimulation of tissue inhibitor of matrix metalloprotease-1 (TIMP-1). Foam cell formation and the deposition of LDL cholesterol is also decreased by

adiponectin through inhibition the expression of class-A scavenger receptor (SR-A) (223).

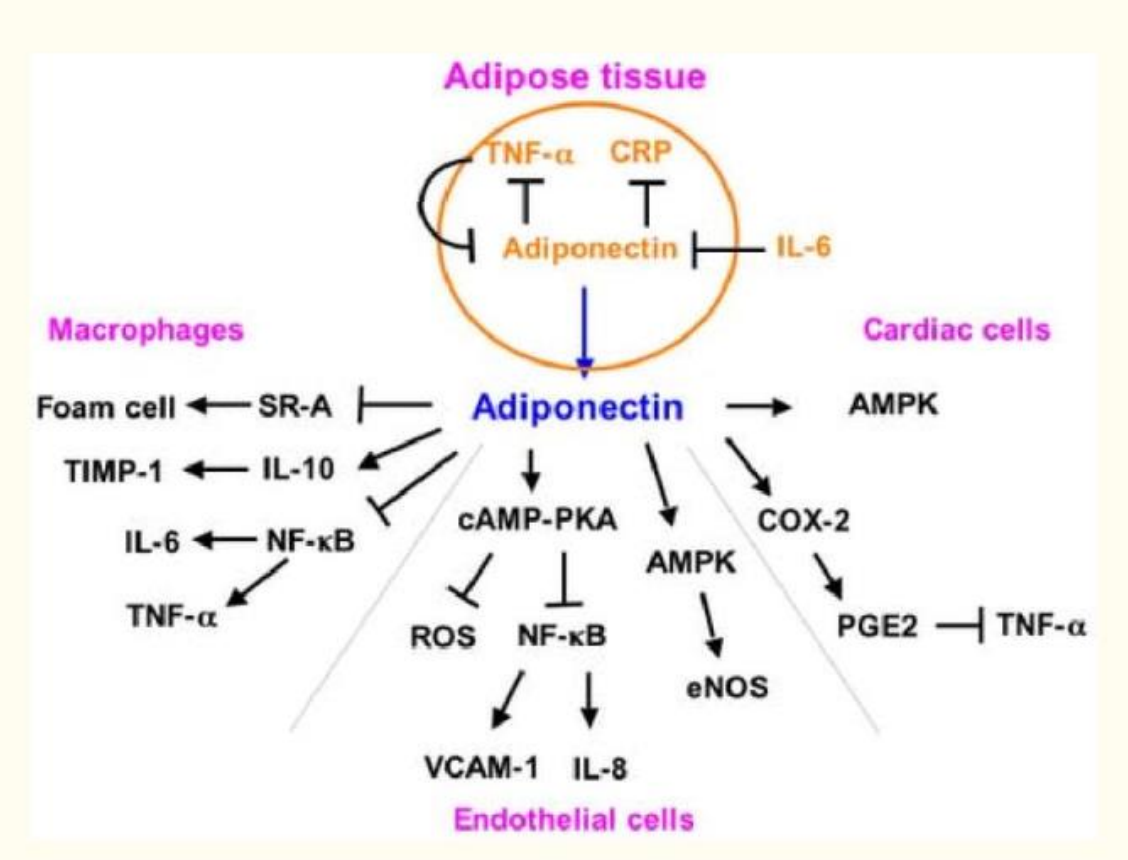

Figure 9. Anti-inflammatory effects of adiponectin (223)

The anti-atherogenic properties of adiponectin are in large part due to ist suppressory effects on signalling molecules involved in the formation of atherosclerotic plaque, including SR-A (223), ICAM-1, VCAM-1, E-selectin (224) and NF-κB (225).

Due to its anti-inflammatory and anti-atherogenic effects, adiponectin is also cardioprotective. Hypoadiponectinamia in men with serum levels below 4 µg/ml is independently associated with a 2-fold higher prevalence of coronary artery disease

(CAD) (226). Furthermore, low adiponectin levels contribute to vulnerability of atherosclerotic plaque in coronary vessels and correlate with the occurence of more severe coronary lesions both in patients with stable CAD and with an acute coronary syndrome (ACS) (227).

Measuring adiponectin levels adds prognostic value in determining the risk for CAD and ACS, such as MI (228). As there is significant co-occurence of CAD, ACS and type 2 diabetes, these associations are important as they underline that hypoadiponectinaemia is a significant factor linking type 2 diabetes with CAD, atherosclerorotic plaque vulnerability and rupture and ACS (229).

The reduction in the rate of MI with thaizolidinediones in patients with CAD, as demonstrated with pioglitazone in the PROactive trial, may be in part due to the adiponectin-increasing effects of this drug class (230).

Adiponectin also plays a major role in essential hypertension. In non-diabetic individuals, levels are significantly lower in hypertensive individuals as compared to normotensive individuals (231). Lower adiponectin levels are associated with a higher risk for hypertension regardless of sex, age and BMI (232). Adiponectin also correlates with the progression of hypertension (233). These associations also hold for type 2 diabetics (234).

Mechanistically, adiponectin exerts ist blood-pressure regulating effects through increased production of NO (235) and by decreasing the sympatic output of the CNS (236). Studies in mice demonstrate that decreased adiponectin directly contributes to

obesity-associated hypertension and replenishment of adiponectin significantly normalizes blood pressure (237).

Serum adiponectin levels are also associated with diabetic microangiopathy and retinopathy incidence and severity (238), diabetic polyneuropathy (239), diabetic foot (240) and nephropathy (241).

8.2 Leptin

Leptin was discovered in 1994 through positional cloning of the obese (ob) gene in mice and ist human analogue. In the seminal paper, it was suggested that leptin acts as an adipostat as a key regulator of the size of adipose tissue (90). Often called the master hormone of energy homeostatis, leptin is the central regulator of adipose tissue stores by integrating energy expenditure with food intake (242).

8.2.1 Production and metabolism

In humans, leptin is encoded by a gene located on the chromosome 7, which codes for a protein with 167 amino acids with a molecular weight of 16 kDa (90, 243). The primary production site of leptin are white adipocytes of the subcutaneous adipose tissue (242). Minor concentrations are also secreted from other organs such as stomach (244).

As adiponectin, leptin secretion pattern also follows a circadian rhytmus both in healthy lean and obese and type 2 diabetic individuals, levels generally peaking during the night and being lowest in the afternoon (245).

Generally, circulating leptin levels reflect body adiposity and serve as a signal of body energy stores to the brain. Leptin concentration in the cerebral spinal fluid directly correlates with circulating plasma levels and body fat levels.

However, the brain transporter proteins for leptin are saturable, and the efficiency of the transport over the blood-brain barrier decreases with higher circulating plasma levels, suggesting a leptin resistance in obese individuals (246).

Leptin secretion is also sensitive to acute perturbances in food intake. Relative or absolute leptin deficiency causes neuroendocrine effects highly similar to those of starvation. Prolonged fasting causes a significant fall in leptin, and restoration of leptin levels reverses all the effects of fasting on the gonadal, thyroid and adrenal axes, suggesting that leptin is the key regulator of starvation-induced effects (247).

In reverse, food intake or insulin injection transiently increase the production of leptin (248). Nevertheless, circulating levels correlate mostly with long-term energy stores reflected in the amount of body fat stores (249).

As with adiponectin, women have higher circulating levels of leptin compared to men, which is due to the suppressive effects of androgens and stimulatory effects of estrogens on its secretion (250, 251) as well as due to normally higher levels of subcutaneous versus visceral adipose tissue in women (252).

8.2.2 Leptin and other body hormones

Given that leptin regulates key pathways in conservation or dissipation of body energy, its production is influenced by other hormones involved in energy metabolism. Post-prandial insulin secretion up-regulates leptin production, sending a signal to the brain that the body is fed (248). In states of prolonged hyperinsulinemia under in rats, there is an increase in serum leptin levels and a decrease in food intake (253).

Similarly, in healthy humans, overfeeding leads to a rise in leptin levels. Short-term overfeeding for 12 hours increases leptin levels by 40%, which is transient and dissappears once a normal feeding pattern is again established. To the contrary, long-term overfeeding, such that produces a 10% body weight gain, causes a significant 3-fold increase in basal leptin levels in healthy individuals, where the rise in levels was correlated with increase in body fat levels. This underpins that leptin levels are sensitive to both acute and chronic changes in energy intake and that long-term leptin levels follow changes in body fat levels (253). However, even overfeeding for only a couple of days can induce leptin resistance, which goes hand in hand with insulin resistance (254).

The interaction between the insulin-leptin route is demonstrated with changes in the production rate of leptin with overfeeding with different macronutrient composition. Overfeeding specifically on carbohydrates, which typically leads to a rise in insulin levels, has the most pronounced impact on leptin levels, whereas fat, which typically does not lead to insulin secretion, does not confer any change (256).

Concomitant changes in insulin and leptin levels as reaction to overfeeding do not come as surprise, as both hormones signal to the body that it is fed. However, in the context of obesity, hyprephagia and chronic energy and nutrient excess, insulin and leptin resistance develop, as there is reduced sensibility to the physiological signal despite higher circulating levels of the hormones (246).

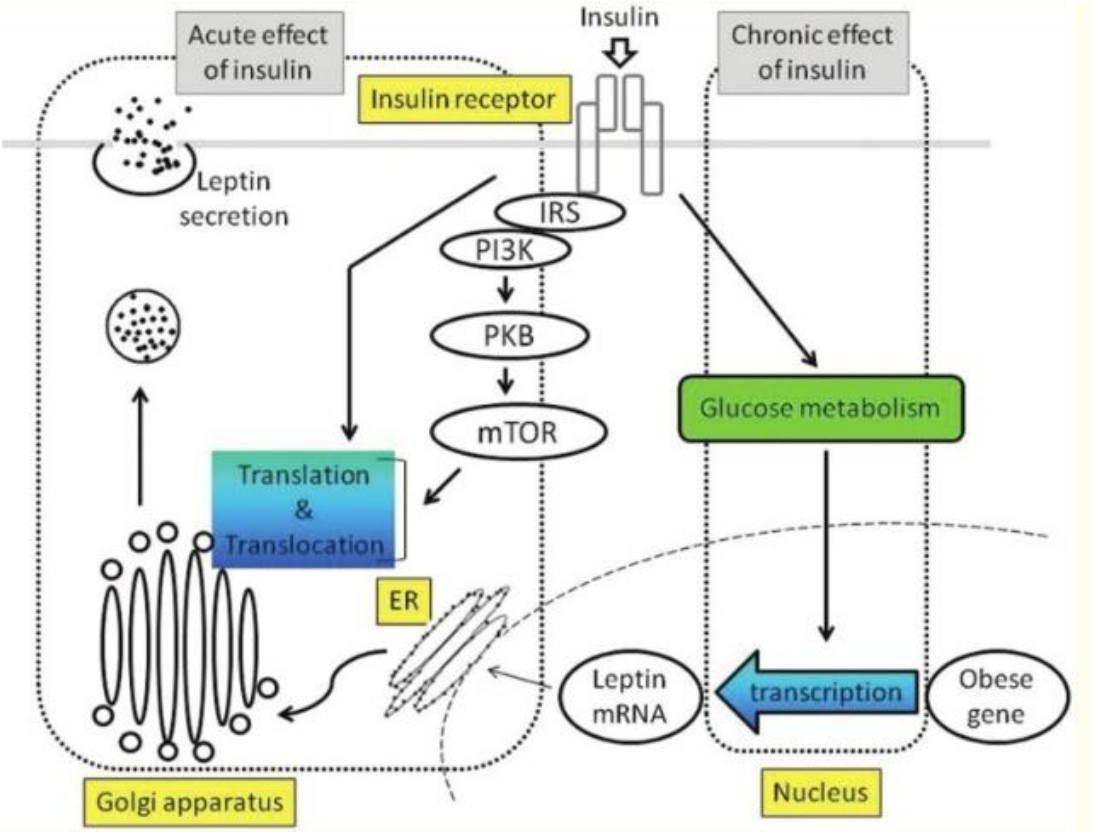

Figure 10. Relationship between insulin levels and leptin production in adipocytes. Acute effects are mediated through the PI3K/PKB/mTOR pathway at the post-transcriptional level, while chronic effects are mediated through mediated glucose metabolism which alters the transcription of the encoding gene. IRS: Insulin receptor substrate, PI3K: Phosphoinositide 3-kinase, PKB: Protein kinase B, mTOR: mammalian target of rapamycin, ER: Endoplasmic reticulum (257).

Leptin and glucocorticoids also interact through multiple and complex feedback loops. Leptin modulates the expression of corticotropin-releasing hormone (CRH) in the hypothalamus. In addition, it also interacts with adenocorticotropin (ACTH) in the adrenal glands. Glucocorticoids and leptin also directly influence each others production. Interestingly, glucocorticoids and leptin follow contrary patterns in their circadian rhytmus. Glucocorticoids act directly at the adipocyte level and stimulate the production of leptin.

This is evidenced by the hyperleptinemia of patients with Cushing's syndrome or those who are treated with synthetic corticosteroids for a long time. However, in parallel, glucocorticoids suppress leptin effects at the CNS level and concomitantly potentiate the effects of the anti-satiety, hyperphagia-promoting hormone neuropeptide Y (NPY) (258-260). As obesity and associated metabolic diseases are often associated with hypercorticolism, this state might contribute to the etiology of leptin resistance (261).

Leptin also interacts with cytokines directly involved in acute and chronic inflammation, many of which are secreted to a considerable degree also from the adipose tissue. Leptin leads in vitro to secretion of CRP at the hepatic and endothelial level and modulates ist levels *in vivo*. In reverse, CRP can bind leptin directly and inactivate ist biological activity. Furthermore, CRP promotes the development of leptin resistance (262). Similarly, leptin is also associated with increased levels of the pro-inflammatory cytokine IL-6, IL-1 beta and TNF-alpha (263, 264).

8.2.3 Receptors and signalling

There are six different leptin receptors which have been identified so far. All six receptors are encoded by the same Lepr gene, and result from alterantive mRNA splicing. All six receptors differ express the same leptin-binding N-terminal extracellular domain (265, 266).

The intracellular C-terminal domain of the receptors is different and is the basis for the classification of the receptors: LEPRa, c, d and f express a short-length intracellular domain, LEPRb the full-length domain containing 300 amino acids, and LEPRe is the secreted form of the receptor (267).

The biological effects of leptin are primarily driven by its signalling in the CNS. At the receptor level, leptin signals occur trough the LEPRb receptor, which is widely expressed in the CNS, but also occurs in peripheral tissues. While the role of central receptors is well-characterized, peripheral receptors are still not fully elucidated (267-272). The dependency of leptin signaling on LEPRb receptors becomes evident in mice deficient with the receptor, which display the same phenotype as mice deficient in all receptors or leptin itself. The exact role of short-domain receptors is not well understood (267).

LEPRb does not contain independent enzymatic activity, but is coupled with the tyrosin kinase Janus kinase 2 (JAK2), which is located on the intracellular side of the receptor and is activated upon leptin binding. JAK2 phosphorylates tyrosine residues of the

LEPRb, which leads to binding of a number of downstream signaling molecules and the activation of different cellular pathways, including JAK2/STAT3, JAK2/STAT5, PI3K/IRS/AKT, and SHP2/ERK. Additional signalling has also been suggested through the calcium calmodulin-dependent protein kinase kinase (CaMKK2)/5'-AMP-activated protein kinase (AMPK)/acetyle-CoA carboxylase (ACC) pathway, but this remains to be well-characterized (273).

LEPRb in the CNS are primarily located in neurons of the hypothalamus, which are distributed across different regions. Neurons of the arcuate nucleus (ARC) are best characterized, but other centers in the hypothalamus, including the ventral premammillary nucleus (PMV), medial preoptic nucleus (MEPO), dorsomedial (DMH), ventromedial (VMH), paraventricular hypothalamic nucleus (PVH), and lateral hypothalamic area (LHA) also contain high levels of LEPRb-expressing neurons (273).

There are two kinds of ARC neurons (273):

1. Pro-opiomelanocortin (POMC) neurons, which express the anorixogenic peptide POMC, which leads to synthesis of alpha-melanocyte stimulating hormone (MSH), which binds to melanocortin-3 receptor (MC3R) and melanocortin-4 receptor (MC4R). MC3R and MC4R-related signalling exerts anorexigenic (i.e., appetite suppressing) effects.
2. Agouti-related protein (AgRP) neurons, which express the orexeigenic (i.e., appetite stimulating) NPY and AgRP. These neuropeptides block the function of MSH and melanocortin receptors. Leptin inhibits the neuronal activity of AgRP and the secretion of NPY and AgRP.

These first order-neurons then project to second-order neurons, which are located in different CNS regions. The resulting signalling cascade leads to a plethora of effects in different organs, controlling energy homeostasis by tightly regulating appetite and energy expenditure. In obese and diabetic individuals, there is a number of mechanisms which contribute to leptin resistance, which is characterized by impaired leptin transport from the blood into the CNS, defect LEPRb signalling and neural circuit dysfunction. The causes of leptin resistance are multifold, and are related to hyperleptinemia, inflammation and endoplasmatic reticulum stress, all of which are seen in obese and diabetic individuals (273).

8.2.4 Relevance of leptin in diabetes

As a central regulator of appetite and energy expenditure, leptin is of high relevance in diabetes and associated metabolic disorders, which go hand in hand with conditions of chronic energy excess due to hyperphagia and/or physical inactivity.

Mice deficient in leptin display a marked obesity phenotype which is characterized by hyperinsulinemia, insulin resistance, impaired glucose control and diabetes (274-276), effects which are completely reversed once exogenous leptin is administered (277). In obese and diabetic humans, hyperleptinemia is associated with leptin resistance, i.e. decreased responsiveness to leptin signalling (278).

Apart from its effects on energy homeostasis, leptin also directly influences glucose metabolism. The anti-diabetic effects of leptin are primarily mediated centrally by its neuronal effects in the ARC region. Restoration of leptin signalling results in a significant improvement in glucose metabolism in diabetes (279).

In humans suffering from complete leptin deficiency due to underlying mutations in the encoding gene or states of relative leptin deficiency such as due to lipodystrophy, long-term negative energy balance and weight loss or neuroendocrine dysfunction seen in hypothalamic amenorrhea in chronically overexercising individuals, administration of exogenous leptin reverses all the metabolic and neuroendocrine adaptations. However, in conditions characterized by normal or increased leptin levels, leptin does not produce the same effects.

In obese individuals, the administration of exogenous leptin has produced discouraging results in terms of weight loss or amelioration of metabolism, which is explained by leptin resistance in such individuals. While the repletion of leptin is therefore a viable strategy in diabetes caused by leptin deficiency, it is not a promissing strategy in humans whose diabetes is caused by obesity. The development and implementation of leptin-sensitizing interventions in such individuals is therefore needed. As hyperleptinemia is associated with leptin resistance and reduction of leptin levels in obese individuals leads to impaired CNS leptin signalling, a reduction in chronically high leptin levels is posed to reduce the incidence of overt diabetes in prediabetic and ameliorate glucose control in diabetic individuals through increased leptin sensitivity (279-281).

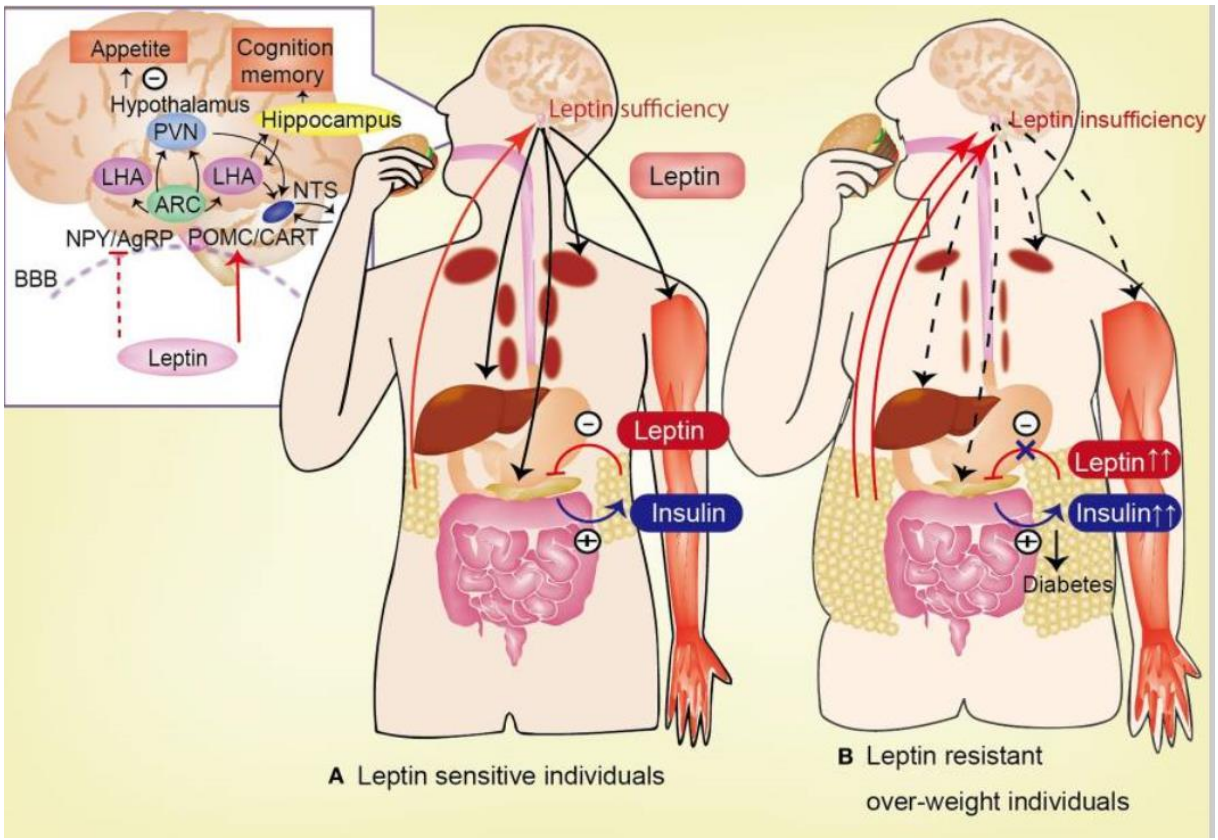

Figure 11. Leptin effects in leptin sensitive and leptin resistant individuals (279)

8.3 Other adipocytokines

Resistin is a relatively newly discovered adipocytokine which is primarily secreted by macrophages (282). In healthy individuals, resistin helps to keep glucose homeostasis in check. However, in obese and diabetic individuals, it is associated with deregulation of glucose metabolism and impaired glucose utilization. Resistin is suggested to play an important role in the development of insulin resistance and type 2 diabetes mellitus in obese individuals (283) and it is also relevant in diabetic complications, primarily in the development of CVD through various mechanisms, including chronic inflammation, endothelial dysfunction, thrombocyte hyperaggregability, and smooth muscle dysfunction. Interventions that reduce the levels of resistin have a growing importance in the treatment of diabetes (284).

Plasminogen activator inhibitor-1 (PAI-1) is primarily synthetized in VAT, but it can also be produced in ectopic fat tissue, for example in the liver. The physiological role of this adipokine is inhibition of fibrinolysis (285). In 1986, it was established that there is a significant and independent association between PAI-1 levels and obesity and insulin levels and that weight loss leads to a concomitant insulin sensitivity increase and PAI-1 reduction (286). A large meta-analysis of observational studies showed an increase in risk for diabetes development with an increase in PAI-1 levels (287). In peripheral blood, PAI-1 inhibits the action of tissue plasminogen activator and therefore inhibits the fibrinolytic system. In peripheral tissue, PAI-1 suppresses the activation of matrix metalloproteinases (MMPs) by plasmin which is formed from plasminogen by the enzyme urokinase. Therefore, plaques that are formed in the presence of increased PAI-

1 levels are particularly instable and prone to rupture (288). Reducing the levels of PAI-1 in diabetics is therefore of clinical relevance (289).

IL-6 and TNF-alpha are key regulators of inflammation and are significantly increased in obesity and diabetes. The grade of obesity in diabetics correlates with the degree of inflammation. These cytokines are primarily secreted by macrophages infiltrating the adipose tissue and are acute-phase response markers and mediators of inflammation (290). TNF-alpha is elevated both locally and systematically in obesity and directly correlates with development of insulin resistance. Blocking TNF-alpha leads to a significant amelioration of glucose utlization in insulin-dependent tissues (291). Circulating FFAs, which are elevated in obesity, stimulate the production of TNF-alpha in macrophages, and and TNF-alpha directly potentiates lipolysis in adipocytes, which further elevates FFA levels (292). Furthermore, TNF-alpha also stimulates hepatic lipogenesis, resulting in hepatic accumulation of ectopic fat (293). These effects combined result in a rapid onset of insulin resistance and glucose metabolism dearrangement. IL-6 is increased up to threefold in obesity and precedes and is an independent risk factor for the development of insulin resistance, diabetes and CVD (294). Reducing IL-6 and TNF-alpha levels in diabetic attenuates chronic inflammation and is an important step in achieving better clinical outcomes.

9. Therapy of type 2 diabetes: Looking for novel interventions

Lifestlye modification is the cornerstone of diabetes prevention and management. Non-pharmacological interventions, such as exercise and diet modification, are the first choice in attempts to hault the progression of pre-diabetes to overt disease, although drugs, such as metformin, are also often prescribed (4). Once full-blown disease develops, lifestyle measures are implemented in conjuction with pharmacotherapy for blood glucose control and in order to hault the disease progression and avoid complications (3).

Although major developments have been achieved, the majority of pre-diabetics will progress to diabetes over time and the disease mostly takes a course of deteriorating glucose control and complications (4).

A major challenge in pharmacotherapy of diabetes is due to poor adherence, which is strongly associated with side effects. A retrospective cohort study conducted in the UK showed that only about 15% of patients are adherent to all of their diabetes medications. Non-adherence to pharmacotherapy was found to be consistent across all oral anti-diabetics, and there was a clear association between non-adherence and poor glycemic control (295). Adherence increases with better tolerability of medication, e.g. adherence to DPP-4 inhibitors is greater than to sulfonylureas and thiazolidinediones due to better tolerability (296).

An additional problem is the fact that the majority of pre-diabetic and diabetic patients is multimorbid and is dependent on poly-pharmacotherapy. That is why several

associations, such as the American Diabetes Association (297) and the American Association of Clinical Endocrinologists (298) have issued recommendations for treatment goals for blood pressure, lipid levels and other parameters besides glucose and HbA1c levels.

As for glycemic control, achieving a HbA1c level <7.0% is the treatment goal, as reducing levels below this threshold significantly reduces complications and mortality, but it is difficult to achieve even with intensive pharmacotherapy (297).

With the ever-growing global burden of diabetes, there is an urgent need to identify interventions that are effective, affordable and easy to implement and adhere to which can be integrated into current standards of care in order to improve the outcomes of patients.

Given the well-established role of adipocytokines in prediabetes and diabetes, it is essential to examine the effects of these interventions on adipocytokines. During my doctoral thesis, I conducted systematic reviews and meta-analyses on three interventions: omega-3/fish oil supplementation, vitamin D supplementation, and exercise on adipokine levels.

9.1 Omega-3 fatty acids/fish oil

Polyunsaturated fatty acids (PUFAs) are classified as omega-3 (n-3) or omega-6 (n-6), depending on whether the first double bound in their chemical structure is located on the third or the sixth carbon from their terminal methyl group. These two groups play essential and often opposite roles in the body, so that maintaining a physiological balance between them is essential.

Biologically, n-6 PUFAs, primarily the arachidonic acid, serve as precursos molecules for the synthesis of pro-inflammatory eicosanoids. To the contrary, n-3 PUFAs, including the eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), yield the synthesis of less biologically active signalling molecules and have therefore anti-inflammatory effects.

N-3 PUFAs reduce the production of inflammatory cytokines, such as IL-1 and TNF-alpha (299). They also reduce the synthesis of reactive oxygen species (ROS) by leukocytes (300), are precursor molecules for the production of anti-inflammatory resolvins (301) and protectins, which have both anti-inflammatory and neuroprotective properties (302).

EPA and DHA are also integral components of the cell membrane and are essential for its integrity and fluidity (303). Many of the health benefits prescribed to these fatty acids are explained by the displacement of n-6 PUFAs from the phospholipids of the cell membrane, including blood cells, heart muscle cells and neurons. This changes the

physical characteristics of the cell membrane, which in turn, impacts on receptor signalling, resulting in changes at cellular and tissue level (304). Through regulation of transcription factors they also affect the expression of genes involved in energy metabolism, cell cycle regulation and cell survival and differentiation (305).

The most abundant dietary n-3 PUFA is alpha-linolenic acid (ALA), which can be converted into EPA and DHA by elongase and desaturase enzymes, but the conversion process is inefficient so that minimal amounts of EPA and DHA can be synthetized through this pathway (306). EPA and DHA are abundantly found in marine food, such as fatty fish, and supplementing with fish oil is the most effective way to increase their intake. Supplements containing EPA and DHA have increased in popularity; in the US alone, more than 34.7 billion USD have been spent on these supplements in 2016 (307).

The beneficial properties of these fatty acids are suggested to be beneficial in metabolic disease and diabetes. Pre-clinical studies demonstrate that supplementing EPA and DHA or fish oil enriched with them attenuates high-calorie induced insulin resistance, exhibits positive effects on the muscle-liver glucoregulatory axis resulting in increased muscle utilization in muscles and decreased hepatic gluconeogenesis, has supressing properties on chronic macrophage-mediated inflammation in adipose tissue, and ameliorate oxidative stress.

Furthermore, these fatty acids exhibit hepatoprotective effects by suppressing the accummulation of lipids in the liver often seen in metabolic conditions. Interestingly, EPA and DHA also specifically ameliorate inflammation and insulin resistance

associated with aging by reducing acute phase inflammatory cytokines, enhacning muscle protein quality and reducing and ameliorating mitochondrial function (308).

Observational studies in humans suggest that there is an inverse relationship between n-3 PUFA intake and insulin resistance (309), even in populations with normally high consumption of fatty fish, such as Eskimos (310). However, randomized controlled trials showed mixed results on glycemic control, incidence of type 2 diabetes and diabetic complications (308). Given the importance of adipokines in all of these processes, examining the effects of EHA and DPA on adipokine levels in a meta-analysis is of great interest.

9.2 Vitamin D

Cholecalciferol, also known as calciol or vitamin D, is a fat-soluble vitamin involved in many physiological processes in the body, including calcium homeostasis, bone turnover, immune system regulation and glucose and lipid metabolism (311). The human body synthesizes calciol in the skin from the steroid precursor 7-dehydrocholesterol with the help of UVB sunlight. Calciol is then transported in to the blood, where it is bound to vitamin D bounding protein (DBP) and transported to the liver. In the liver, calciol is hydroxylized into 25-hydroxy-vitamin D (25(OH) vitamin D or calcidiol). Calcidiol is then release into the blood stream and is bound again to DBP.

Calcidiol represents the main storage form of vitamin D, as potential shortcomings in the natural synthesis due to sun exposure must be compensated. A part of calcidiol is hydroxylized in the kidney to 1,25-hydroxy-vitamin D (calcitriol), which is biologically the most active form of vitamin D (312).

Levels of vitamin D are suggested to be inversely associated with body fat mass, blood pressure, blood glucose levels and incidence of type 2 diabetes (313). Numerous cross-sectional studies showed an inverse association between serum vitamin D levels and impaired glucose tolerance and type 2 diabetes (314-316). The prospective observational Nurses Health Study showed that an intake of vitamin D of at least 800 UI/day reduced the risk of type 2 diabetes by 33% in otherwise healthy women (317). Interestingly, some studies also show seasonal variations in glucoregulation of type 2 diabetic patients with no changes in pharmacotherapy, with poorest glucose regulation in the winter and lowest HbA1c levels in the summer (318).

The effects of vitamin D in type 2 diabetes are multi-faceted. It is implicated in the synthesis and secretion of insulin from pancreatic beta-cells, as both the vitamin D receptor (VDR) and DBP are found in pancreatic isles secreting insulin. Insulin secretion is stimulated through vitamin D-dependent calcium signalling pathways. Studies have shown that vitamin D is essential for insulin release in glucose tolerance, and that an induced deficiency automatically leads to insulin secretion reduction and impaired glucose tolerance. Vitamin D supplementation has been shown to ameliorate insulin secretion in early typ 2 diabetes but not in advanced disease (319).

Apart from its effects on insulin secretion, vitamin D is also essential in maintaining the undergoing of proper insulin action in target tissues, including the liver, muscles and adipose tissue.

VDR is ubiqutously found in insulin-sensitive tissues and upon binding of vitamin D, a complex is formed with the retinoid X-receptor (RXR). The 1,25(OH)2-vitamin D-VDR-RXR complex binds to the promoter region of the human insulin receptor gene and stimulates the insulin receptor expression. Furthermore, vitamin D supplemention was also found to ameliorate glucose metabolism by affecting the sirtuin 1-insulin receptor substrate-GLUT4 signalling pathway. Vitamin D also exerts its positive effects through stimulation of PPAR- δ, thereby modulating fatty acids release from the adipose tissue and reducing FFA-induced insulin resistance. It also increases intracellular calcium concentration in the muscles, thereby leading to higher translocation of GLUT4 on the cell surface and a higher glucose uptake. In vitamin D deficient individuals, insulin resistance is partly mediated through parathormone, and replenishing vitamin D reverses these dearrangements. Vitamin D also improves insulin sensitivity through the inhibition of the renin-angiotensin-aldosteron system (RAAS). Hepatic lipogenesis and gluconeogenesis are also suppressed by vitamin D.

In case of deficiency, there is accummulation of ectopic fat in the muscle, which leads to reduced insulin sensitivity, which can be reversed by correcting the serum levels (319).

Vitamin D also exerts anti-inflammatory effects by suppressing the release of pro-inflammatory cytokines from macrophages and monocytes, largely by downregulating the release of TNF-alpha and NF-κB and the expression of Toll-like receptors 2 and 4 (319).

Due to its lipophilic nature, vitamin D accumulates substantially in adipose tissue. In healthy humans, about a fifth of vitamin D is stored in adipose tissue, while the rest is consumed or metabolized (320).

Adipose tissue serves as a buffering system, which stores incoming vitamin D upon intake and releases small amounts of vitamin D under fasting conditions (321). VDR is expressed widely preadipocytes and adipocytes and both in SAT and VAT. In the adipose tissue, vitamin D has several roles, including the regulation of gene expression, adipogenesis, secretion of adipokines and adipocyte energy metabolism. In obesity, vitamin D is sequestered in the adipose tissue both due to expanding fat mass and tissue dysfunction. Consequently, obese individuals, such as the majority of prediabetics and type 2 diabetics, have lower circulating vitamin D levels than lean individuals. Correcting the levels of vitamin D, such as through supplementation, can correct the deficiency, but even in individuals with normal serum levels, supplementing vitamin D is suggested to ameliorate adipose tissue function (322). Yet, the exact effects of vitamin D supplementation on adipokine levels in prediabetic and diabetic individuals is unknown.

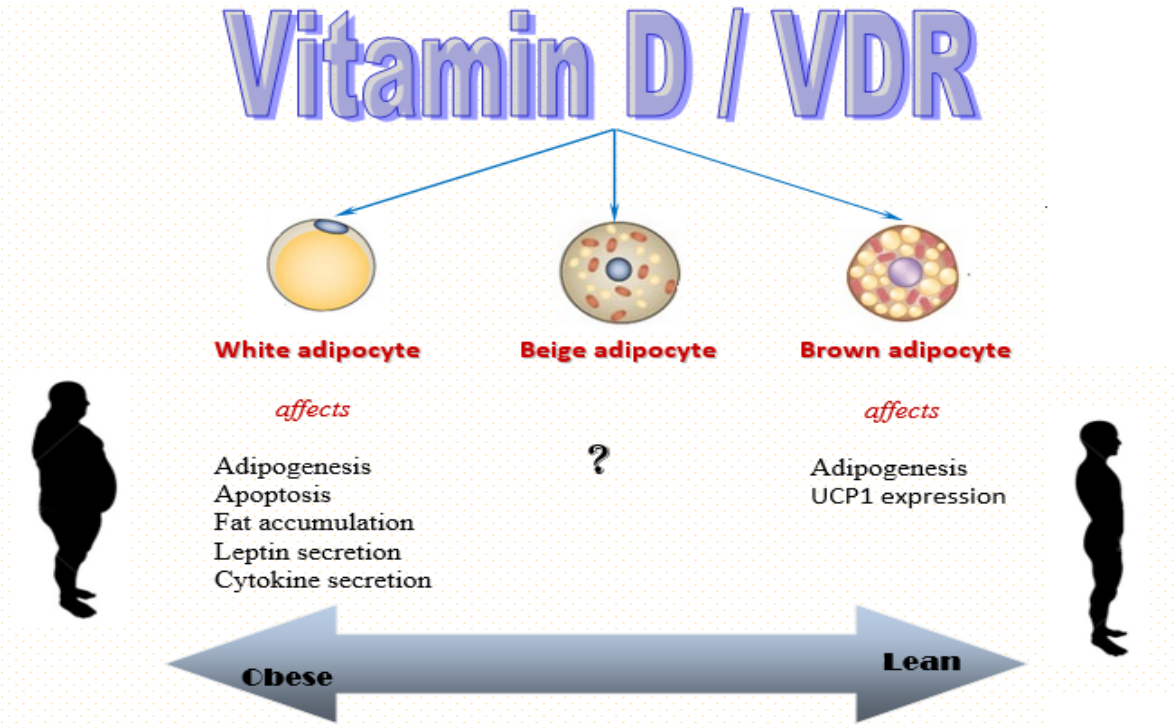

Figure 12. The role of vitamin D in adipose tissue (322).

9.3 Physical exercise

Physical exercise is structured, planned and repetitive physical activity in which energy expenditure is increased above the resting energy expenditure with a final goal of attainment of physical fitness (323).

There are several forms of exercise, but the main ones are aerobic and resistance exercise. According to the The American College of Sports Medicine (ACSM), aerobic exercise is any activity in which large muscle groups are used, can be executed continously and is rhytmic in nature.

Energy requirements for areobic exercise are primarily covered by the aerobic energy system, and the ultimate goal of this form of exercise is enhancement of cardiorespiratory fitness (324).

Resistance exercise is performed with the ultimate goal of attaining muscle strength, i.e. the ability of muscle to exert force, or muscle endurance, that is the ability of muscle to continue to perform without fatigue. It can be performed with e.g., weight machines, free weights, elastic bands or one´s own body weight. Energy is primarily delievered through the anerobic system, which results in the formation of lactate (324).

Physical exercise is the cornerstone of diabetes prevention and management. The American Diabetes Association recommends that individuals with diabetes acquire at least 150 min of moderate to vigorous aerobic exercise per week and engage in a minimum of 2 weekly resistance exercise sessions (325). However, the majority of diabetic individuals or those at risk do not engage in regular exercise activities. For instance, one study reported that only 55% of diabetic performed walking as exercise on a regular basis and only 12% engaged in some form of resistance exercise (326).

Exercise is associated with both acute and chronic effects in diabetics.

During aerobic exercise, circulating blood glucose is taken up by active muscles during exercise once muscle glycogen stores are depleted, helping to clear excessive glucose from the blood stream. This uptake of glucose is mediated by muscle contractions and is

independent of insulin, so that even in individuals with strong insulin resistance, there is normal uptake of glucose by the working muscle.

Interestingly, post-exercise, this increased glucose uptake persists for hours, and is accompanied by insulin-dependent uptake due to increased insulin sensitivity related to higher expression of GLUT4 transporter in the muscle tissue (327-332). In healthy individuals, the increased glucose uptake is compensated by increased hepatic glucose output. However, in diabetic individuals, this mechanism is impaired raising concerns of a possible hypoglycemia Nevertheless, due to a concomitant fall in insulin levels with exercise, the risk of hypoglycemia is minimal (333, 334).

Similarly, resistance exercise acutely improves fasting glucose levels for at least 24 hours (335). Evidence suggest that a combination of resistance and aerobic exercise is the best at producing positive effects on blood glucose control (336). The increased insulin sensitivity seen with exercise is not solely due to insulin´s effects at the muscle level, as hepatic insulin sensitivity is ameliorated as well. This may be in part due to decreased ectopic fat in the liver, which is normally found in high lvels in persons with diabetes and is associated with hepatic insulin resistance (337).

Exercise is also associated with increased muscular capacity for fat oxidation, which is an important component in enhancing insulin sensitivity, as increased circulation of free fatty acid contributes significantly to insulin resitance (338, 339).

Regular exercise also exerts a wide range of positive chronic effects in individuals with diabetes. It leads to long-term improvement of blood glucose control, reduced HbA1c

levels, improved insulin resistance and a shift toward greater fat oxidation, which is usually lower in favor of carbohydrate utilization in diabetes.

Exercise also improves body composition by increasing skeletal muscle mass and decreasing fat mass. An improvement in blood lipids, such as a decrease in LDL cholesterol is often observed, especially when accompanied by weight loss.

Aerobic exercise decreases blood pressure over time, especially the systolic blood pressure. In general, diabetic individuals who exercise regularly have a lower risk of cardiovascular and overall mortality, and an improved psychological well-being. Exercise also reduces the incidence of type 2 diabetes in high-risk individuals, such as those with prediabetes (340).

Reduced ardiovascular fitness is significantly associated with increased cardiovascular mortality. Diabetics, in comparison to healthy individuals, have a lower cardiorespiratory fitness, even without obesity and with recently diagnosed disease. Therefore, ehnacing cardiovascular fitness in this population is essential, given that cardiovascular mortality is the leading cause of death. This is caused by insulin resistance, decreased limb blood flow, decreased myocardial perfusion and muscular mitochondrial dysfunction. These maladaptations can be alleviated with regular exercise (341).

Exercise also induces a number of positive changes at the adipose tissue level. It increases lipolysis and free fatty acid release from adipocytes, reduces adipocyte size,

decreases adiposity, enhances the expression of GLUT4 and potentially leads to beaging of WAT. These effects are independent of weight loss (342).

With regards to the effects on adipokines, the effects of exercise are inconsistent and it is especially not known whether different exercise modalities have different effects.

10. Methodology: systematic review and meta-analysis

The history of meta-analysis did not begin in medical, but rather in social sciences when the psychologist Glass used „the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings“ in 1976 (343). Meta-analysis is a type of systematic review which uses a quantitive approach to systematically evaluate the results of existing research from multiple studies.

Typically, meta-analysis uses results from randomized controlled trials (RCTs) in order to evaluate the effect of a certain intervention on an outcome. The primary value of meta-analysis in comparison to a single RCT is the more precise estimate of the effect of the intervention on the outcome. In addition, it can provide useful information on different responses to a certain intervention according to different characteristics of that intervention and on individuality or generalization of results across different population subgroups. A meta-analysis can provide answers to questions which cannot be answered by a single study, bring clarity to the effects of an intervention with inconsistent or conflicting results in individual studies, circumvent the caveat of false positive or false negative results, overcome the problem of small sample sizes and inadequate statistical

strength and lead to new hypotheses. Meta-analysis represents the highest form of evidence in the hierarchy of evidence-based medicine (344, 345).

Figure 13. Hierarchy of evidence-based medicine (344)

The Cochrane Collaboration is the international organization which has set the standards for conducting high-quality systematic review and meta-analysis and „whose primary aim ist o help people make well-informed decisions about health care by preparing, maintaining and promoting the accessibility of systematic reviews of the evidence that underpins them“. The Cochrane Collaboration also clearly defines the need for systematic reviews by healthcare providers in order to make well-informed, evidence-based decisions as with the ever-growing wealth of medical research it

becomes very difficult to appraise and interpret the evidence from single studies for healthcare recommendations (346).

The PRISMA statement (Preferred Reporting Items for Systematic reviews and Meta-analyses) defines the reporting standards and has replaced the QUORUM Statement (347).

According to Cochrane Collaboration (346) systematic review is characterized by:

1. A clearly stated set of objectives with pre-defined eligibility criteria for studies to be included in the review;
2. An explicit, reproducible methodology;
3. A systematic search that attempts to identify all studies that would meet the eligibility criteria;
4. An assesment of the validity of the findings of the included studies, e.g. through an assessment of risk of bias; and
5. A systematic presentation and synthesis of the characteristics and findings of the included studies.

In accordance with these characteristics, meta-analysis is performed in following steps (344, 346-349):

1. Literature search: Searching for relevant studies in several electronic databases (.e.g. PubMed, EMBASE, Cochrane Library) according to a prespecified research question with regards to participants, interventions, outcomes and study design. In addition, hand searching in the identified papers and in meta-analysis and reviews can identify additional eligible studies. A thorough literature search is essential, as it minimizes the risk of bias and the findings depend on it.

2. Assessing the risk of bias: The Cochrane risk of bias tool provides a comprehensive tool to assess for different sources of bias in every individual study: random sequence generation (selection bia), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias.
3. Testing for study hetereogeneity: The degree of dissimilarity between the individual study results is referred to as statistical hetereogeneity. With higher study hetereogeneity, findings should be interpreted with caution. There are different sources of heterogeneity, including clinical variants (e.g., participant characteristics, interventions) and methodological variants (e.g., study design). Identifying clinical sources of diversity that modify the intervention effect is an important contribution of meta-analysis, as it can result in finding e.g. special patient subpopulations which benefit the most from an intervention. If heterogeneity is driven by methodological diversity only, it points to different degree of bias among studies. Hetereogeneity can be tested with the help of Cochrane´s Q test and the I2 value. Cochrane´s Q test is a chi-squared ($\chi 2$) test of variation among k trials, with k-1 degree of freedom. Due to its limited statistical power to detect heterogeneity, especially with small number of studies, the Q test uses a power level of 0.10 to detect hetereogeneity. To overcome this, the I2 value is used, as this value does not depend on the number of studies used and represents inter-study variability attributable to true heterogeneity versus chance. An I^2 value of >75% representes high study heterogeneity.

4. Statistical analysis: The overall effect size is calculated from individual studies´ effect sizes, which are weighted according to study sample size or study variance. Studies with greater sample size and smaller variance have more weight on the overall effect size. Two statistical models can be used when calculating the effect size: fixed effects model and random effects model. The fixed effects model assumes that all the studies have a true effect size and that the variation is only due to sampling error or chance (intra-study variability).

   On the other hand, the random effects model assumes that the true effect size can vary from study to study and therefore also accounts for inter-study variability. With increasing study heterogeneity, the random effects model should be used. The results are presented graphically with a forest plot.
5. Evaluating the causes of heterogeneity: It is essential to evaluate the underlying sources of heterogeneity. A subgroup analysis according to certain patient or intervention characteristics can offer new research hypotheses which is not found in individual studies. A sensitivity analysis, e.g. through the leave-one-out method, can provide robustness to the results as it tests the dependency of the overall effect size on every single study to assure that the results are not driven by any one individual study.
6. Testing for publication bias: Studies that find a negative effect of an intervention are less likely to be published than those who show a positive effect, and studies with no significant results often remain unpublished. As meta-analysis can only include published studies, this can lead to exaggeration of the actual effect size. This is called publication bias. A commonly used graphical method to inspect for publication bias is the funnel plot method, in which the effect size of every

study is plotted against its precision. If there is no publication bias, the graph results in a symmetrical inverted funnel shape, while asymmetry indicates to publication bias. Other commonly used methods are Egger´s regression test and „trim and fill“ method.

7. Presenting the results according to PRISMA statement.

Although meta-analysis is not new in medical research, there are constant new developments, such as the introduction of network meta-analysis, in which multiple treatment options can be assessed in a single analysis (350), or meta-epidemiology, in which multiple meta-analysis are evaluated in order to assess the risk of bias in RCTs (351). Meta-analysis is the most commonly cited form of medical research (352).

A simple search of “meta-analysis” in PubMed shows 162499 results. Given that it represents the highest form of research in evidence-based medicine, meta-analysis was chosen as methodology to analyse the research questions for this doctoral thesis.

11. Aims of the thesis

The thesis had the aim to synthesize the evidence as to the influence of different interventions on levels of adipocytokines in individuals with prediabetes and type 2 diabetes in form of systematic reviews and meta-analyses of randomized controlled trials. The results were published in peer-reviewed journals. The scientific rationale for each individual systematic review and meta-analysis is provided in the specific paper.

Omega-3 fatty acids

The aim of this systematic review and meta-analysis was to synthesize the effects of supplementing with EPA, DHA, both EPA and DHA or EPA/DHA containing fish oil at different doses and length of supplementation on adipocytokine levels (PAI-1, adiponectin, leptin, resistin, TNF-alpha, IL-6) in prediabetics and type 2 diabetics.

Vitamin D

In this systematic review and meta-analysis, the aim was to investigate the effects of supplementation with vitamin D and its analogues (calcidiol, ergocalciferol, cholecalciferol) regardless of the route of administration (oral, intramuscular injection) on adipocytokines (adiponectin, leptin, TNF-alpha, IL-6) in individuals suffering with prediabetes and type 2 diabetes.

Exercise

The aim of the exercise part was to evaluate the effects of different modalities of exercise (aerobic, resistance, combined aerobic and resistance) on levels of adiponectin and leptin in the target population. In order to differentiraly characterize the interventions, subgroup analyses were conducted according to the length of the intervention, concomitant employement of dietary intervention and the number of training sessions per week.

12. Results

The results of the mentioned analyses were published in peer-reviewed journals. The abstracts of the publications are provided below.

Paper 1: Effects of Omega-3 Supplementation on Adipocytokines in Prediabetes and Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis of Randomized Controlled Trials

Published in: Diabetes Metab J. 2018;42(2):101-116

Background: The objective of this systematic review and meta-analysis was to determine the effects of omega-3 supplementation on adipocytokine levels in adult prediabetic and diabetic individuals.

Methods: We searched PubMed, Medline, EMBASE, Scopus, Web of Science, Google Scholar, Cochrane Trial Register, World Health Organization Clinical Trial Registry Platform, and Clinicaltrial.gov Registry from inception to August 1, 2017 for randomized controlled trials. Pooled effects of interventions were assessed as mean difference using random effects model. We conducted a sensitivity, publication bias and subgroup analysis.

Results: Fourteen studies individuals (n=685) were included in the meta-analysis. Omega-3 supplementation increased levels of adiponectin (0.48 μg/mL; 95% confidence interval [CI], 0.27 to 0.68; P<0.00001, n=10 trials), but effects disappeared after sensitivity analysis. Tumor necrosis factor α (TNF-α) levels were reduced (-1.71 pg/mL; 95% CI, -3.38 to -0.14; P=0.03, n=8 trials). Treatment duration shorter than 12 weeks was associated with greater reduction than longer treatment duration. Levels of other adipocytokines were not significantly affected. Publication bias could generally not be excluded.

Conclusion: Eicosapentaenoic acid and docosahexaenoic acid supplementation may increase adiponectin and reduce TNF-α levels in this population group. However, due to overall study heterogeneity and potential publication bias, a cautious interpretation is needed.

Paper 2: Effects of Vitamin D Supplementation on Adipocytokine Levels in Patients with Abnormal Glucose Metabolism: Systematic Review and Meta-Analysis of Randomized Controlled Trials

Published in: J Pharmacol & Clin Res 2018;5(5):555674

Objective: The objective of this systematic review and meta-analysis was to investigate the effects of vitamin D supplementation on circulating levels of adipocytokines in prediabetic and diabetic individuals

Materials and Methods: The following databases were searched from randomized controlled trials: PubMed, Medline, Scopus, EMBASE, Web of Science, Google Scholar, Cochrane Trial Register, WHO Clinical Trial Registry Platform and Clinicaltrial.gov Registry until December 2017. We assessed pooled effects of interventions as mean difference by applying the random effects model. The Cochrane Collaboration's tool was used to investigate the risk of bias. To exclude that the overall effect sizes depended on individual studies, we conducted sensitivity analysis through the leave-one-out method. We also inspected for publication bias.

Results: Our meta-analysis included 16 studies with 1058 individuals. Supplementing vitamin D significantly reduced levels of IL-6 (MD: 1.67pg/ml (95% CI-2,53, -0.80), p= 0.0002, I2= 99%), and the effects remained after sensitivity analysis. Levels of adiponectin, leptin and tumor necrosis factor-alpha (TNF-alpha) were not significantly changed. Overall study heterogeneity was high. We could not exclude publication bias.

Conclusion: Vitamin D supplementation can reduce levels of IL-6 in prediabetic and diabetic individuals but does not affect other adipocytokines. However, a cautious interpretation of our results is warranted.

Paper 3: Exercise Increases Adiponectin and Reduces Leptin Levels in Prediabetic and Diabetic Individuals: Systematic Review and Meta-Analysis of Randomized Controlled Trials

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It is speculated that lifestyle interventions known to improve diabetic metabolic state may exert their effects via adipokines. The aim of this systematic review and meta-analysis was to evaluate the chronic effects of physical exercise on adiponectin and leptin levels in adult prediabetic and diabetic individuals. PubMed, Embase, Scopus, The Cochrane Library, clinicaltrials.gov, and WHO Clinical Trials Registry were searched for randomized controlled trials. Pooled effects of interventions were assessed as mean difference (MD) with random effects model. Sensitivity analysis was conducted to test data robustness and subgroup analysis for study heterogeneity. Twenty-two trials with 2996 individuals were included in the meta-analysis. Physical exercise increased levels of adiponectin (MD: 0.42 μg/mL; 95% confidence interval (CI), 0.23, 0.60, $p < 0.00001$, $n = 19$ trials) and reduced leptin levels (MD: -1.89 ng/mL; 95% CI, -2.64, -1.14, $p < 0.00001$, $n = 14$ trials). These results were robust and remained significant after sensitivity analysis. Study heterogeneity was generally high. As for physical exercise modalities, aerobic exercise, but not other modalities, increased adiponectin and reduced leptin levels. In conclusion, physical exercise and, specifically, aerobic exercise, leads to higher adiponectin and lower leptin levels in prediabetic and diabetic adults. However, cautious interpretation of current findings is warranted.

13. Discussion

Paper 1:

The findings of this systematic review and meta-analysis are that, omega-3 fatty acid supplementation increases adiponectin levels and reduces TNF-alpha levels in prediabetes and diabetes, but has no significant effects on levels of PAI-1, resistin, leptin and IL-6.

The effect on adiponectin was statistically significant but with very high study hetereogeneity and dependent on results of one study (353), in which pharmacotherapy with pioglitazone was also conducted. There were no effects observed once this study was removed in the sensitivitiy analysis. Pioglitazone alone also leads to increases in serum adiponectin in individuals with type 2 diabetes (354, 355). This suggests that the results of the meta-analysis could have been driven by pioglitazone co-administration in this single study. Both thiazolidinediones and omega-3 fatty acids are PPAR-γ agonists. Activation of the PPAR- γ pathway is essential in stimulating adiponectin production and secretion in adipocyte. *In vitro* studies demonstrate that inhibition of the pathway completely abolishes this stimulatory effects and that thiazolidinediones and omega-3 fatty acids show additive effects (356, 357).

A meta-analysis from 2013 (358) found that supplementing with fish oil can moderately increase adiponectin levels. However, studies included in this meta-analysis only employed fish oil as supplement, but not pure EPA or DHA supplementation, and also included trials with fish meal feeding. Also, the analysis was not confined to individuals with abnormal glucose metabolism and the effect found was modest: adiponectin was increased by 0.37 μg/mL, which would correspond to reduced incidence of type 2

diabetes by 3% (359). This meta-analysis also found considerable and uneaxplainable study hetereogeneity.

Another similar meta-analysis from 2014 (360), which was also not restricted to individuals with prediabetes or type 2 diabetes, found that omega-3 can increase circulating adiponectin levels. However, the interventions used were very heterogeneous, e.g. besides fish oil, different plant oils were used as well. The analysis also revealed significant publication bias.

A narrative review from 2013 (361) also comes to the conclusion that „*Current evidence suggests a positive, dose-dependent relationship between omega-3 fatty acid intake and circulating levels of adiponectin*". However, the studies reviewed included both human (mixed RCTs and observational trials) and rodent studies. Also, the level of evidence associated with a narrative review is lower than with a meta-analysis.

Two meta-analysis published later than ours (360, 361) including only RCTs with individuals with type 2 diabetes, but not prediabetes found that, adiponectin can moderately increase circulating adiponectin levels.

Bahreini et al. (360) also included RCTs with alternative forms of omega-3 supplementation in their meta-analysis, such as milled flaxseeds, fish feeding and ALA supplementation. It cannot be excluded that other bioactive components in flaxseeds or fish also have an impact on adiponectin levels. Also, the rate of ALA conversion to EPA and DHA in the body is highly variant between individuals. This meta-analysis also found significant study hetereogeneity. Also, in contrast to our study, the authors found significant differences in effect size according to length of supplementation, but they used a different cut-off value (8 weeks vs. 12 weeks in our analysis).

Farimani et al. (361) found that the increase in adiponectin was non-significant. They also reported that omega-3 non-significantly reduced leptin supplementation. Hariri et al. (362) showed in their meta-analysis, which included both healthy and individuals with medical conditions, that the reducing effect on leptin is only found in non-obese individuals. In our meta-analysis, results were not stratified by BMI status of individuals. However, studies included in our analysis enrolled overweight and obese individuals according to mean BMI. Therefore, it is plausible that the the absence of effect on leptin in our analysis might be explained by the BMI status of individuals.

In our analysis, omega-3 fatty acid supplementation reduced TNF-alpha levels and the effects were sustained after sensitivity analysis; however, with considerable study hetereogeneity. In the subgroup analysis, supplementation lasting shorter than 12 weeks resulted in higher decreases in TNF-alpha levels than longer than 12 weeks, but different omega-3 dosages did not produce different effects.

Omega-3 fatty acids can reduce concentrations of pro-inflammatory cytokines, including TNF-alpha, in chemically induced models of type 2 diabetes (363). This evidence is supported by our and other meta-analyses. A meta-analysis from 2014 (364) found that TNF-alpha levels can be significantly reduced by supplementing omega-3 fatty acids in subjects with chronic non-autoimmune disease (including type 2 diabetics). However, the effect was predominantly seen in subjects with BMI lower than 30 kg/m^2, and contrary to our findings, longer supplementation produced greater lowering effect. A recent meta-analysis from 2018 (365) also showed a reducing effect.

Paper 2:

This meta-analysis showed that supplementing vitamin D reduced IL-6 levels by 1.67 pg/ml. No effects were found regarding levels of adiponectin, leptin and TNF-alpha.

Mixed results have been found in several meta-analysis as to the effects of vitamin D suppelementation on inflammatory markers. Contrary to our findings, a recent meta-analysis (366) found no effects of vitamin D supplementation on chronic low-grade inflammation, as evident by CRP and IL-6 levels, in middle aged and older adults. However, this analysis also included individuals with other medical conditions and no overt clinical disease. Similarly, meta-analyses from 2016 showed no effects on TNF-alpha and IL-6 (367) and adiponectin and leptin (368) in overweight and obese individuals. This is in line with findings of Zuk et al. (369) who conclude that there is no established benefit of supplementing vitamin D on inflammatory markers in conditions of overweight and obesity.

Two meta-analyses evaluated the effects of vitamin D supplementation in type 2 diabetes. Yu et al. (370) found no effects on levels of TNF-alpha and IL-6. Mousa et al. (371) report in their meta-analysis, however, a reduction in TNF-alpha and an increase in leptin, but no effects on interleukin-6 and adiponectin. Interestingly, their meta-regression and subgroup analysis showed that age, sex, body mass index, duration of diabetes, baseline vitamin D status, and dose and duration of supplementation did not modify the results.

In a meta-analysis from 2016 (372), supplementing vitamin D with less than 1000 IU/day led to significant increases in leptin levels in diabetics. In our analysis, supplementation dose regularly exceeded this cut-off, but in one trial, which employed a dose of 400 IU/day, leptin was significantly increased.

Taken together, there is no strong evidence to support the supplementation of vitamin D in order to modulate levels of adipocytokines in prediabetes and diabetes. The suggested benefits of vitamin D supplementation on insulin resistance and glycemic control are therefore probably not mediated through changes in adiponectin levels. The main question remaining is if these effects are only seen when correcting deficiency or are also found in individuals with apparently normal serum levels of vitamin D. Danescu et al. (373) state that "*both animal and human studies support the notion that adequate vitamin D supplementation may decrease the incidence of type 1 and possibly also of type 2 diabetes mellitus and may improve the metabolic control in the diabetes state. However, the exact mechanisms are not clear and need further investigation*." According to Martin et al. (374), there are optimal levels of serum vitamin D for people at risk of diabetes, those with diabetes, and those without diabetes.

Paper 3:

This meta-analysis showed that exercise, specifically aerobic exercise, reduces leptin levels and increased adiponectin levels in prediabetic and diabetic individuals.

Our findings are in line with meta-analysis of Yu et al. (375), which included overweight and obese individuals. Hayashino et al. (376) reported a non-significant increase in adiponectin levels in type 2 diabetics across different exercise modalities, and a reduction in leptin levels, which was significant only in aerobic exercise. In comparison to ours, their analysis was confined only to type 2 diabetics, included much less subjects and also allowed for drug co-treatment.

However, according to Rostas et al. (377), resistance exercise, but not aerobic exercise, reduces leptin levels in middle-aged and old obese subjects, while no effects on adiponectin were found. Interestingly, the reduction in leptin was seen also in absence of diet or weight loss. This supports our findings, where the subgroup analysis showed that the reduction in leptin was also observed without dietary co-intervention, but significantly higher reduction was found with employment of diet. Given that a combination of diet and exercise generates a larger energy net deficit than either alone, this leads to acute negative energy balance short-term and also long-term through loss of adipose tissue. Leptin levels are sensitive to both short-term and long-term energy perturbations and are reduced with caloric deficit and/or fat loss (378). Exercise alone can therefore lead to reduction in leptin levels, but the effects are certainly greater when dietary intervention is added to the program. This is in line with guidelines (63, 298), which recommend exercise in conjuction with diet for prevention and management of type 2 diabetes.

An RCT published after our meta-analysis (378), which employed combined aerobic and resistance exercise for 12 weeks in overweight men, showed that exercise downregulates the expression of pro-inflammatory transcripts in white subcutaneous adipose tissue, which were initially increased in dysglycemic individuals. In addition, exercise increased the expression of adiponectin and decreased the expression of leptin in the adipose tissue and these changes were also reflected in plasma levels. The changes in adiponectin, leptin and other adipokines correlated with improvements in insulin sensitivity with exercise. The authors conclude that the improvements seen in insulin sensitivity with exercise intervention are mediated through adipose tissue function. Similarly, intensive lifestyle intervention consisting of diet and exercise over 2 years resulted in a pronounced increase in adiponectin levels in obese wemen (379). Finally, Gilbertson et al. (380) showed combining exercise with diet leads to significant improvement in adiponectin/leptin ratio, accompanied by improved insulin sensitivity.

Overall, there is strong evidence to support implementing exercise as an effective way to induce positive changes in levels of two key adipokines, adiponectin and leptin, especially when combined with dietary intervention.

14. Conclusion

This dissertation adds important evidence to the effects of several intervention types on adipocytokine levels in individuals with abnormal glucose metabolism.

Although meta-analysis represents the highest form of evidence in evidence-based medicine, there are several limitations to consider when interpreting the results. The results of the meta-analysis are driven by the studies included in the analysis. A thorough literature search was performed for every meta-analysis, but it cannot be excluded that there are trials which were not identified by the search strategy. Study populations included were generally heterogenous in terms of characteristics, such as sex, age and BMI. Interventions employed were also heterogenous, e.g. with regards to intervention duration or form and dosage of supplementation. Studies also generally did not monitor adherence to intervention, which is important, as patient adherence in chronic disease, such as type 2 diabetes, is often an issue. Results were often accompanied by high study hetereogeneity or were driven by results of single trials. Generally, publication bias could also not be excluded, which makes it impossible to exclude that unpublished results would have changed the outcome of the analyses.

There are several research gaps to address in future trials, including:

- As diabetes is a progressive disease, and metabolic dysfuncion is generally easier to reverse or halt at early stages of disease, are outcomes dependent on disease duration and severity?

- Are outcomes different in individuals with co-existing medical conditions, which are highly relevant in diabetes, and in which per se adipocytokines play big role, e.g. cardiovascular disease?

- How does pharmacological co-treatment influence adipocytokine levels?

- Can combining several lifestyle interventions, e.g. diet + exercise + omega-3 supplementation, produce synergistic effects?

- How do changes in adipocytokine levels relate to weight loss and changes in body composition?

- How do changing adipocytokine levels translate into clinically defined outcomes, e.g. glucose levels or insulin resistance?

Further evidence generation is crucial in order to optimize existing and develop novel interventions to halt the global diabetes burden.

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Effects of Omega-3 Supplementation on Adipocytokines in Prediabetes and Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background: The objective of this systematic review and meta-analysis was to determine the effects of omega-3 supplementation on adipocytokine levels in adult prediabetic and diabetic individuals.
Methods: We searched PubMed, Medline, EMBASE, Scopus, Web of Science, Google Scholar, Cochrane Trial Register, World Health Organization Clinical Trial Registry Platform, and Clinicaltrial.gov Registry from inception to August 1, 2017 for randomized controlled trials. Pooled effects of interventions were assessed as mean difference using random effects model. We conducted a sensitivity, publication bias and subgroup analysis.
Results: Fourteen studies individuals (n=685) were included in the meta-analysis. Omega-3 supplementation increased levels of adiponectin (0.48 μg/mL; 95% confidence interval [CI], 0.27 to 0.68; P<0.00001, n=10 trials), but effects disappeared after sensitivity analysis. Tumor necrosis factor α (TNF-α) levels were reduced (–1.71; 95% CI, –3.38 to –0.14; P=0.03, n=8 trials). Treatment duration shorter than 12 weeks was associated with greater reduction than longer treatment duration. Levels of other adipocytokines were not significantly affected. Publication bias could generally not be excluded.
Conclusion: Eicosapentaenoic acid and docosahexaenoic acid supplementation may increase adiponectin and reduce TNF-α levels in this population group. However, due to overall study heterogeneity and potential publication bias, a cautious interpretation is needed.

Keywords: Adipokines; Diabetes mellitus; Fatty acids, omega-3

INTRODUCTION

Global rates of diabetes mellitus have reached epidemic proportions and are associated with an ever-growing health and socioeconomic burden. The World Health Organization (WHO) estimates that the number of diabetic individuals rose from 108 million in 1980 to 422 million in 2014, whereas low- and middle-income countries have experienced a particularly high increase in diabetes prevalence [1]. With this enormous increase in global rates of diabetes also rises the economic cost, e.g., US data show that the total economic cost of diagnosed cases of diabetes amounted to USD 174 billion in 2007 and rose to 245 billion in 2012 [2]. A large part of the growing diabetes rates can be explained by the enormous increase in the global burden of obesity [3]. According to data from WHO, more than 1.9 billion adults were overweight in 2014; of these 600 million were obese [4]. Diabetes is associated with a significantly increased risk for all-cause mortality [5]. From the

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pathological perspective, there are two main types of diabetes: type 1 diabetes mellitus (T1DM), which is characterized by pancreatic β-cell secretion deficiency, and type 2 diabetes mellitus (T2DM), which is preceded by prediabetes in most individuals [6], and is caused by a complex interplay between insulin resistance and β-cell dysfunction [7].

Increased body adiposity leads to a greater risk of developing T2DM even with at normal body mass index (BMI) [8]. Dysfunction at the cellular level within the adipose tissue has been linked to insulin resistance and T2DM [9]. It is now well-established that adipose tissue is a metabolically highly active organ, secreting a plethora of molecules, with more than 600 identified so far [10]. These biologically active molecules, collectively known as adipocytokines, have been suggested to play a significant role in insulin resistance [11], β-cell dysfunction [12], as well as the occurrence of diabetes [13] and associated comorbidities [14,15].

Omega-3 fatty acids, a group of polyunsaturated fatty acids, have gained increasing popularity among general population and clinicians for their suggested positive modulatory effects on a variety of physiological function. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) represent the two major types of omega-3 fatty acids. EPA and DHA are mainly gained from seafood consumption, such as from fatty fish. These fatty acids are essential, as they cannot be produced by the human body, but have to be obtained by consuming marine food, such as fatty fish and fish oil. Supplementing omega-3 fatty acids was estimated to costs of 34.7 billion USD in 2016 [16]. These supplements have been used in managing a variety of clinical conditions and are believed to have a plethora of health benefits [17].

Regarding the effects of omega-3 fatty acids on the risk of developing diabetes, studies have shown mixed results [18,19]. Positive effects have been reported in relation to diabetic complications [20]. Omega-3 fatty acids have also been associated with favourable outcomes on adiponectin and leptin [21] and a range of inflammatory cytokines [22].

Given their ever-increasing popularity and the emerging evidence suggesting positive modulatory effects on signalling molecules, including adypocytokines, it is of high relevance to investigate the influence of omega-3 fatty acid supplementation on adipocytokine plasma concentration in prediabetic and diabetic individuals.

METHODS

Literature search

PubMed, Medline, EMBASE, Scopus, Web of Science, Google Scholar, Cochrane Trial Register, WHO Clinical Trial Registry Platform, and Clinicaltrial.gov Registry were used to systematically search for randomized controlled trials. We did not use any language restriction in the search. The literature was searched from inception to August 1, 2017. Key words included, among others: fatty acids, omega-3, fish oil, EPA, DHA, adipokines, adipocytokines, leptin, adiponectin, and clinical trial. In addition, related articles in electronic databases were also searched. Retrieved articles, systematic reviews and meta-analyses were searched manually in order to identify any overlooked additional potentially relevant trials.

Study selection

We used the following inclusion criteria: (1) intervention involving supplementation with EPA, DHA, both EPA and DHA, and fish oil; (2) randomized controlled trials with parallel or cross-over design; (3) involving adult (≥18 years) human subjects diagnosed with any of the following clinical conditions: insulin resistance, impaired glucose tolerance, impaired fasting glucose, or T2DM; (4) a minimum of 4 weeks intervention period; (5) encompassed the evaluation the outcome of interest to this meta-analysis; and (6) reported post-intervention mean values or change from baseline values with standard deviation (SD).

We applied the following exclusion criteria: (1) non-interventional studies, (2) uncontrolled studies, (3) lack of sufficient information on baseline or follow-up plasma concentration values of the selected adipocytokines, and (4) reviews, conference abstracts, commentaries, case reports, or duplicate publication from the same study.

Risk of bias assessment

We used Cochrane Collaboration's tool for assessing the risk of bias in randomized trials in order to assess the risk of bias of included studies (low, unclear, high) with respect to following study characteristics: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and others [23].

Data extraction and statistical analysis

From every study included, the two authors independently extracted the following information into a data spread sheet: family name of the first author of the study; publication year; characteristics of trial participants (number, age and gender); duration of the intervention; definition of the intervention and respective control; and assessed outcomes.

We extracted values of group means and corresponding SD. Where medians or interquartile ranges were reported instead of means, we used formulas proposed by Hozo et al. [24] to calculate the means and SD values. Where standard error of the mean (SEM) was only reported, SD was estimated using the following formula: SD=SEM×square root (n), where n is the number of subjects. The primary end point was change in circulating levels of adipocytokines, which were reported as changes between the values of arithmetic means at the end of the study-baseline [25]. SD of mean differences (MDs) were calculated as SD=square root $[(SD_{baseline})^2+(SD_{end\ of\ treatment})^2-(2r\times SD_{baseline}\times SD_{end\ of\ treatment})]$ for each group, assuming that $r=0.5$ [26].

We used the software Review Manager 5.3 as provided by the Cochrane Collaboration [27]. We applied the inverse-variance, random effects model of DerSimonian and Laird [28] to calculate the pooled estimates of the weighted MDs between the intervention and control groups, because this model incorporates between-study variability and provides a more conservative estimate of the average effect size.

The standard chi-square test was used as a statistical measure of heterogeneity between the different studies. The I^2 value was applied to determine the magnitude of inconsistency [29], calculated as $I^2=[(Q-df)]/Q\times100\%$, Q being the χ^2 value and df the corresponding degree of freedom. An I^2 value of greater than 50.0 % was defined as a cut-off to determine considerable heterogeneity between the included studies.

Sensitivity analysis through the leave-one-out method was employed to explore whether the dependency of the results on individual studies when these reported substantially different effect-sizes than the other studies.

We used the funnel-plot method to test for publication bias. In this method, the difference in mean changes were plotted against their standard errors to measure the precision of the studies. Where there was any disagreement between the authors, the data were revisited and agreed on by discussion by the authors.

RESULTS

Literature search and study characteristics

After applying all the selection criteria, 15 studies were included in the systematic review [30-44]. These studies reported the following outcomes: plasminogen-activator inhibitor 1 (PAI-1), adiponectin, leptin, resistin, tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6). One study [42] had a cross-over design, and could not be included in the quantitative synthesis (meta-analysis) in Review Manager; hence, 14 studies with parallel design were included in the meta-analysis [30-41,43,44]. Fig. 1 shows a detailed overview of the search strategy. Fig. 2A represents the overall risk of bias summary according to the defined characteristics, and Fig. 2B provides an overview across all individual studies included in the systematic review.

The studies included in the meta-analysis included a total of 29 treatment arms, comprising a total of 685 trial participants

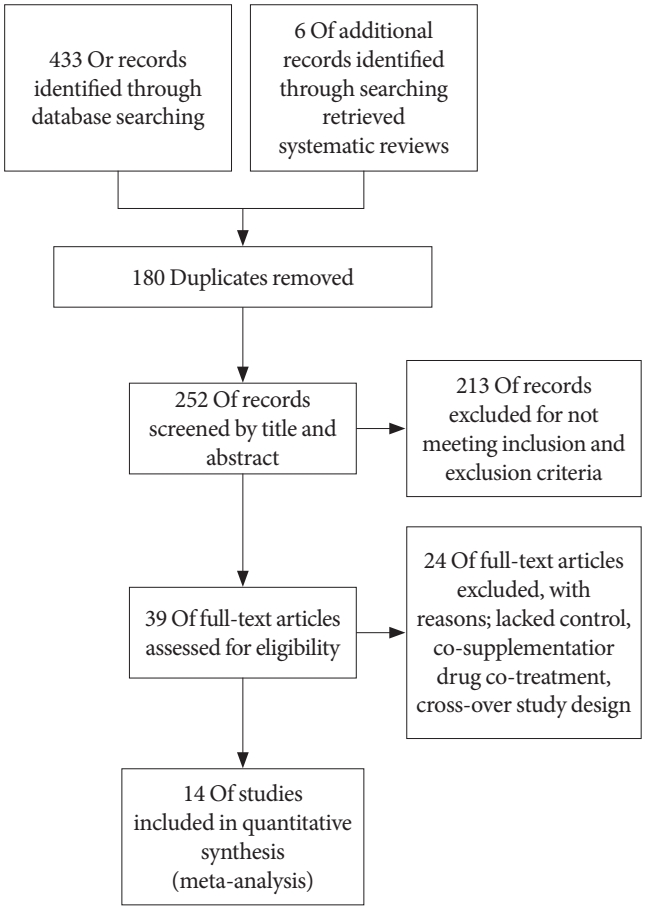


Fig. 1. Flow diagram.

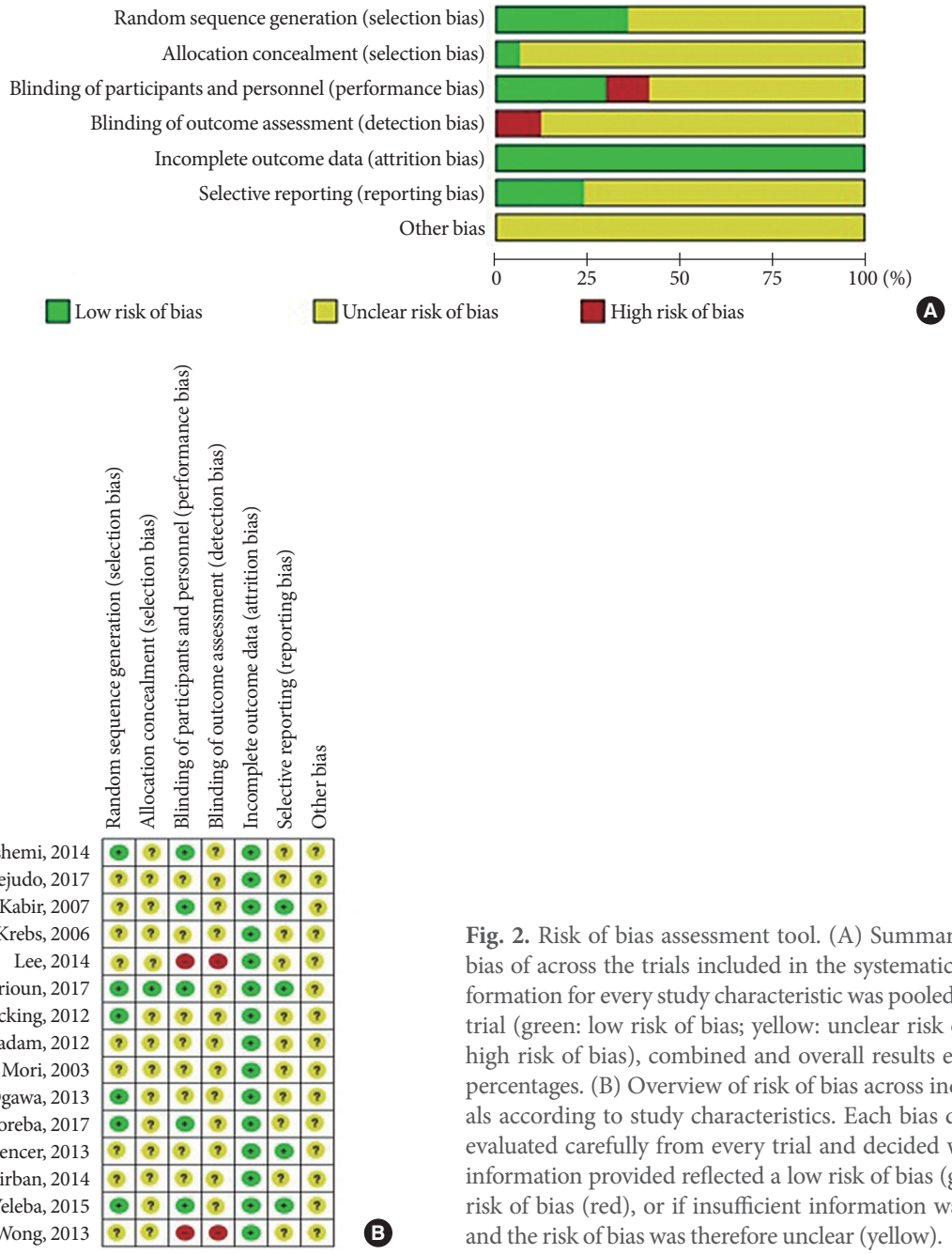


Fig. 2. Risk of bias assessment tool. (A) Summary of risk of bias of across the trials included in the systematic review. Information for every study characteristic was pooled from every trial (green: low risk of bias; yellow: unclear risk of bias; red: high risk of bias), combined and overall results expressed in percentages. (B) Overview of risk of bias across individual trials according to study characteristics. Each bias domain was evaluated carefully from every trial and decided whether the information provided reflected a low risk of bias (green), high risk of bias (red), or if insufficient information was provided and the risk of bias was therefore unclear (yellow).

(Table 1). Most studies involved subjects with T2DM; Krebs et al. [33], Spencer et al. [41], and Wong et al. [44] enrolled patients with prediabetes. One study [33] was conducted only with female subjects, otherwise both sexes were represented. The trial duration ranged from 6 to 24 weeks. Interventions in most studies consisted of omega-3 supplementation alone, in two studies [33,44] intended weight loss was a part of the intervention. Most studies used both EPA and DHA in the intervention, one study [30] used only EPA, and in one study EPA and DHA [38] were two different treatment arms, so that effect sizes were pooled separately.

Influence of omega-3 supplementation on plasma concentrations of adipocytokines

Two studies reported PAI-1 levels. Kabir et al. [32] used IU/mL as unit, and conversion was done to ng/mL [45]. Omega-3

Table 1. General characteristics of randomized controlled trials included in the systematic review

Reference	Sample size, % female subjects	Age, yr	BMI, kg/m^2	Duration, design	Disease, medication	Intervention (daily EPA/DHA dose)	Control group	Outcome, parameters
Hashemi et al. (2014) [30]	70 (I: 41, C: 36)	I: 44±5.0 C: 45±4.1	I: 27.9±1.7 C: 27.8±1.6	12 Weeks, parallel	T2DM subjects; treated with biguanides and sulfonylureas (76%), sulfonylureas (16%) or biguanides alone (8%)	EPA pearls (2,000 mg)	Placebo (corn oil)	Adiponectin
Jacobo-Cejudo et al. (2017) [31]	54 (I: 70.6, C: 83.9)	I: 50.4±6.3 C: 48.1±6.9	I: 25.6±2.4 C: 26.0±1.6	24 Weeks, parallel	T2DM; therapy with glibenclamide+metformin or metformin alone, received no additional dietary or lifestyle advice	Fish oil (320 mg EPA, 200 mg DHA)	Placebo (cornstarch)	Adiponectin, leptin, resistin
Kabir et al. (2007) [32]	26 (all female)	I: 55±2.0 C: 55±1.0	30±2.0 in both groups	2 Months, parallel	Postmenopausal T2DM women without hypertriglyceridemia; 3 patients treated only with diet, 23 taking oral hypoglycemic treatment (7 only biguanides, 16 sulfonylureas and biguanides), 5 lipid-lowering agents, 6 hormone replacement therapy	Fish oil (1,080 mg EPA, 720 mg DHA)	Placebo (paraffin oil)	Adiponectin, leptin, TNF-α, IL-6, PAI-1
Krebs et al. (2006) [33]	67 (all female)	44.7±13.2	35.0±5.5	24 Weeks, parallel	Overweight or obese women with hyperinsulinemia	Fish oil (1,300 mg EPA, 2,900 mg DHA)+weight loss intervention (10% of body weight loss in 12 weeks, 12 weeks weight maintenance)	5 g oil/day totaling 2.8 g linoleic acid and 1.4 g oleic acid+weight loss intervention (10% of body weight loss in 12 weeks, 12 weeks weight maintenance)	Adiponectin, leptin, TNF-α, IL-6
Lee et al. (2014) [34]	37 (I: 62.5, C: 71.4)	I: 56.2±8.7 C: 59.9±9.8	I: 33.2±4.8 C: 34.8±5.3	8 Weeks, parallel	Early stage T2DM (no evidence of end-stage organ damage secondary to diabetes)	Fish oil (3,580 mg EPA, 2,440 mg DHA)	Placebo (corn oil)	Leptin
Mazaherioun et al. (2017) [35]	85 (I: 34.09, C: 41.4)	I: 51.5±7.45 C: 50.56±7.21	I: 29.22±3.58 C: 29.21±2.90	10 Weeks, parallel	T2DM; no insulin, thiazolidinediones or anti-obesity medications	Omega-3 capsules (1,800 mg EPA, 900 mg DHA)	Placebo (paraffin)	Adiponectin
Mocking et al. (2012) [36]	24 (I: 62, C: 42)	I: 53.1±13.8 C: 55.0±8.6	I: 29.3±5.1 C: 29.8±4.8	12 Weeks, parallel	Diabetic patients with major depressive disorder (determined by the Composite International Diagnostic Interview standards); therapy with antidepressants for at least 2 months, diabetes treated with diet only, hypoglycemics, insulin, or both	Fish oil (at least 900 mg ethyl-EPA)	Placebo (rapeseed oil+medium chain triglycerides)	TNF-α, IL-6
Malekshahi Moghadam et al. (2012) [37]	84 (50% in both groups)	I: 55.36±9.88 C: 52.96±10.72	I: 27.72±3.47 C: 27.51±3.16	8 Weeks, parallel	T2DM subjects (at least 2 years since diagnosis), all receiving anti-hyperglycemic treatment	Omega-3 capsules (1,548 mg EPA, 828 mg DHA)	Placebo (sunflower oil)	TNF-α
Mori et al. (2003) [38][a]	51 (I_1: 17,6, I_2: 27,7, C: 25)	I_1: 61.2±2.3 I_2: 60.9±1.9 C: 61.5±1.9	I_1: 30.6±0.7 I_2: 27.9±0.8 C: 29.9±1.0	6 Weeks, parallel	Non-smoking, treated-hypertensive, T2DM men and postmenopausal women; antihypertensive therapy for a minimum of 3 months, oral hypoglycemic, but no insulin therapy	I_1: 4 g EPA-capsules per day (96% EPA ethyl ester) I_2: 4 g DHA per day (92% DHA ethyl ester)	Placebo (olive oil)	TNF-α, IL-6

(*Continued to the next page*)

Table 1. Continued

Reference	Sample size, % female subjects	Age, yr	BMI, kg/m^2	Duration, design	Disease, medication	Intervention (daily EPA/DHA dose)	Control group	Outcome, parameters
Ogawa et al. (2013) [39]	26 (I: 69, C: 82)	I: 79.5±8.6 C: 81.2±7.6	I: 19.9±4.0 C: 20.1±3.6	3 Mllel	Bedridden elderly T2DM patients	Liquid diet rich in EPA (25 mg/100 kcal) and DHA (17 mg/100 kcal)[b]	Liquid diet lacking EPA and DHA	Adiponectin, TNF-α, IL-6
Poreba et al. (2017) [40]	74 (I: 38,9, C: 31.6)	I: 64.4±6.7 C: 66.7±6.8	I: 30.9 (27.9–34.7) C: 31.1 (28.1–32.7)[c]	3 Months, parallel	T2DM patients with a history of coronary artery disease or peripheral artery disease	Omega-3 drink (1,000 mg EPA, 1,000 mg DHA)	Drink without omega-3	Adiponectin, leptin, TNF-α, IL-6
Spencer et al. (2013) [41]	33 (I: 68,4, C: 64,2)	I: 48.8±2.3 C: 53.3±2.2	I: 33.4±2.3 C: 33.4±1.1	12 Weeks, parallel	Non-diabetic subjects with either impaired fasting glucose, impaired glucose tolerance (23 subjects), or at least three signs of metabolic syndrome	Fish oil (1,860 mg EPA, 1,500 mg DHA)	Placebo (corn oil)	Adiponectin, leptin, TNF-α, I L-6, PAI-1, resistin
Stirban et al. (2014) [42]	34, No specification of sex	56.8±8.3	31.2±4.1	6 Weeks, cross-over	T2DM (duration 9.8± 6.6 years), no history of major cardiovascular events or a surgical or interventional history within the previous 6 months; treated with low dose aspirin (n=20), ACE-inhibitors or AR blockers (n=24), calcium channel blockers (n=5), β-blockers (n=15), diuretics (n=13), and statins (n=13), all withdrawn before measurements; diabetes medication: diet (n=3), oral therapy (n=16), insulin (n=4), oral hypoglycemic plus insulin (n=10), and incretin mimetics (n=1)	Omega-3 capsules (920 mg EPA, 760 mg DHA)	Placebo (olive oil)	Adiponectin, leptin
Veleba et al. (2015) [43]	29, 34	I: 59.5 C: 62.0	I: 34.0 C: 30.9	24 Weeks, parallel	T2DM (at least 3 months from diagnosis); metformin as monotherapy as a stable dose (0.5–3.0 g/day) for at least 1 month	Omega-3 concentrate (750 mg EPA, 2,000 mg DHA)	Placebo (corn oil)	Adiponectin, leptin
Wong et al. (2013) [44]	25, 44	60±4.0	I: 34±2.0 C: 33±1.0	16 Weeks, parallel	Middle-aged, centrally obese, normotensive, dyslipidemic (elevated triglycerides, low HDL), insulin resistant adults; 6 subjects receiving lipid-lowering therapy (4 atorvastatin, 2 rosuvastatin), 5 anti-hypertensive treatment (2 on ACE inhibitors, 2 on AR blockers, 1 on β1-blocker)	Fish oil (1,840 mg EPA-ethyl ester, 1,520 mg ethyl-ester)+weight loss intervention (12 weeks hypocaloric diet with energy deficit of at least 1,900 kJ/day, followed by 4 weeks of weight maintenance with energy intake increased by 460 kJ/day)	Weight loss alone (protocol as in intervention group)	Adiponectin

Values are presented as mean±standard deviation.

BMI, body mass index; I, intervention group; C, control group; T2DM, type 2 diabetic mellitus; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; PAI-1, plasminogen-activator inhibitor 1; ACE, angiotensin-converting-enzyme; AR, angiotensin II receptor; HDL, high density lipoprotein.

[a]EPA and DHA were used in two separate treatment arms, [b]The patients consumed 1,015.4±231.1 kcal/day, [c]Values are presented as median (interquartile range).

supplementation did not significantly affect PAI-1 levels (MD, −11.47 ng/mL; 95% confidence interval [CI], −23.52 to 0.57; $P=0.06$, $I^2=11\%$) (Fig. 3).

Effect sizes for adiponectin were pooled from a total of 10 studies. Omega-3 supplementation significantly increased plasma adiponectin concentration (MD, 0.48 μg/mL; 95% CI,

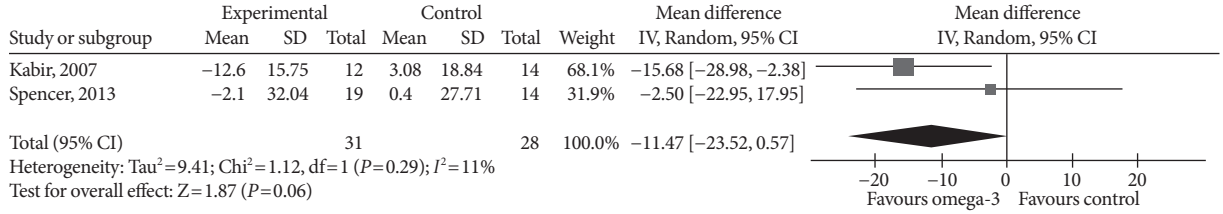


Fig. 3. Influence of omega-3 fatty acids supplementation on plasminogen-activator inhibitor 1 levels (ng/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for two randomized controlled trials. The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups. SD, standard deviation; IV, interval variable.

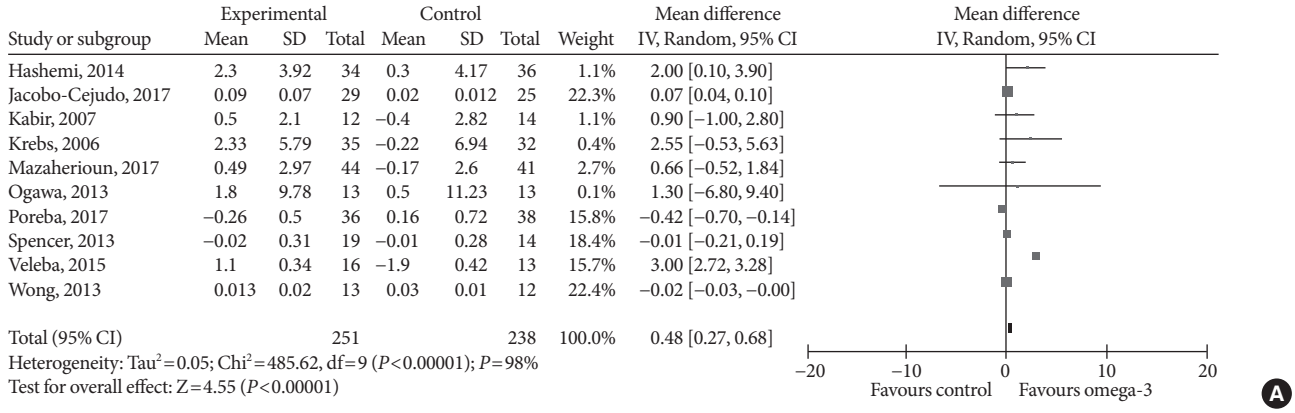


Fig. 4. Influence of omega-3 fatty acids supplementation on adiponectin levels (μg/mL). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for 10 randomized controlled trials. The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups. As opposed to graphs for all other outcome parameters, the labels of the X-axis are different, because an increase in adiponectin levels is seen as favorable. (B) Meta-analysis after eliminating Veleba et al. [43] as part of the leave-one-out sensitivity analysis (the trial was given a relative weight of 0.0%). SD, standard deviation; IV, interval variable.

0.27 to 0.68; $P<0.00001$, $I^2=98\%$) (Fig. 4A). For the sensitivity analysis, we eliminated Veleba et al. [43], because this study reported an effect which was significantly greater than that of other individual studies. No effects of omega-3 supplementation on adiponectin concentration were observed (MD, 0.00 μg/mL; 95% CI, −0.09 to 0.08; $P=0.98$, $I^2=85\%$) (Fig. 4B) in this sensitivity analysis.

With regards to effects on resistin, omega-3 supplementation did not affect its levels in a statistically significant way (MD, −0.77 ng/mL; 95% CI, −2.44 to 0.97; $P=0.4$, $I^2=100\%$) (Fig. 5).

Supplementing omega-3 fatty acids did not significantly alter

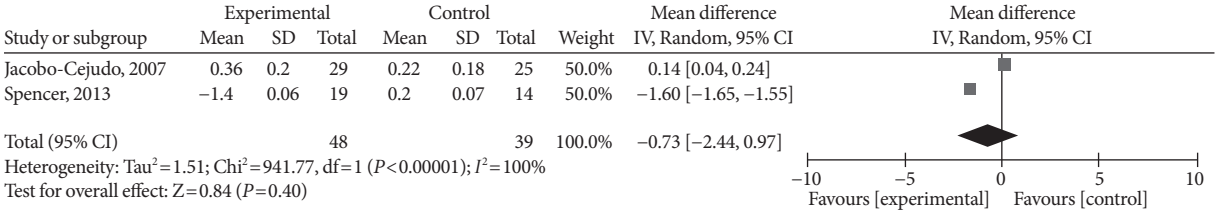


Fig. 5. Influence of omega-3 fatty acids supplementation on resisting levels (ng/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for two randomized controlled trials. The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups. SD, standard deviation; IV, interval variable.

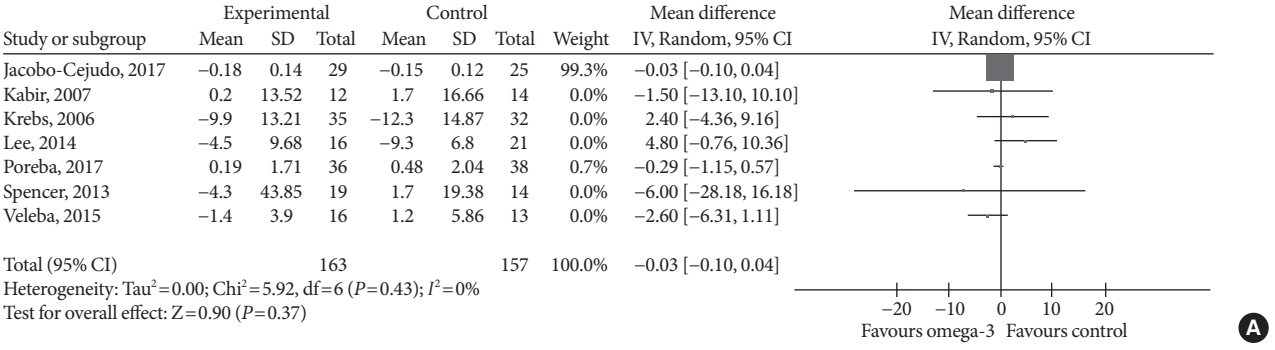


Fig. 6. Influence of omega-3 fatty acids supplementation on leptin levels (ng/mL). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for seven randomized controlled trials. The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. Notice the absence of the black colored diamond at the bottom as in other graphs because of the magnitude of the pooled mean difference with 95% CI for all study groups. (B) Meta-analysis after eliminating Spencer et al. [41] as part of the leave-one-out sensitivity analysis (the trial was given a relative weight of 0.0%). SD, standard deviation; IV, interval variable.

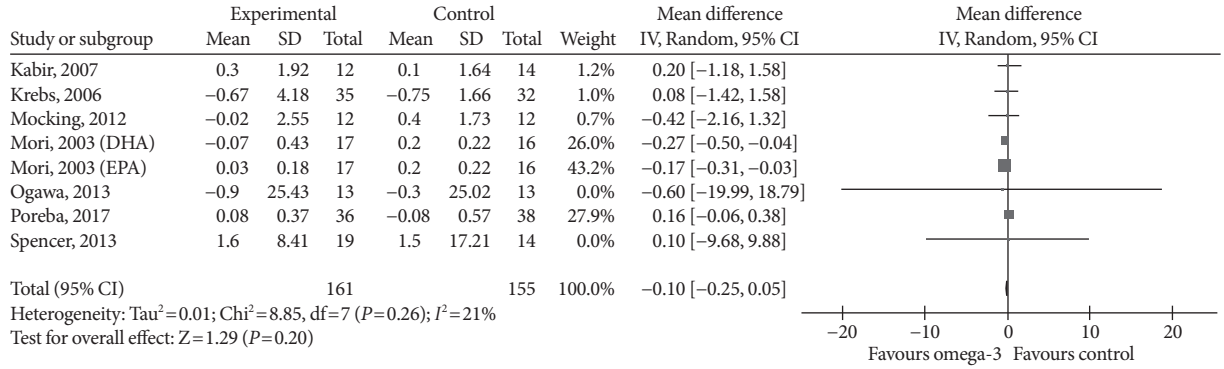


Fig. 7. Influence of omega-3 fatty acids supplementation on interleukin 6 levels (pg/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for eight intervention effects pooled from seven randomized controlled trials (two separate effects were pooled for docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA] treatment arms from Mori et al. [38]). The green colored square represents the point estimate of the effect of the intervention for each intervention. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the interventions in the meta-analysis. SD, standard deviation; IV, interval variable.

leptin levels (MD, –0.03 ng/mL; 95% CI, –0.10 to 0.04; $P=0.37$, $I^2=0\%$) (Fig. 6A). As the effect reported in Spencer et al. [41] was considerably different compared with that of other individual studies, we eliminated it from the analysis, this did result in a significant change in effect size (MD, –0.08 ng/mL; 95% CI, –0.49 to 0.34; $P=0.72$, $I^2=11\%$) (Fig. 6B).

Omega-3 supplementation also exerted an effect on plasma concentrations of inflammatory cytokines IL-6 and TNF-α. The effects on IL-6 did not reach statistical significance (MD, –0.10 pg/mL; 95% CI, –0.25 to 0.05; $P=0.2$, $I^2=21\%$) (Fig. 7), but TNF-α levels significantly reduced (MD, –1.71; 95% CI, –3.38 to –0.14; $P=0.03$, $I^2=86\%$) (Fig. 8A), this effect also remained after removing Spencer et al. [41] for the sensitivity analysis, as this was the only study which reported an increase in TNF-α levels in the primary analysis (MD, –2.01 pg/mL; 95% CI, –3.55 to –0.47; $P=0.01$, $I^2=87\%$) (Fig. 8B).

Subgroup-analysis

As adiponectin and TNF-α were the only two outcomes whose blood levels were significantly affected by omega-3 supplementation in the primary analysis and we found considerable study heterogeneity, we conducted a subgroup analysis by dose (low ≤2,000 mg, high >2,000 mg) and treatment duration (<12, ≥12 weeks) to investigate whether the effect of the intervention in these outcomes varied between the subgroups.

For adiponectin, omega-3 significantly increased its levels when supplemented in high dose (MD, 0.62 μg/mL; 95% CI, 0.40 to 0.85; $P<0.00001$, $I^2=99\%$); low-dose supplementation did not lead to significant changes (MD, 0.48 μg/mL; 95% CI, –0.73 to 1.69; $P=0.44$, $I^2=67\%$; test for subgroup differences: $P=0.82$, $I^2=0\%$) (Fig. 9A). As for treatment duration, we found significant effects in the subgroup ≥12 weeks (MD, 0.47 μg/mL; 95% CI, 0.26 to 0.68; $P<0.00001$, $I^2=99\%$), while shorter treatment duration did not produce statistically significant effects (MD, 0.73 μg/mL; 95% CI, –0.28 to 1.73; $P=0.16$, $I^2=0\%$; test for subgroup differences: $P=0.62$, $I^2=0\%$) (Fig. 9B).

For TNF-α, omega-3 did not significantly change the levels neither in the low-dose (MD, –0.04 pg/mL; 95% CI, –0.10 to 0.02; $P=0.17$, $I^2=0\%$) nor in the high-dose group (MD, –2.31 pg/mL; 95% CI, –5.55 to 0.93; $P=0.16$, $I^2=88\%$; test for subgroup differences: $P=0.17$, $I^2=46.9\%$) (Fig. 10A). Supplementing omega-3 for less than 12 weeks significantly reduced TNF-α levels (MD, –4.71 pg/mL; 95% CI, –6.01 to –3.41; $P<0.00001$, $I^2=0\%$); whilst no statistically significant effects were found with longer supplementation (MD, –0.16 pg/mL; 95% CI, –0.96 to 0.64; $P=0.7$, $I^2=46\%$; test for subgroup differences: $P<0.00001$, $I^2=97.1\%$) (Fig. 10B).

Publication bias

Visual inspection of funnel plots for adiponectin, leptin, IL-6, and TNF-α (Fig. 11) revealed a moderate asymmetry in all of the outcomes, so that a publication bias cannot be excluded and we cannot ascertain that a non-publication of negative or inconclusive data did not influence our meta-analysis.

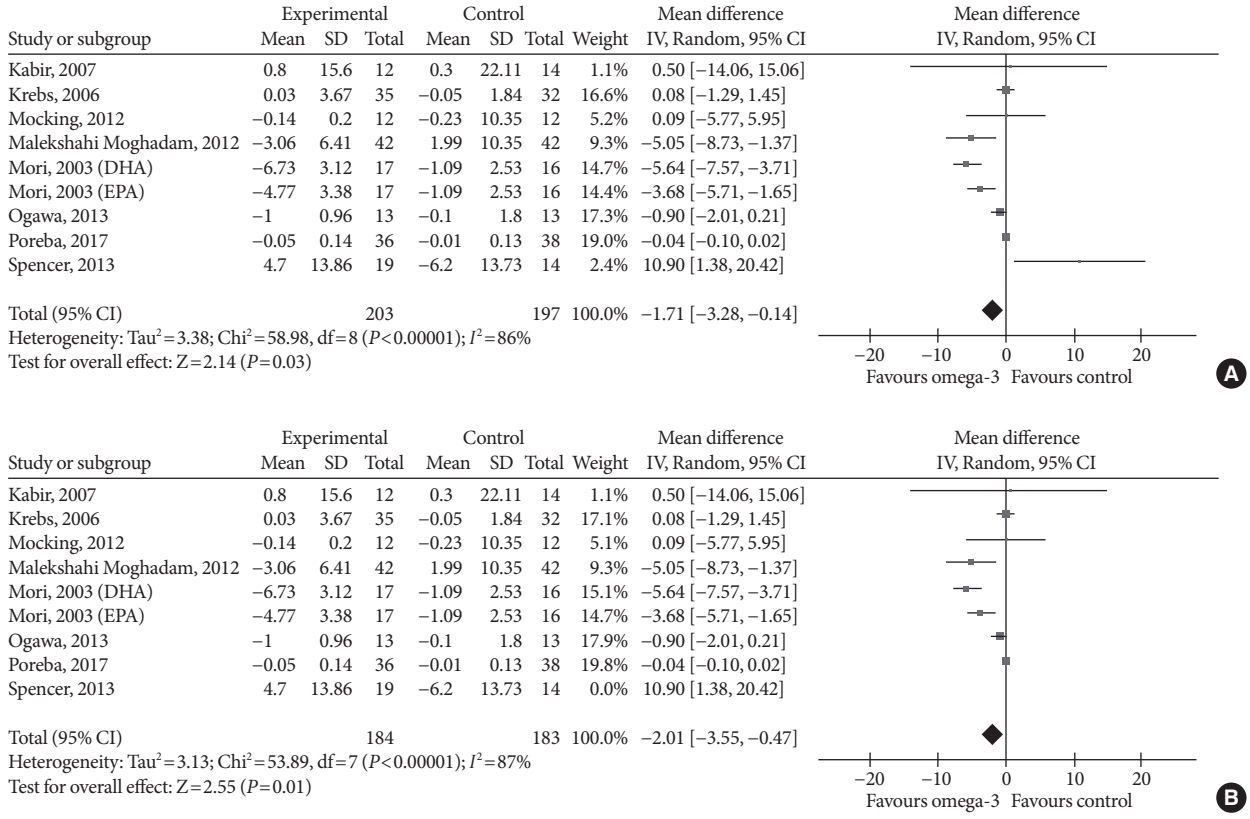
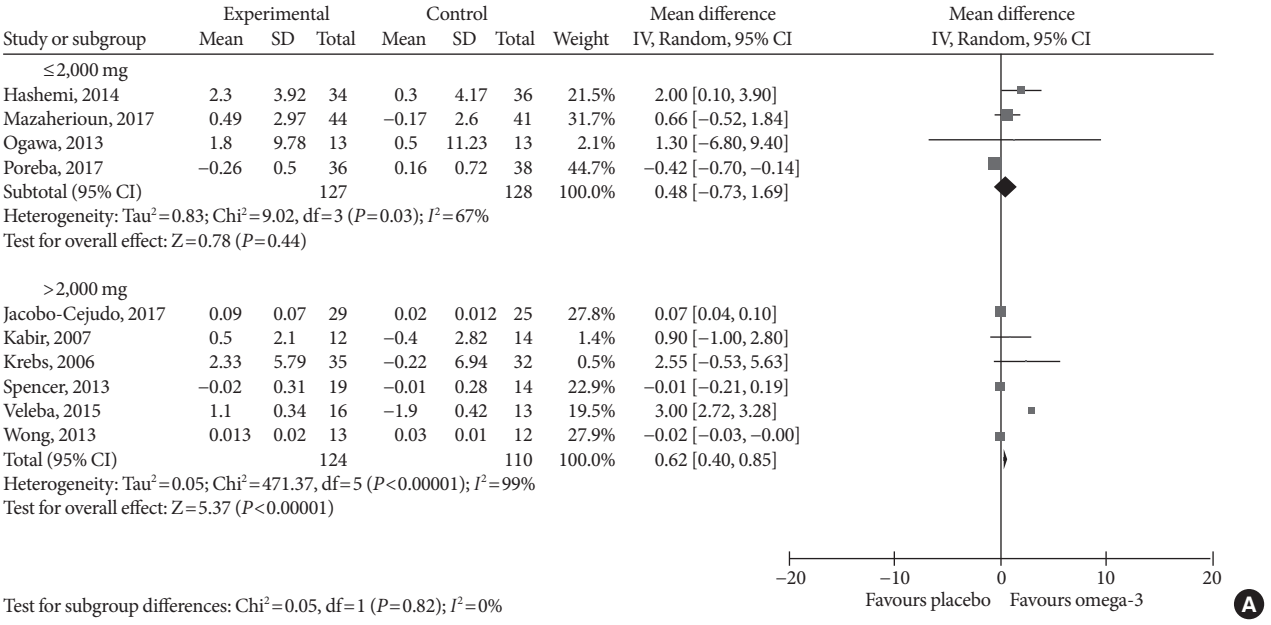


Fig. 8. Influence of omega-3 fatty acids supplementation on tumor necrosis factor α levels (pg/mL). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CIs) for nine intervention effects pooled from eight randomized controlled trials (two separate effects were pooled for docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA] treatment arms from Mori et al. [38]). The green colored square represents the point estimate of the effect of the intervention for each intervention. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the interventions in the meta-analysis. (B) Meta-analysis after eliminating Spencer et al. [41] as part of the leave-one-out sensitivity analysis (the trial was given a relative weight of 0.0%). SD, standard deviation; IV, interval variable.

DISCUSSION

To the best of our knowledge, this is the first meta-analysis to investigate the results of omega-3 supplementation on adipocytokine levels in prediabetic and diabetic individuals. We found that omega-3 supplementation has no statistically significant effects on levels of PAI-1, resistin, leptin, and IL-6. We found that omega-3 can significantly increase adiponectin levels, but study heterogeneity was very high in this analysis (I^2=98%). Moreover, this effect was dependent on one individual study, as it completely disappeared once we conducted sensitivity analysis. Regarding TNF-α, omega-3 supplementation reduced its levels both in the primary as well in the sensitivity analysis, but with considerable study heterogeneity in both analyses. We conducted a subgroup analysis based on omega-3 dose and treatment duration for these two outcomes in order to elucidate whether the effects of omega-3 supplementation in the primary outcomes varied between the subgroups via a test of interaction (indicating effect modification). We found that for TNF-α, omega-3 supplementation has different effects in subgroups with different treatment durations while using 12 weeks as the cut-off value, i.e., there was significant effect modification based on treatment duration. Interestingly, lower treatment duration significantly reduced TNF-α levels as compared to longer treatment duration. We found no effect modification in the dose subgroup analysis for TNF-α levels, nor for dose or treatment duration for adiponectin levels.

PAI-1's associations with obesity and diabetes have been established in the 1980s [46], and it is nowadays well established that elevated levels of PAI-1 are associated with the develop-



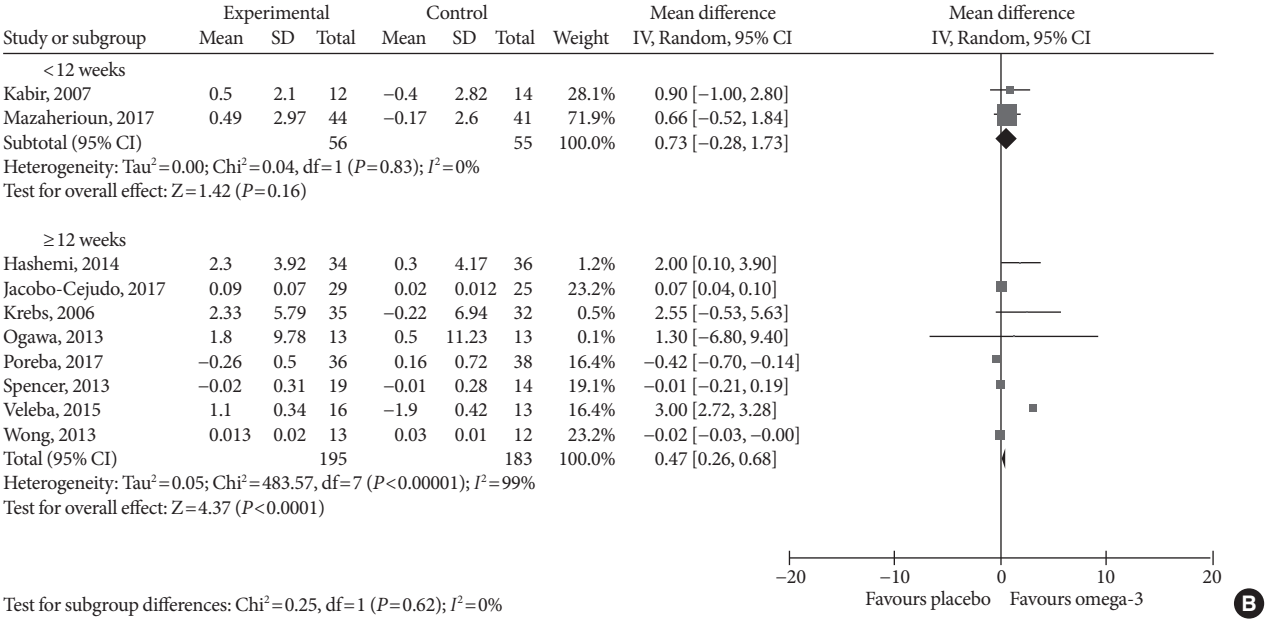


Fig. 9. Subgroup analysis on the influence of omega-3 fatty acids supplementation according to (A) omega-3 dose and (B) treatment duration for adiponectin (μg/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CIs). The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups. SD, standard deviation; IV, interval variable.

ment of T2DM [47] and cardiovascular events and mortality [48]. Intervention to reduce the levels of PAI-1 have therefore been recognized as a priority in this population [49].

Leptin could be righteously called the master hormone of all adipokines, because of the variety of physiological functions that it controls and they are all of great relevance in diabetes [50]. Body fat mass is the single most important determinant of leptin levels in individuals, but insulin resistance that occurs

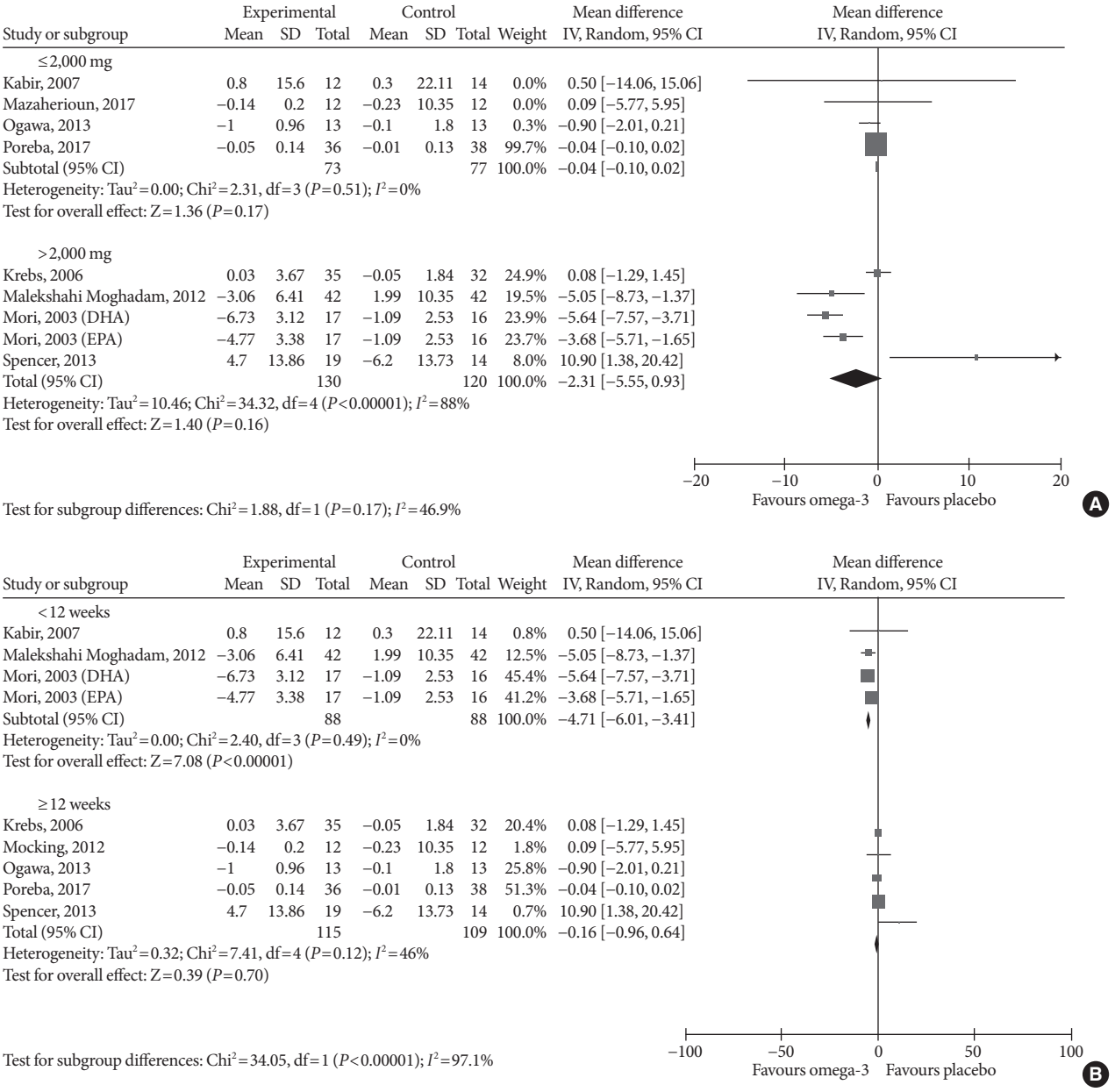


Fig. 10. Subgroup analysis on the influence of omega-3 fatty acids supplementation according to (A) omega-3 dose and (B) treatment duration for tumor necrosis factor α (pg/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CIs). The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups. SD, standard deviation; IV, interval variable; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

in T2DM was found to be associated with higher leptin levels independently of body fat mass [51]. Hyperleptinaemia is a marker of leptin resistance, a pathophysiological condition where tissues do not respond to leptin signaling, which further potentiates the metabolic and cardiovascular disarrangements that occur in face of diabetes [52]. Consequently, reducing blood leptin levels is suggested to ameliorate leptin sensitivity [53], but a recently conducted meta-analysis, which was not only constrained to prediabetic and diabetic individuals, found that moderate, but significant reduction in leptin levels was

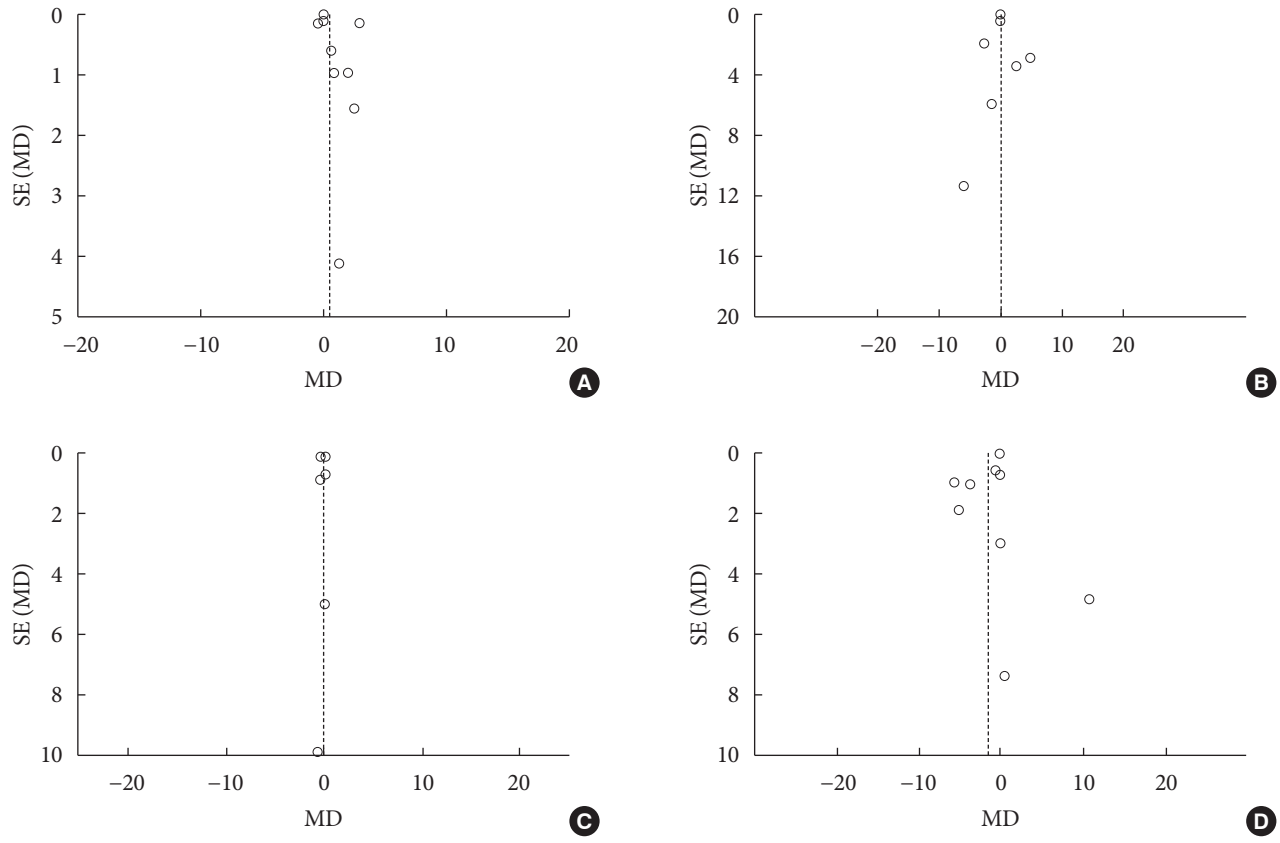


Fig. 11. Funnel plot displaying study precision against the mean difference (MD) effect estimate with 95% confidence interval for (A) adiponectin, (B) leptin, (C) interleukin 6, and (D) tumor necrosis factor α. SE, standard error.

achieved with omega-3 fatty acid supplementation in non-obese individuals, whereas smaller and non-significant effects were observed in obese individuals [54], but the grade of reduction was greater than the one revealed in our analysis in both groups.

Adiponectin circulates in relatively high concentrations (μg/mL) as compared to other adipokines, and has unique physiological properties with a profound impact on glucose and fatty acid metabolism as well as the cardiovascular system, which has placed it in the center of interest of the scientific community. Hypoadiponectinaemia paradoxically occurs in obesity and diabetes, and due to its insulin-sensitizing and anti-inflammatory properties, increasing circulating levels of adiponectin is believed to result to improved metabolic and cardiovascular functions [55]. Our results are in line with those previously reported in meta-analysis [56] and narrative reviews [21]. Interestingly, the increase in adiponectin levels we observed was largely dependent on one study and there was unexplained study heterogeneity, as we could not infer effect modification neither to treatment duration nor omega-3 dose used. The unexplained heterogeneity in adiponectin levels was also found in previous meta-analysis [56].

Resistin is a relatively newly discovered adipocytokine which is suggested to exert very important effects that link obesity to insulin resistance and T2DM [57], and evidence also amounts with regards to its role in mechanisms leading to cardiovascular disease, including inflammation, endothelial dysfunction, thrombosis, angiogenesis, and smooth muscle function. Reducing the levels of resistin is therefore seen as a promising strategy [58]. To our knowledge, this is the first meta-analysis that investigated the effects of omega-3 supplementation on resistin, and even though the effect was non-significant with a very high degree of study heterogeneity, our results suggest a potential modulatory role of omega-3 fatty acids on this interesting adipocytokine.

TNF-α and IL-6 are one of the main proinflammatory cytokines that are also secreted to a large part from the adipose tissue which is infiltrated by macrophages and other immune

cells and their levels are elevated in diabetic individuals, resulting to inflammation [59]. Inflammation not only contributes to the etiology of diabetes, but once the disease has set on, it aggravates its clinical course and is associated with a range of complications [60]. The reducing effects of omega-3 fatty acids on these two cytokines might be explained by their general anti-inflammatory properties [23]. The reduction in subclinical inflammation by supplementing EPA and DHA along with potential positive modulation of other physiological pathways that are brought about by changes in plasma concentrations of herein investigates cytokines may contribute to improved management of patients suffering from prediabetes or T2DM. Interestingly, our work identified that for TNF-α, treatment duration infers effect modification, where shorter treatment duration is associated with higher reductions in TNF-α levels.

However, our systematic review and meta-analysis has several limitations. In general, most studies which were included did not provide sufficient information to be able to assess the risk of bias across many of the pre-set out criteria. Based on the I^2 measure of greater than 50%, substantial study heterogeneity was found across outcome parameters investigated. The population analyzed was also heterogeneous, as the patients enrolled differed in terms of general characteristics, such as age, BMI, male/female ratio, medication use. Omega-3 supplementation also varied across the included studies with regards to the dose, duration, and EPA/DHA ratio. Moreover, intentional weight loss was a part of intervention in two studies, whereas the remaining studies only included omega-3 supplementation as intervention. This is important, as weight loss is known to affect a variety of metabolic parameters. Furthermore, two outcome parameters (PAI-1 and resistin) were inspected on the basis of only two studies with a small sample size. Finally, we could not exclude publication bias in our meta-analysis. These limitations implicate that a cautious interpretation of the results of the present systematic review and meta-analysis is necessary.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Effects of Vitamin D Supplementation on Adipocytokine Levels in Patients with Abnormal Glucose Metabolism: Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Abstract

Objective: The objective of this systematic review and meta-analysis was to investigate the effects of vitamin D supplementation on circulating levels of adipocytokines in prediabetic and diabetic individuals

Materials and Methods: The following databases were searched from randomized controlled trials: PubMed, Medline, Scopus, EMBASE, Web of Science, Google Scholar, Cochrane Trial Register, WHO Clinical Trial Registry Platform and Clinicaltrial.gov Registry until December 2017. We assessed pooled effects of interventions as mean difference by applying the random effects model. The Cochrane Collaboration's tool was used to investigate the risk of bias. To exclude that the overall effect sizes depended on individual studies, we conducted sensitivity analysis through the leave-one-out method. We also inspected for publication bias.

Results: Our meta-analysis included 16 studies with 1058 individuals. Supplementing vitamin D significantly reduced levels of IL-6 (MD: 1.67pg/ml (95% CI-2,53, -0.80), p= 0.0002, I2= 99%), and the effects remained after sensitivity analysis. Levels of adiponectin, leptin and tumor necrosis factor-alpha (TNF-alpha) were not significantly changed. Overall study heterogeneity was high. We could not exclude publication bias.

Conclusion: Vitamin D supplementation can reduce levels of IL-6 in prediabetic and diabetic individuals, but does not affect other adipocytokines. However, a cautious interpretation of our results is warranted.

Keywords: Diabetes Mellitus; Adipokines; Vitamin D

Introduction

Diabetes mellitus is increasing at alarming rates worldwide. This condition is associated with serious health consequences and an enormous socioeconomic burden. According to recent estimates, the number of individuals suffering from diabetes rose from 108 million in 1980 to 422 million in 2014, i.e. a 4-fold increase. Somewhat higher increases have been observed in men as compared to women (4.3% to 9.0% and 5.0% to 7.9%, respectively) [1]. The economic implications of managing diabetes are enormous [2]. For instance, in the US alone, the cost of diagnosed diabetes amounted to 245 billion USD in 2012 [3]. Diabetes is associated with significant mortality, and affected individuals have a higher risk of all-cause mortality compared to non-diseased individuals [4]. The increasing prevalence of diabetes noted in the past decades goes hand in hand with the ever growing number of overweight and obese individuals. High prevalence of overweight and obesity globally along with low levels of physical activity are associated with significant part of the world´s diabetes burden [5]. According to the World Health Organization, more than 1.9 billion adults were overweight in 2014, out of which 600 million were obese [6].

The vast majority of individuals living with diabetes develop metabolic abnormalities, such as insulin resistance before developing full-blown disease. These metabolic disturbances, apparent as impaired fasting glucose and impaired glucose tolerance, collectively known as pre-diabetes, are present potentially in as up to 70% of individuals who eventually develop true diabetes [7]. Insulin resistance is the key pathological characteristic of abnormal glucose metabolism [8]. Over an observational period of 3-5 years, approximately one out of four individuals with prediabetes progresses to develop overt diabetes, but long-term most prediabetic individuals are likely to become diabetic [9]. The exact molecular mechanisms that lead from prediabetes to frank diabetes are not clear [10]. Pancreatic beta-cell failure is believed to represent the crucial step in this

process [11], and occurs within a short time period [12-14]. Changes in glucose concentration, insulin sensitivity and insulin secretion are mostly subtle in prediabetic individuals, and can go on for more than 10 years, followed by a sharp deterioration of glucose metabolism and diabetes development within 2 years [14].

Background

Body mass index [15] and body fat levels [16] are both strong predictors of insulin resistance. Other anthropometric indicators, such as waist circumference and waist/hip ratio, which are indicative of central adiposity, are also associated with the incidence of diabetes [17]. Clearly, a common denominating factor of the vast majority of prediabetic and diabetic individuals is adiposity. A paradigm shift has been brought about in the past decades as to the physiological role of adipose tissue. As large number of compounds secreted from the adipose tissue has been identified, it is now well-established that adipose tissue functions as a highly functional endocrine organ [18]. More than 600 bioactive compounds which are secreted from the adipose tissue have been identified so far [19].

Adipocytokines play a pivotal role in the main hallmark of abnormal glucose metabolism, i.e. insulin resistance [20-26], but they appear to play a major role in diabetes-associated beta-cell failure as well [27]. Indeed, dysregulated adipocytokine profile has been linked with increased incidence of diabetes in prospective studies [27,28]. The importance of adipose tissue function in susceptibility to develop diabetes becomes apparent when normal weight diabetic individuals are accounted for [29], as in this group of patients dysregulated adipocytokine profile is of great importance [30]. In addition, adipocytokines can explain a great part of comorbidities typically occurring in patients with abnormal glucose metabolism. They are suggested to be at the metabolic cross-roads connecting insulin resistance and atherosclerosis [31]. This is particularly important, as cardiovascular disease is the most common cause of death in diabetic individuals [32,33]. Furthermore, they are associated with microvascular complications in diabetic individuals [34,35], and consequently diabetic nephropathy [36-39] and retinopathy [39], with emerging evidence as to their role in diabetic neuropathy as well [40-41]. Vitamin D is a fat-soluble vitamin with a plethora of physiological functions in the body [42,43]. Globally, inadequate vitamin D levels are widely present, bearing serious health implications [44]. Given its profound metabolic effects, the significance of vitamin D in diabetes has been extensively discussed [45-47]. Although there is evidence that vitamin D deficiency is associated with a higher risk for diabetes and that maintaining adequate levels seems to exert a protective effect [48-51], there are mixed results as to the effects of vitamin D on glycemic parameters in prediabetic [52-55] and diabetic individuals [56-59], with emerging importance with respect to common diabetic complications [60-66].

Vitamin D has important physiological signalling roles in the adipose tissue [67-69], but its receptors have also been identified in infilitrating immune cells [70,71]. Correlations between vitamin D and adipoctyokine concentrations such as leptin [72,73], adiponectin [73,74] and interleukin-6 [75]have already been reported, but less is clear about the effects in individuals with disturbances in glucose metabolism specifically. Recent results from the Jackson Heart Study demonstrated that the associations between vitamin D status and risk factors for cardiovascular disease are partially mediated through circulating adipocytokines [76]. Importantly, vitamin D as a fat-soluble molecule accumulates in the fat mass in obese individuals [77], so that is plausible that its effects on secretion of adipocytokines from adipose tissue might be observed particularly in this group. In light of this compelling evidence, the aim of this systematic review and meta-analysis was to investigate the effects of vitamin D on plasma adipocytokine concentrations in individuals suffering with prediabetes and type 2 diabetes, as these effects may be of clinical relevance.

Methodology

Literature Search

A systematic literature search of randomized controlled trials was conducted in the following electronic databases: PubMed, Medline, EMBASE, Scopus, Web of Science, Google Scholar, Cochrane Trial Register, WHO Clinical Trial Registry Platform and Clinicaltrial.gov Registry. No language restrictions were applied in the search. The literature was searched from inception to December 2017. We used the following search words, among others: vitamin D, cholecalciferol, calcitriol, diabetes, prediabetes, adipokines, adipocytokines. Retrieved articles were hand searched in the parts introduction, discussion and reference list to identify additional potentially relevant trials. Additionally, we also searched retrieved reviews and meta-analyses for any potentially overlooked article.

Inclusion Criteria

Any randomised controlled trial which fulfilled the following criteria was included in the analysis:

a) Intervention involving supplementation with vitamin D or its analogues (calcidiol, ergocalciferol, cholecalciferol) regardless of the route of administration (oral, intramuscular)

b) Parallel or cross-over design

c) Involving adult (minimum 18 years of age) human subjects with insulin resistance, impaired glucose tolerance, impaired fasting glucose, or type 2 diabetes

d) An intervention period with a minimum duration of 4 weeks

e) Included an assessment of an outcome of interest, i.e. plasma concentrations of one or more adipocytokines

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f) Reported post-intervention mean values or change from baseline values with standard deviation. The included studies had to be original research (not a review or conference abstract)

Risk of Bias Assessment

The Cochrane Collaboration's tool for assessing the risk of bias in randomized trials was applied to evaluate the risk of bias of the studies included in the meta-analysis by determining either low, unclear or high risk of bias to the following study characteristics: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) [78].

Data Extraction and Analysis

Relevant variables which were predetermined from each included trial were collected independently by both authors, including sample size, sex distribution (expressed as % female participants), age, BMI, study duration and design, clinical condition and medication, vitamin D intervention (daily dose, vitamin D chemical form, route of administration), comparison group and outcomes. Data was independently extracted by the two authors.

Group means and corresponding standard deviations (SD) were extracted for the intervention and comparison group from every trial. In the case that the trial reported medians or interquartile ranges, we calculated the mean values and standard deviations [79]. Where standard error of the mean (SEM) reported instead, we calculated the SD on the basis of the following formula: SD = SEM × square root (n), where n is the number of subjects in the group. When logarithmically transformed data were reported, we used formulas suggested by Higgins [80], to calculate raw data.

Changes in serum concentrations of adipocytokines were the primary outcome for our meta-analysis and were extracted as change from baseline. Where change was not reported, we calculated it as the difference between the arithmetic means post intervention – baseline [81]. SD of mean differences were calculated as SD = square root [(SDbaseline)2+(SDend of treatment)2-(2r×SDbaseline×SDend of treatment)] for each group, assuming that r=0.5 [82].

We used the software Review Manager 5.3 as provided by the Cochrane Collaboration for all statistical analysis [83]. The inverse-variance, random effects model was applied to compare post-mean values [84].

Heterogeneity was assessed by $\chi 2$ and I2. An I2-value of greater than 50% was taken to represent considerable heterogeneity between the analyzed trials and significance was set at $p < 0.05$. In the case that a considerably different effect size was found in any one study, a sensitivity analysis was performed through the leave-one-out method to confirm that the overall effect size was not driven by any single study. To inspect for publication bias, a funnel plot of the data (mean changes against the corresponding standard errors) was applied.

Results

Literature Search, Study Selection and Study Characteristics

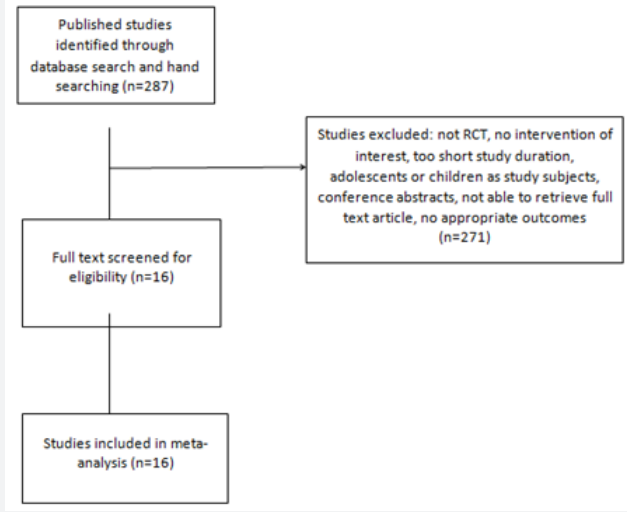

Figure 1: Flow diagram.

Using the above described search strategy, a total of 287 studies were identified. After deduplication and applying the selection criteria, a total of 16 studies were included. Figure 1 provides a detailed overview of study selection flow. The full text was screened for quantitative data availability and all screened 16 studies [85-101] were included in the final analysis, with 1058 subjects altogether. All studies used a mixed population of males and females; two did not report sex distribution of participants [96,101]. According to the BMI, the study populations were either overweight (25-30 kg/m^2) or obese (30-35 kg/m2). A total of 4 studies enrolled individuals with prediabetes [90,91,98,101] all other studies involved type 2 diabetic patients. The study duration ranged between 8 weeks and 6 months, whereas Duta 90 reported a significantly longer follow-up when compared to other studies included in the analysis.

Only one study 100 used paracalcitol and another study 85 calcitriol as supplement. Otherwise, cholecalciferol (vitamin D^3) was used, out of which in 11 studies as oral supplement (in 1 study as a single bolus dose) and in 3 studies in form of fortified dough. In most studies, vitamin D was used as a sole supplement; however, in 3 studies [90,91,93] participants received calcium alongside vitamin D. Outcomes identified were concentrations of adiponectin, leptin, TNF-alpha and IL-6.

Figure 2a provides an overview of risk of bias distribution according to the above described characteristics, Figure 2b shows risk of bias assessment across individual studies. Overall,

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the studies were of low or unclear risk of bias; high risk of bias was only identified in a small percentage in three study characteristics (random sequence, blinding of participants and personnel, incomplete outcome data).

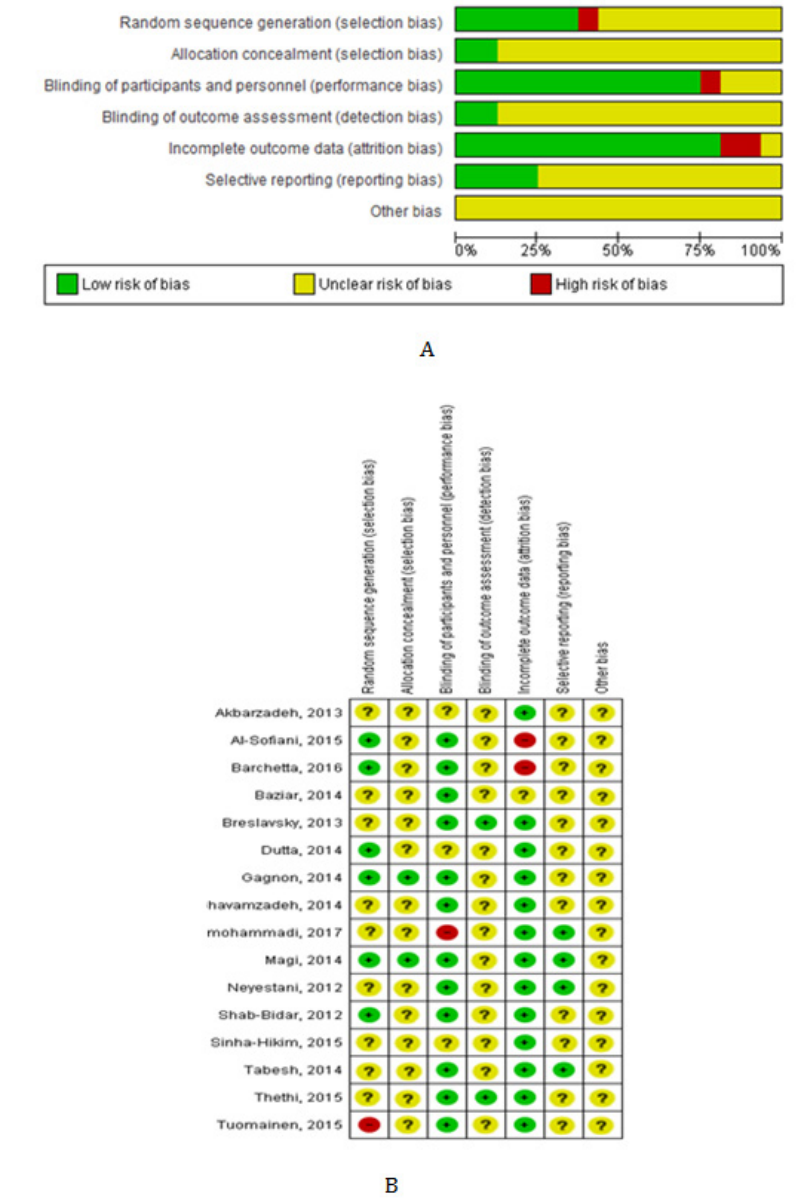

Figure 2: Risk of bias assessment. Each bias domain was evaluated carefully from every trial and decided whether the information provided reflected a low risk of bias (green), high risk of bias (red), or if insufficient information was provided and the risk of bias was therefore unclear (yellow). (A) Summary of risk of bias according to characteristics. The results were pooled from every trial, combined and general results expressed as percentages. (B) Risk of bias in individual trials.

Influence of Vitamin D Supplementation on Adipocytokine Levels (Quantitative Analysis)

The current meta-analysis of 8 RCTs suggests no effects of vitamin D supplementation on circulating levels of adiponectin, as shown in Figure 3 (MD: 0.00µg/ml (95% CI -0.00, 0.00), p=0.25, I2=73%). With regards to effects on leptin (Figure 4a), pooled effect size from 5 RCT found a non-significant increase in serum concentration with vitamin D supplementation (MD: 0.82 ng/ml (95% CI -4.34, 5.98), p=0.76, I2= 40%). The magnitude of the effect augmented after removing Tabesh [99] from the analysis (MD: 1.73 ng/ml (95% CI -3.30, 6.75), p=0.50, I2=38%) (Figure 4b). However, a non-significant reduction in leptin levels was seen once we removed Ghavamzadeh 92 from the analysis, and this also significantly reduced study inconsistency (MD= -0.09 ng/ml (95% CI -2.99, 2.82), p=0.95, I2=3%) (Figure 4c). Taken overall, it is inconclusive whether vitamin D supplementation affects circulating leptin levels.

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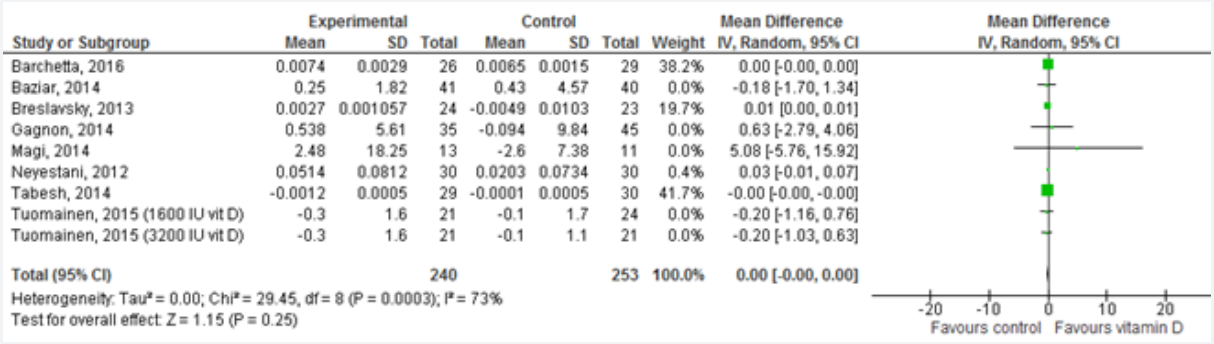

Figure 3: Effects of vitamin D supplementation on adiponectin levels (μg/ml). Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 10 pooled effect sizes from 9 randomized controlled trials (two separate effect sizes were pooled for two different dosing regimens of vitamin D in Toumainen [101]). The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups, but in this meta-analysis, its absent because there are no effects. Notice the labelling of the X-axis is different as compared to other outcomes, because an increase in adiponectin levels would be seen as favourable.

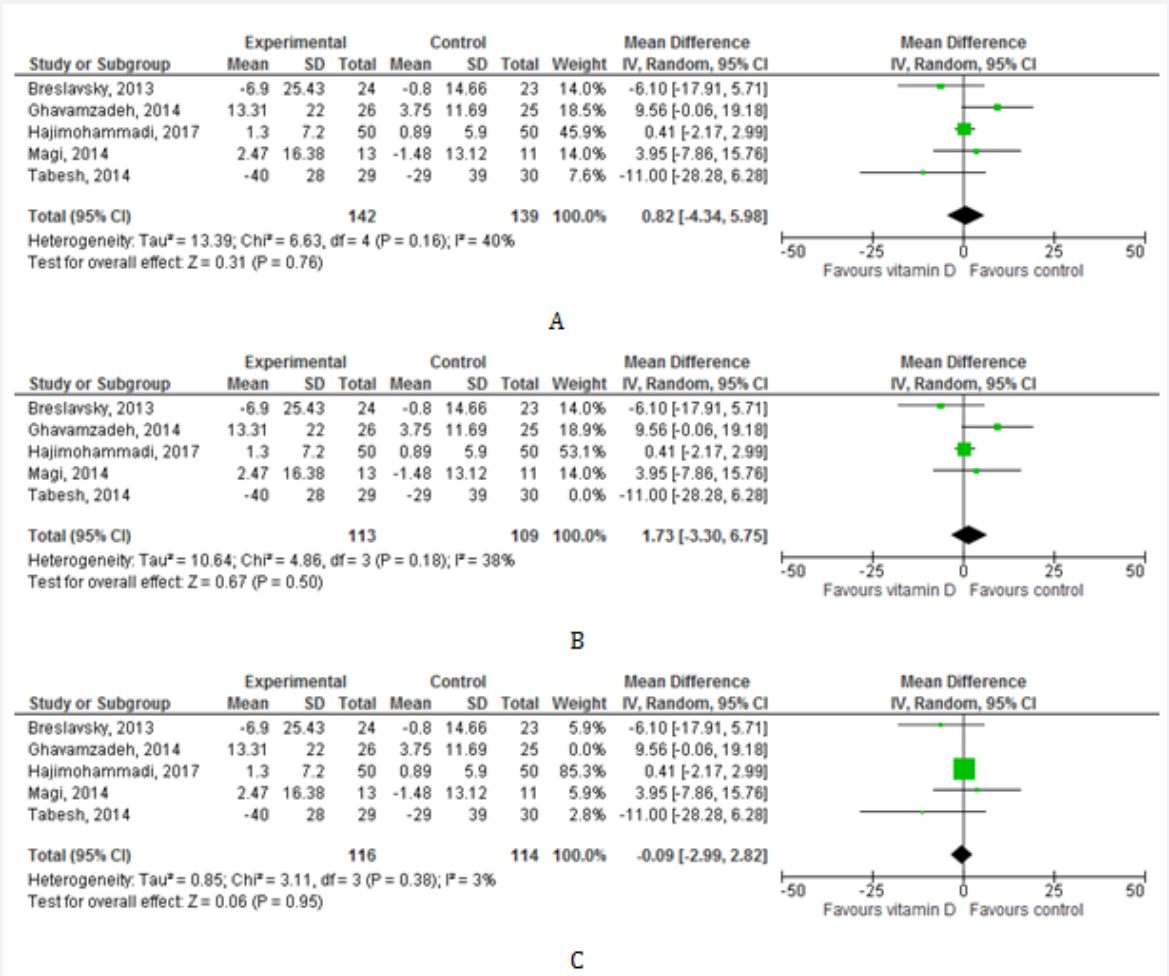

Figure 4: Effects of vitamin D supplementation on leptin levels (ng/ml). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 5 randomized controlled trials. The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. (B) Eliminating Tabesh [99] and (C) Ghavamzadeh [92] in leave-one-out sensitivity analysis (the trials were given a relative weight of 0.0%).

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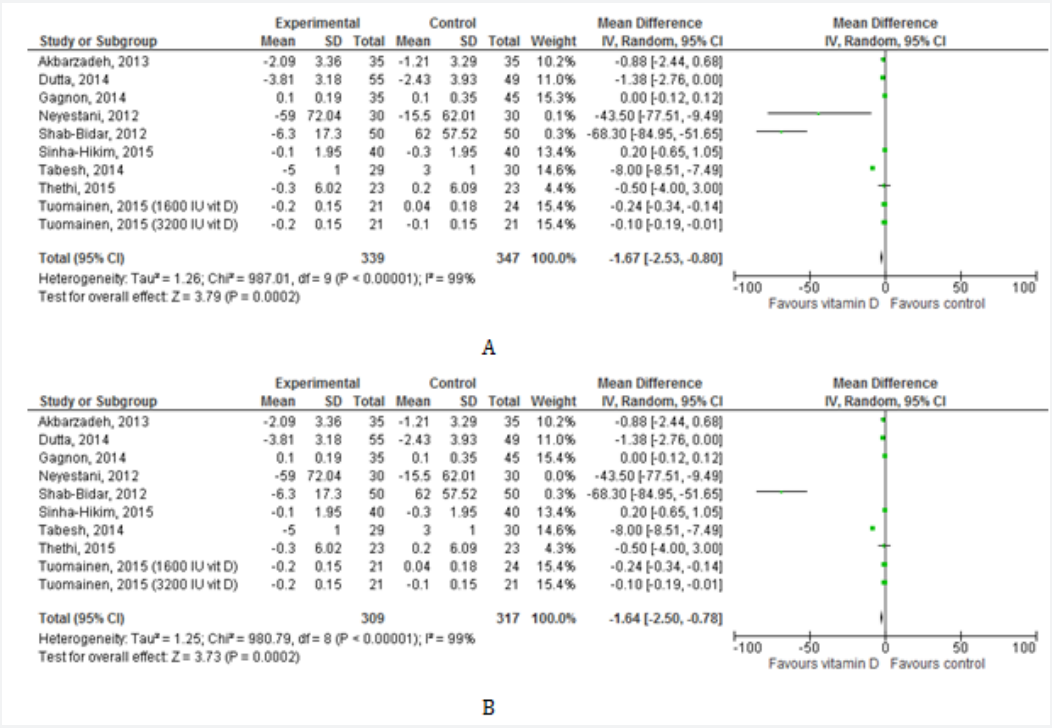

Figure 5: Effects of vitamin D supplementation on IL-6 levels (pg/ml). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 10 intervention effects pooled from 9 randomized controlled trials (two separate effect sizes were pooled for two different dosing regimens of vitamin D in Toumainen [101]). The green colored square represents the point estimate of the effect of the intervention for each intervention. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the interventions in the meta-analysis. (B) Eliminating Neyestani [96] and (C) Shab-Bidar [97] in leave-one-out sensitivity analysis (the trials were given a relative weight of 0.0%).

Table 1: Characteristics of randomized controlled trials included.

Reference	Sample Size, % Female	Age (Years, Mean ± SD)	BMI (kg/m2, Mean ± SD)	Duration, design	Clinical condition/ Medication	Vit D Intervention	Comparison group	Outcome Parameters
Akbarzadeh [85]	70, 50%	Vit D: 53.8 ± 8.9, control: 52.4 ± 7.8	Vit D: 28.3 ± 4.4, control: 27.0 ± 3.4	12 weeks, parallel	Type 2 diabetes, Metformin, glybenclamide	0,5 µg Calcitriol (=1,25 dihydroxy-cholecalciferol) per day, orally	Placebo	IL-6
Al-Sofiani [86]	20; 25%	Vit D: 54.8 ± 9.16, control: 55 ± 11.99	Vit D: 28.8 ± 2.0, control: 33.5 ± 2.2	12 weeks, parallel	Type 2 diabetes, HbA1c ≥ 6, hypovitaminosis D, insulin resistance (HOMA-IR ≥ 2), hypoglycemic agents, lipid lowering drugs, antihypertensives	5.000 IU vit D^3 per day, orally	Placebo	Adiponectin, leptin, TNF-alpha, IL-6
Barchetta, 2016 [87]	55; Vit D: 30%, control: 40%	Vit D: 57.4 ± 10.7, control: 59.8 ± 9.1	Vit D: 29.3 ± 4.4, control: 30.8 ± 4.5	24 weeks, parallel	Type 2 diabetes, NAFLD, oral antidiabetics, insulin, statins, antihypertensives	2.000 IU vit D_3 daily, orally	Placebo	Adiponectin
Baziar [88]	81; Vit D: 31.7, control: 35.0	Vit D: 50.34 ± 6.71 control: 52.75 ± 6.34	Vit D: 27.33 ± 1.64, control: 27.25 ± 1.35	8 weeks, parallel	Type 2 diabetes, hypovitaminosis D; Hypoglycemic agents, excluding insulin and thiazolidinediones	50.000 Vit D^3 per week, orally	Placebo	Adiponectin

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Breslavsky [89]	47; Vit D: 54.2, control: 52.2	Vit D: 66.8 ± 9.2, control: 65.8 ± 9.7	Vit D: 27.9 ± 5.2, control: 30.6 ± 5.1	12 months, parallel	Type 2 diabetes, Oral antidiabetics: Metformin, Sulfonylurea, Repaglinide, DDP-4 inhibitors, insulin, Antihypertensives: ACEIs/ARBs, Diuretics, Beta blockers, CCBs	1.000 IU Vit D^3 per day, orally	Placebo	Leptin, adiponectin
Dutta [90]	104, Vit D: 63,2%, control: 54,3%	Vit D: 48.37 ±10.47, control: 47.4 ± 11.51	Vit D: 26.32 ± 4.52, control: 26.83 ± 4.63	At least one year follow up (Mean duration of follow up: Vit D: 28.2±8.83 months, control: 29.15±7.69 months), parallel	Prediabetes, hypovitaminosis D	60.000 vit D^3 per week, then once monthly, orally; along with 1250 mg calcium carbonate per day (500 mg elemental calcium)	1250 mg calcium carbonate per day (500 mg elemental calcium)	TNF-alpha, IL-6
Gagnon [91]	80; vit D: 71, control: 67	Vit D: 53.8 ± 11.9, control: 55.3 ± 11.1	Vit D: 31.1. ± 5.7, control: 31.9 ± 6.2	6 months, parallel	Multiethnic prediabetic individuals, hypovitaminosis D, no medications known to affect glucose and mineral metabolism over the last 3 months, no pharmacological treatment for obesity	Initially 2.000 IU vit D^3 and 1200 mg calcium per day, orally; dose of vitamin D increased by 2,000 IU every 2 months to reach a target of ≥75 nmol/L (final dose: 2.000-6.000 IU daily)	Placebo	Adiponectin, TNF-alpha, IL-6
Ghavamzadeh [92]	51; 58.8%	Vit D: 49.28 ± 2.00, control: 52.26 ± 2.09	Vit D: 28.9 ± 0.86, control: 27.9 ± 0.93	14 weeks, parallel	Type 2 diabetes, no other diseases, hypoglycemic agents, no insulin therapy	400 IU vit D^3 per day, orally	Placebo	Leptin, TNF-alpha
Hajimohammadi [93]	100, vit D: 62, C: 52	Vit D: 52.4 ± 8.4, C: 52.6 ± 6.3	Vit D: 28.6 ± 4.0, C: 30.0 ± 4.2	12 weeks, parallel	Type 2 diabetes	Fortified doogh: 1000 IU D3 + 340 mg calcium per day, orally	Plain doogh	Leptin
Magi [95]	30, Vit D: 35,7%, control: 12,5%	69	29	24 weeks, parallel	Type 2 diabetes, diabetic foot complications, 60 years and older	Single bolus dose of vit D^3 (300.000 IU), orally	Placebo	Leptin, adiponectin, TNF-alpha
Neyestani [96]	60, NR	Vit D: 51.5 ± 5.4, control: 50.8 ± 6.7	Vit D: 29.2 ± 4.4, control: 29.9 ± 4.7	12 weeks, parallel	Type 2 diabetes, not taking steroidal antiinflammatory or anticoagulant medications; no insulin therapy	Fortified doogh: 500 IU vit D3 daily, orally	Plain yoghurt drink or doogh	Adiponectin, IL-1 beta, IL-6, TNF-alpha, RBP-4
Shab-Bidar [97]	100; Vit D: 52%, control: 62%	30-60 years	NR	12 weeks, parallel	Type 2 diabetes, no insulin therapy, no weight reduction program, no history of other diseases	Fortified doogh: 500 IU vit D^3 twice daily (=1000 IU vit D3 per day), orally	Plain doogh	IL-6, TNF-alpha

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Sinha-Hikim [98]	80; Vit D: 70, control: 70	Vit D: 51.6 ± 7.7, control: 52.4 ± 6.7	Vit D: 32.5 ± 4.4, control: 32.9 ± 4.5	12 months, parallel	Pre-diabetes, hypovitaminosis D, no medication	Average 85 300 IU ± 16 000 (range 64 700–134 000) Vit D^3 per week, orally	Placebo	TNF-alpha, IL-6
Tabesh [99]	59; vit D: 48, control: 53	Vit D: 50.2 ± 6.6, control: 51.0 ± 6.1	NR	8 weeks, parallel	Type 2 diabetes, hypovitaminosis D, hypoglycemic agents	50.000 IU Vit D^3 per week, orally	Placebo	Leptin, adiponectin, TNF-alpha, IL-6
Thethi [100]	55; Vit D: 35.7, control: 29.6	Vit D: 64 ± 9, control: 61 ± 10	NR	12 weeks, parallel	Type 2 diabetes, Chronic kidney disease stage 3 or 4, hypoglycemic agents, ACEIs/ ARBs, Statins	Paracalcitol 1 mcg daily, orally	Placebo	TNF-alpha, IL-6
Tuomainen [101]	66; NR	65.7 ± 7.0	29.4 ± 2.7	5 months, parallel	Prediabetic individuals, at least 60 years of age, overweight but not severely obese (BMI between 25 and 35), serum 25(OH) D^3 concentration <75 nmol/L	Two groups: 40 µg (equivalent to 1600 IU) or 80 µg (3200 IU) vit D^3 per day, orally	Placebo	Adiponectin, IL-6

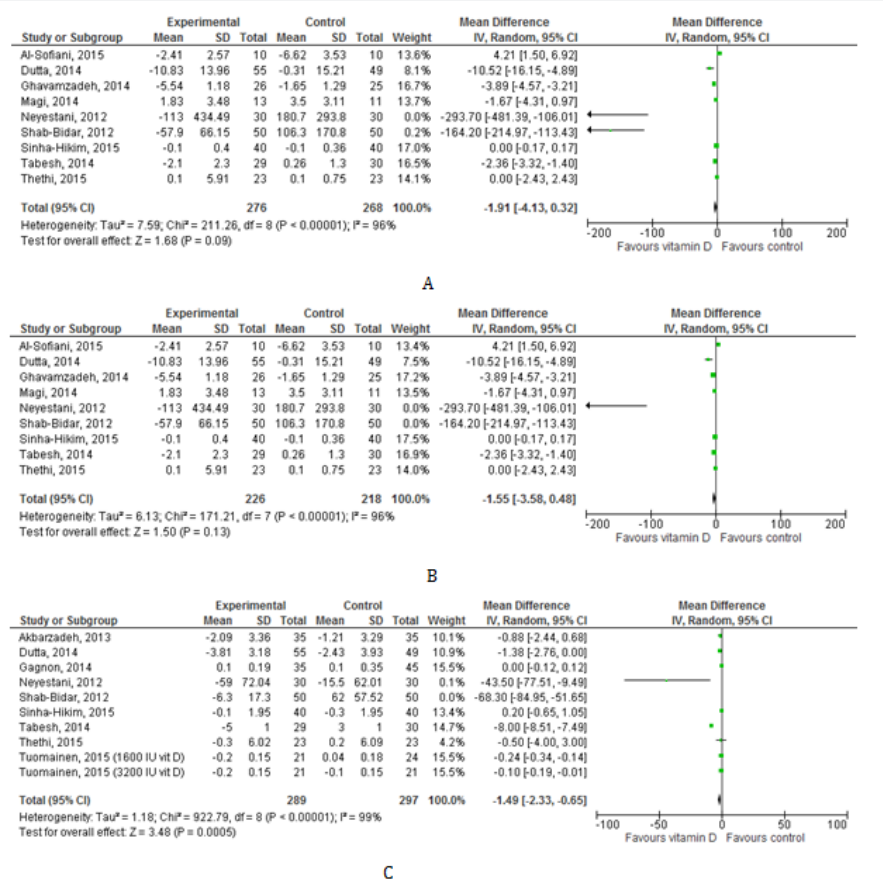

Figure 6: Effects of vitamin D supplementation on TNF-alpha levels (pg/ml). (A) Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 9 randomized controlled. The green colored square represents the point estimate of the effect of the intervention for each intervention. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the interventions in the meta-analysis. (B) Eliminating Shab-Bidar [97] as part of the leave-one-out sensitivity analysis (the trial was given a relative weight of 0.0%).

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Vitamin D supplementation significantly reduced levels of IL-6, but with considerable study heterogeneity (MD: -1.67 pg/ml (95% CI -2,53, -0.80), p= 0.0002, I2= 99%) (Figure 5a). Removing Neyestani 96 (MD: -1.64 pg/ml (95% CI -2.50, -0.78), p= 0.0002, I2= 99%) (Figure 5b) and Shab-Bidar 97 (MD: -1.49 pg/ml (95% CI -2.33, -0.65), p= 0.0005, I2= 99%) (Figure 5c) from the analysis did not significantly change the effect size, which can be explained by the very low relative weights these studies had in the original meta-analysis. TNF-alpha levels were also reduced by vitamin D supplementation, but the effects were not statistically significant and study heterogeneity was high (MD: -1.91 pg/ml (95% CI -4.13, 0.32), p= 0.09, I2= 96%) (Figure 6a). No significant changes in effect size were seen once we removed Shab-Bidar 97 for the sensitivity analysis (MD: -1.55 pg/ml (95% CI -3.58, 0.48), p= 0.13, I2= 96%) (Figure 6b) (Table 1).

Inspection Of Publication Bias

The visual inspection of funnel plots (Figure 7) showed an asymmetry for the inspected outcomes, except for leptin. This implies that for most of the outcomes inspected in present work, a publication bias cannot be excluded with certainty.

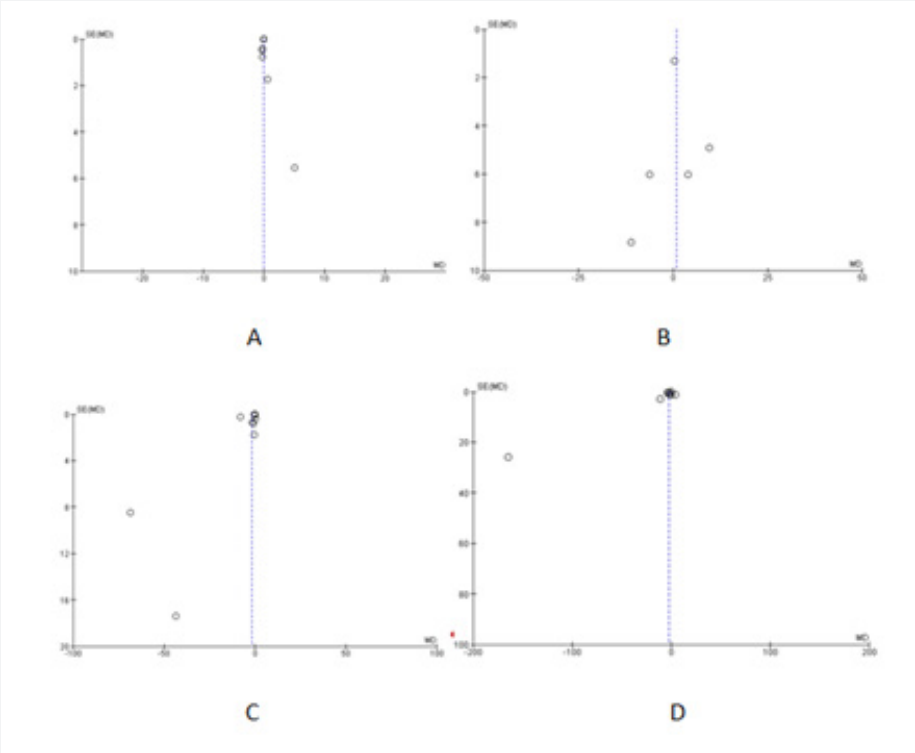

Figure 7: Funnel plot showing study precision against the mean difference effect estimate with 95% confidence interval for (A) adiponectin, (B) leptin, (C) IL-6 and (D) TNF-alpha. SE = standard error.

Discussion

The prevalence of inadequate vitamin D levels in populations across the globe go hand-in-hand with the ever-growing diabetes burden. However, the extent to which reaching and maintaining adequate vitamin D levels affect glucose metabolism and prevent the development of its pathological dysregulation is not entirely known. While it is plausible that being deficient bares a higher risk for diabetes [102], supplementation has yielded mixed results in studies in diseases individuals [52-66]. Given this inconsistency and the emerging wealth of evidence regarding the importance of adipocytokines in prediabetes and diabetes, we set out to investigate the effects of vitamin D supplementation in this group.

In the present systematic review and meta-analysis, we demonstrate that vitamin D can indeed affect circulating levels of some adipocytokines. In general, we observed no effects on adiponectin, while leptin levels as outcome was sensitive to individual studies and overall inconclusive, but after reducing study inconsistency to the lowest level, the size of the effect was negligible. These results are in line with another recent meta-analysis, which also found no significant effects of vitamin D supplementation on levels of adiponectin and leptin, though this meta-analysis was not restricted solely to prediabetic and diabetic individuals [103].

On the other hand, IL-6 and TNF-alpha levels were reduced by vitamin D supplementation, although the results were statistically significant only for IL-6. This is in contrast with a previous meta-analysis involving overweight and obese individuals which found no effects of supplementing vitamin D on these inflammatory biomarkers [104]. A study employing high-sensitivity assay

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found the upper 95th perecentile reference limit for IL-6 to be 4.45pg/ml, and for TNF-alpha 2.53pg/ml [105]. However, in prediabetic individuals a 4-fold and 10-fold increase in levels of IL-6 and TNF-alpha was described, respectively [106], and 10-fold and 50-fold in obese diabetic individuals [107]. Therefore, the effect size we found, i.e. less than 2 pg/ml reduction for both cytokines, is unlikely to be of clinical relevance that would bring about significant amelioration of complications associated with increased levels of these pro-inflammatory cytokines.

Our study has several limitations. Overall, we observed high study heterogeneity. Most studies did not provide sufficient information in order to be able to judge on the risk of bias across all of the predetermined criteria. Also, the studies included did not take into account seasonal variations in vitamin D status. Sun exposure is suggested to be associated with serum 25-hydroxy vitamin D levels more strongly than vitamin D supplementation [108]. The studies included populations with mixed characteristics in terms of sex distribution, age, body weight, vitamin D status, co-pathologies and medication use. Also, vitamin D supplementation interventions were heterogeneous among the studies, including chemical form, supplement type, intervention duration and dose. Calcium supplementation also formed part of the intervention in some studies; calcium is suggested to exert effects on body weight [109], which in turn could also have an influence on adipocytokine levels. In addition, we could not exclude publication bias in most of the outcomes analysed. Given these limitations, the results of the current meta-analysis should be interpreted with caution.

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medical sciences

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Review

Exercise Increases Adiponectin and Reduces Leptin Levels in Prediabetic and Diabetic Individuals: Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Abstract: It is speculated that lifestyle interventions known to improve diabetic metabolic state may exert their effects via adipokines. The aim of this systematic review and meta-analysis was to evaluate the chronic effects of physical exercise on adiponectin and leptin levels in adult prediabetic and diabetic individuals. PubMed, Embase, Scopus, The Cochrane Library, clinicaltrials.gov, and WHO Clinical Trials Registry were searched for randomized controlled trials. Pooled effects of interventions were assessed as mean difference (MD) with random effects model. Sensitivity analysis was conducted to test data robustness and subgroup analysis for study heterogeneity. Twenty-two trials with 2996 individuals were included in the meta-analysis. Physical exercise increased levels of adiponectin (MD: 0.42 µg/mL; 95% confidence interval (CI), 0.23, 0.60, $p < 0.00001$, $n = 19$ trials) and reduced leptin levels (MD: −1.89 ng/mL; 95% CI, −2.64, −1.14, $p < 0.00001$, $n = 14$ trials). These results were robust and remained significant after sensitivity analysis. Study heterogeneity was generally high. As for physical exercise modalities, aerobic exercise, but not other modalities, increased adiponectin and reduced leptin levels. In conclusion, physical exercise and, specifically, aerobic exercise, leads to higher adiponectin and lower leptin levels in prediabetic and diabetic adults. However, cautious interpretation of current findings is warranted.

Keywords: exercise; adipokines; diabetes mellitus

1. Introduction

Diabetes mellitus represents a major global public burden. The number of diseased individuals has quadrupled between 1980 and 2014, rising from 108 million to 422 million [1]. Recent data demonstrate that about 150 million people worldwide suffer from diabetes, a number which is expected to double by 2025 [2]. Diabetic individuals are at a high risk of developing a range of complications, including heart disease, retinopathy, nephropathy, neuropathy, and diabetic foot complications [2]. An estimated 1.8 million deaths in 2012 were due to diabetes worldwide, whilst an additional 2.2 million deaths were associated with complications arising from higher-than-optimal blood glucose [1]. It is projected that, in 2030, diabetes mellitus will be the seventh leading cause of death globally [3]. Overweight and obesity and physical inactivity are major risk factors for developing the disease, as 9 out of 10 diabetic individuals in the United States are overweight or obese, and 4 out of 10 are physically inactive [4]. The pathological dysregulations that eventually lead to diabetes are preceded by prediabetes in most individuals [5]. This early phase represents a major opportunity for preventive interventions.

The seminal Diabetes Prevention Program (DPP) showed that a lifestyle intervention including a physical activity component could significantly reduce the incidence of diabetes in high risk individuals, and the effects were even greater than with pharmacotherapy based on metformin [6]. In general, engaging in regular physical activity can reduce the risk of developing diabetes by 30–50% [7]. Even moderate intensity of physical activity, such as brisk walking, seems to offer protective benefits [8]. However, most individuals who are at risk or are already diagnosed with diabetes are not physically active on a regular basis [9]. Regular physical activity is also associated with improved glucose control in individuals already diagnosed with the disease. Physical exercise can reduce glycated haemoglobin HbA(1c) significantly, even in the absence of body weight changes [10], while higher intensity exercise is suggested to offer additional benefits on glycemic control and cardiorespiratory fitness [11]. Different training modalities are employed as strategies for managing abnormal glucose metabolism, including aerobic exercise, resistance exercise, and combined exercise. Both training modalities are important. For instance, aerobic exercise can prevent or change the course of peripheral diabetic neuropathy [12] and improve the cardiac autonomic nervous system function [13]. Resistance exercise was found to alter body composition in favor of lean muscle vs. adipose tissue which results in increased peripheral insulin sensitivity, among a plethora of other mechanisms [14]. In a randomized controlled trial, a combination of aerobic and resistance exercise was better in improving glycemic control than each modality alone [15]. Similar findings were found in a recent meta-analysis, where combined exercise was not only superior for glycemic control, but also in improving blood lipids in patients with diabetes [16]. However, another meta-analysis found that engaging in some form of physical activity is more important than choosing the type itself [17], which is important, given the rates of physically inactive diabetic and prediabetic individuals [18].

The physiological benefits of regular physical activity, as well as guidelines and recommendations on the type and amount, are provided in different guidelines for prevention and treatment of diabetes [18–20].

Adipose tissue is not considered an inert energy storage system any more. Since the discovery of leptin in 1994 [21], adipose tissue is regarded as a highly active endocrine system secreting a plethora of signaling molecules collectively known as adipokines [22]. Physical exercise has been found to affect adipose tissue, especially visceral adipose tissue, even in the absence of weight loss, and the effects are suggested to be even greater than with dietary restriction [23]. Leptin regulates a wide range of physiological mechanisms important for obesity and metabolic disorders, including energy balance, neuroendocrine function, and metabolic pathways. Leptin levels are primarily associated with the amount of fat tissue and energy balance. Leptin exerts is effects by binding to its receptor (ObR) in the hypothalamus, activating several signal transduction pathways, such as Janus kinase/signal transducer and activator of transcription-3 (JAK-STAT3), which is involved in the regulation of energy homeostasis, and phosphatidylinositol 3-kinase (PI3K), involved in the regulation of food intake and glucose homeostasis. Ultimately, leptin induces decreased food intake and increased energy expenditure, i.e., it has weight-reducing effects. However, in the state of leptin resistance, which is often found in type 2 diabetes, leptin cannot exert its effects, making these individuals resistant to the weight-reducing effects, even in the presence of hyperleptinemia [24]. Adiponectin is a hormone with anti-inflammatory and cardioprotective functions. Under normal conditions, it is secreted exclusively from adipose tissue. It is found abundantly in the plasma, accounting for 0.01% of plasma proteins in humans. Adiponectin exerts its effects by binding the receptors AdipoR1 and AdipoR2. The anti-inflammatory and cardioprotective properties are mostly due to inhibiting the expression of adhesion molecules, thereby reducing the adherence of monocytes to endothelial cells. In addition, adiponectin reduces plaque formation and increases plaque stability and nitric oxide (NO) production. In the liver, adiponectin reduces glucose output by inhibiting the expression of enzymes for gluconeogenesis. Its expression is paradoxically reduced in obesity, insulin resistance, and type 2 diabetes [25]. Both adipokines are clinically very relevant in prediabetes and diabetes, with the general perception that they exert contrary effects—leptin upregulates proinflammatory pathways which

are associated with type 2 diabetes and cardiovascular disease, while adiponectin downregulates them [26]. Physical exercise has been found to affect adiponectin and leptin levels in a favorable manner [27,28], and the effect may be potentiated with dietary co-intervention [29].

The aim of this systematic review and meta-analysis was to synthesize data on the effects of physical exercise, including different exercise modalities, on adiponectin and leptin levels in prediabetic and diabetic individuals.

2. Materials and Methods

2.1. Literature Search

The following databases were searched until 1 March 2018 for randomized controlled trials: PubMed, Embase, Scopus, The Cochrane Library, clinicaltrials.gov, and WHO Clinical Trials Registry. Key words applied in the literature search were exercise, physical activity, training, adipokines, leptin, adiponectin, prediabetes, and diabetes. The reference sections of retrieved trials were also hand searched in order to identify further potentially relevant trials. Systematic reviews and meta-analysis which were identified were also hand-searched for additional trials. The search strategy for PubMed is provided in the Supplementary Material (Figure SX). Our systematic review is registered in PROSPERO (CRD42018098633).

2.2. Study Selection

Studies were included if they were (i) randomized controlled trials involving adults (minimum 18 years of age) with prediabetes (insulin resistance, impaired glucose tolerance, impaired fasting glucose) or type 2 diabetes; (ii) used physical exercise in supervised form, including different exercise modalities (aerobic, resistance, and concurrent exercise) or provided exercise advice to enrolled individuals; (iii) an intervention time of minimum 4 weeks; iv) evaluated adiponectin and/or leptin as outcomes; and (v) reported change in means or baseline and postintervention means with standard deviations for the intervention and control group, or values from which these could be calculated.

Studies were excluded if they (i) lacked a control group; (ii) included individuals with type 1 diabetes; (iii) involved a confounding co-intervention other than diet, e.g., a drug cotreatment; (iii) lacked sufficient information on the outcomes of interest; (iv) were conference abstracts, reviews, case reports, commentaries; and (v) were duplicate publications.

2.3. Risk of Bias Assessment

The Cochrane risk of bias tool [30] was used to evaluate the risk of bias of included trials (low, unclear, high) for the following study characteristics: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias.

2.4. Data Extraction and Analysis

The following data, that were abstracted from every trial, included first author's last name, publication year, modality of physical exercise, sample size, sex distribution, baseline characteristics (age, body mass index (BMI)), medical condition, number of training sessions per week, intervention duration, dietary co-intervention, and outcome parameters. If several time points were reported for an outcome, then data from the longest follow-up time period was taken. If the trials included multiple different physical exercise interventions, then data were extracted from every intervention arm.

Where reported, changes in group means and corresponding standard deviations (SDs) for levels of adiponectin and leptin were extracted for the intervention and control groups. Otherwise, changes

were calculated as the difference between the post-intervention and baseline mean; in this case, SD was calculated for each group, assuming that r = 0.5 [31], as:

$$\sqrt{[(\text{SDbaseline})2 + (\text{SDend of treatment})2 - (2r \times \text{SDbaseline} \times \text{SDend of treatment})]}$$

If medians or interquartile ranges were reported instead of means, then mean values and SDs were calculated as proposed by Hozo et al. [32].

The statistical analysis was done with Review Manager 5.3 of the Cochrane Collaboration Group [33]. The analysis was done by using the inverse-variance random effects model [34]. Effect size of the intervention was calculated as the pooled estimates of the weighted mean differences (WMD) between the intervention and control groups.

Study heterogeneity was measured by Higgins I^2 statistic [35], where a value higher than 50% was considered to represent considerable heterogeneity.

Subgroup analyses were conducted according to preset criteria: (i) intervention duration, where we applied 12 weeks as cut-off [36]; (ii) dietary co-intervention, as it can modulate the effect of exercise [29]; and (iii) number of training sessions per week, based on recent recommendations of the American Diabetes Association [37].

Sensitivity analysis through the leave-one-out method was employed to verify the robustness of data by removing one trial at a time from the meta-analysis, and recalculating the effects with the remaining trials. In addition, sensitivity analysis was also done by leaving out studies which involved prediabetics, and by excluding trials with two or more defined areas of high risk of bias.

Publication bias was inspected through the funnel plot method, where the differences in mean changes are plotted against their standard errors, in order to determine the precision of the studies.

3. Results

3.1. Characteristics of Included Trials

Once all selection criteria were applied, a total of 22 studies with 2996 individuals were included in the analysis [38–59]. Figure S1 provides an overview of the search strategy.

A total of 19 studies reported adiponectin levels as outcome, while 14 studies reported leptin levels (Table 1). Most studies employed a structured physical exercise program, the majority of which was aerobic exercise. Three studies [39,58,59] provided exercise advice. Multiple different exercise modalities were used in three studies [39,40,51]; therefore, multiple effect sizes were extracted from these studies. Most trials included both sexes; whilst five [38,41,42,45,53] enrolled men only and one [51] women only. The participants were heterogeneous in terms of age (overall range: 36–66 years). Mean BMI of all groups was higher than 25 kg/m^2 or 30 kg/m^2, hence, the participants included were overweight and obese. The absolute majority of trials enrolled individuals with type 2 diabetes, while three [52,53,59] included prediabetic individuals. The number of training sessions per week was in the range of 2–6. The length of intervention duration, in weeks, ranged between six and 104. Five out of 22 studies [44,48,54,58,59] provided dietary treatment as part of the intervention. Figure S2 provides an overview of the risk of bias.

Table 1. Study characteristics. Values expressed are mean ± standard deviation (SD), unless indicated otherwise.

Author	Sample Size; % Female	Age (Years, Mean ± SD)	BMI (kg/m2, Mean ± SD)	Medical Condition	Training Sessions/Week	Intervention Duration in Weeks	Training Characteristics	Dietary Co-Intervention	Change in Outcomes as Compared to Baseline (Mean ± SD) †
Concurrent Exercise									
Annibalini, 2017 [38]	16; 0%	I: 57 ± 9.1, C: 60 ± 6.8	I: 28.3 ± 1.5, C: 29.0 ± 3.8	T2D	3	16	Aerobic: 40–65% VO_{2max}, 30–60 min; Resistance: 2–4 sets of 12–20 repetitions, 40% to 60% of 1-RM	No	Leptin: I: −0.1 ± 0.95, C: −0.1 ± 1.4
Balducci, 2010 [39]	42; I: 36.4%, C: 45%	I: 60.6 ± 9.3, C: 61.1 ± 7.1	I: 30.5 ± 0.9, C: 30.9 ± 1.1	T2D	2	52	Aerobic: 70–80% VO_{2max}, 40 min; Resistance: 80% 1-RM, 40 min	No	Adiponectin: I: 5.54 ± 3.6, C: 0 ± 3.6; leptin: I: −7.29 ± 8.33, C: −2.08 ± 8.33
Jorge, 2011 [40]	24; I: 66.6%, C: 66.6%	I: 57.90 ± 9.82, C: 53.41 ± 9.82	I: 31.24 ± 3.88, C: 29.59 ± 4.90	T2D	3	12	Aerobic: 30 min, intensity set individually acc. to lactate threshold; Resistance: 30 min, circuit training with 7 exercises	No	Adiponectin: I: 0.6 ± 4.76, C: −1.32 ± 4.76
Loimaala, 2009 [41]	48; 0%	I: 53.6 ± 6.2, C: 54.0 ± 5.0	I: 29.3 ± 3.7; 29.8 ± 3.6	T2D	4	104	Aerobic: 70–80% $VO_{2max,}$ at least 30 min; Resistance: 3–4 sets with 10–12 repetitions, 80% 1-RM, at least 30 min	No	Leptin: I: −0.7 ± 3.9, C: 0.5 ± 3.6
Mendham, 2013 [42]	21; 0%	I: 39.5 ± 10.6, C: 36.1 ± 16.1	I: 31.6 ± 3.1, C: 34.5 ± 6.6	T2D	3	12	45 min sessions; Aerobic: 80–85% VO_{2max}; Resistance: free-weights training with 3 exercises; additional boxing session	No	Adiponectin: I: −1.1 ± 3.71, C: −0.2 ± 2.96 leptin: I: −5.71 ± 7.7, C: 0.49 ± 15.06
Okada, 2010 [43]	38; I: 52.3%, C: 35.3%	I: 61.9 ± 8.6, C: 64.5 ± 5.9	I: 25.7 ± 3.2, C: 24.5 ± 2.9	T2D	3–5	12	Aerobic: 40 min, Resistance: 20 min	No	Adiponectin: I: 1.9 ± 3.89, C: 1.8 ± 3.25 leptin: I: 1.2 ± 3.3, C: 1.4 ± 4.9
Aerobic Exercise									
Aas, 2005 [44]	18; I: 33.3%, C: 33.3%	I: 59 (45–67), C: 53 (39–66)[a]	I: 29.8 (26.7–34.5), C: 30.0 (25.8–31.0)[a]	T2D	2	52	60 min, moderate intensity	Hypocaloric diet aimed at weight loss and improved metabolic control	Adiponectin: I: −1.4 ± 0.95, C: −1.1 ± 2.97 leptin: I: −1.17 ± 1.16, C: 3.17 ± 1.44
Balducci, 2010 [39]	40; 40%	I: 64.3 ± 8.1, C: 61.1 ± 7.1	I: 29.4 ± 1.1, C: 30.9 ± 1.1	T2D	2	52	70–80% VO_{2max}, 60 min	No	Adiponectin: I: 5.97 ± 3.6, C: 0 ± 3.62 leptin: I: −4.17 ± 8.33, C: −2.08 ± 8.33
Boudou, 2003 [45]	16; 0%	I: 42.90 ± 5.20, C: 47.9 ± 8.35	I: 28.3 ± 3.90, C: 27.6 ± 4.30	T2D	3	8	2 sessions with continuous intensity: 75% VO_{2max} (45 min), 1 session with alternating intensity: 50–85% VO_{2max} (20 min)	No	Adiponectin: I: −0.3 ± 3.19, C: −0.25 ± 2.36, leptin: I: −0.45 ± 4.45; C: 0.14 ± 3.9
Dede, 2015 [46]	60; I: 50%; C: 53.3%	I: 52.5 ± 7.5, C: 55.5 ± 8.4	I: 30.8 ± 4.6, C: 30.2 ± 4.5	T2D	3	12	Sessions progressed from 15–20 min at 60% of VO_{2max} to 45 min at 75% of VO_{2max}	No	Adiponectin: I: −0.6 ± 0.8, C: −0.2 ± 1.1 leptin: I: −2.8 ± 19.79, C: 1.3 ± 17.16

Table 1. *Cont.*

Author	Sample Size; % Female	Age (Years, Mean ± SD)	BMI (kg/m2, Mean ± SD)	Medical Condition	Training Sessions/Week	Intervention Duration in Weeks	Training Characteristics	Dietary Co-Intervention	Change in Outcomes as Compared to Baseline (Mean ± SD) †
Giannopoulou, 2005 [47]	22; 100%	55.5 ± 1.7	35.9 ± 1.9	T2D	3–4	14	65–70% VO_{2max}, 60 min	No	Adiponectin: I: 1.8 ± 1.37, C: 1.2 ± 1.95 leptin: I: −15.6 ± 8.77, C: −9.8 ± 8.77
Iishi, 2001 [48]	50; I: 60.9%; C: 59.3%	I: 56.0 ± 4.6, C: 57.9 ± 7.6	I: 26.2 ± 3.5, C: 25.4 ± 3.2	T2D	5	6	50% VO_{2max}, 60 min	25- to 27-kcal/kg/d (54% to 58% carbohydrate, 22% to 24% protein, 18% to 20% fat)	Leptin: I: −2.6 ± 0.77, C: −1.1 ± 0.62
Jorge, 2011 [40]	24; I: 58.3%, C: 66.6%	I: 52.09 ± 8.71, C: 53.41 ± 9.82	I: 29.30 ± 2.09, C: 29.59 ± 4.90	T2D	3	12	Intensity set individually acc. to lactate threshold, 60 min	No	Adiponectin: I: 2.2 ± 5.0, C: −1.32 ± 4.76
Kadoglou, 2007a [49]	60; I: 56.6%, C: 60%	I: 59.33 ± 4.76, C: 63.82 ± 7.03	I: 32.1 ± 3.19, C: 31.99 ± 3.41	T2D	4	26	50–75% VO_{2max}, 30–45 min	No	Adiponectin: I: −0.24 ± 0.95, C: 0.35 ± 0–93
Kadoglou, 2007b [50]	46; I: 65.21%, C: 60.86%	I: 56.91 ± 7.09, C: 60.32 ± 9.28	I: 31.14 ± 3.58, C: 28.96 ± 1.03	T2D	4	32	50–80% VO_{2max}, 45–60 min	No	Adiponectin: I: 0.33 ± 1.15, C: −0.6 ± 0.8
Ku, 2010 [51]	31; 100%	I: 55.7 ± 7.0, C: 57.8 ± 8.1	I: 27.1 ± 2.4, C: 27.4 ± 2.8	T2D	5	12	3.6–5.2 METs (1 MET = 3.5 mL O_2/kg/min), 60 min	No	Adiponectin: I: 2.9 ± 2.32, C: 1.99 ± 2.06 leptin: I: −3.73 ± 3.1, C: −0.1 ± 3.52
Marcell, 2005 [52]	34; 60.7%	I: 44.4 ± 6.5, C: 44.1 ± 9.5	I: 32.5 ± 5.3, C: 35.3 ± 3.7	IR	5	16	80–90% of age-predicted maximum heart rate (220 − age), 30 min	No	Adiponectin: I: 0.9 ± 1.0, C: −1.9 ± 1.1
Rokling-Andersen, 2007 [53]	85; 0%	45.1 ± 2.51	I: 28.5 ± 3.3, C: 28.5 ± 3.3	IFG, IGT	3	52	60–80% of measured maximum heart rate, 60 min	No	Adiponectin: I: −0.5 ± 7.35, C: −4.1 ± 8.82 leptin: I: −0.5 ± 4.7, C: 1.0 ± 7.61
Thompson, 2014 [54]	494; I: 66.0%, C: 64.0%	I: 60 ± 10, C: 60 ± 10	I: 31.6 ± 5.6, C: 31.5 ± 5.7	T2D	5	52	Low-intensity walking, 30 min	Hypocaloric diet aiming to produce 5–10% weight loss	Adiponectin: I: 0.96 ± 0.24, C: 0.5 ± 0.04
Zhang, 2017 [55]	32; I: 58,8%, C: 46,6%	I: 47.2 ± 10.5, C: 46.9 ± 11.1	I: 27.6 ± 3.2, C: 27.9 ± 3.8	T2D	5	12	70% of age-predicted maximum heart rate (220 − age), 60 min	No	Adiponectin: I: 1.2 ± 1.81, C: 0.4 ± 1.95 leptin: I: −3.3 ± 6.57, C: −2.1 ± 7.7
Resistance Exercise									
Brooks, 2007 [56]	62; I: 32.2%, C: 38.7%	I: 66 ± 2, C: 66 ± 1	I: 30.9 ± 1.1, C: 31.2 ± 1.0	T2D	3	16	60–80% of 1-RM, 3 sets with 8 repetitions, 35 min	No	Adiponectin: I: 2.3 ± 2.09, C: 1.99 ± 2.06
Jorge, 2011 [40]	24; I: 58.3%, C: C: 66.6%	I: 54.10 ± 8.94, C: 53.41 ± 9.82	I: 31.29 ± 4.08, C: C: 29.59 ± 4.90	T2D	3	12	60 min, circuit training with 7 exercises	No	Adiponectin: I: 0.68 ± 4.21, C: −1.32 ± 4.76

Table 1. *Cont.*

Author	Sample Size; % Female	Age (Years, Mean ± SD)	BMI (kg/m2, Mean ± SD)	Medical Condition	Training Sessions/Week	Intervention Duration in Weeks	Training Characteristics	Dietary Co-Intervention	Change in Outcomes as Compared to Baseline (Mean ± SD) †
Ku, 2010 [51]	29; 100%	I: 55.7 ± 6.2, C: 57.8 ± 8.1	I: 27.1 ± 2.3, C: 27.4 ± 2.8	T2D	5	12	Elastic band exercises with 40–50% of maximal exercise capacity, 3 sets with 15–20 repetitions	No	Adiponectin: I: 2.3 ± 2.09, C: 1.99 ± 2.06 leptin: I: −1.02 ± 2.89, C: −0.1 ± 3.52
Lovrencic, 2015 [57]	140; I: 56.1%; C: 54.1%	I: 58.5 ± 4.8, C: 57.7 ± 6.2	I: 29.44 (4.67), C: 30.64 (4.54)	T2D	6	52	Strength exercises with light to medium intensity, 90 min	No	Adiponectin: I: −0.1 ± 6.42, C: −0.4 ± 3.31
Exercise Advice									
Balducci, 2009 [39]	40; 45%	I: 62.5 ± 7.1, C: 61.1 ± 7.1	I: 30.0 ± 1.0, C: 30.9 ± 1.1	T2D	NA	52	Counseling to perform low-level aerobic physical activity regularly	No	Adiponectin: I: −0.43 ± 6.63, C: 0 ± 3.6 leptin: I: −1.04 ± 2.89, C: −0.1 ± 3.52
Belalcazar, 2015 [58]	1397; I: 57%, C: 57%	I: 57.1 ± 7.1, C: 57.3 ± 7.3	I: 36.4 ± 6.4, C: 36.0 ± 5.9	T2D	NA	52	Advice to increase moderate intensity physical activity to at least 175 min per week	Hypocaloric diet aiming at 7% weight loss, 1200–1500 kcal/day if body weight <114 kg, 1500–1800 kcal/day if body weight ≥114 kg	Adiponectin: I: 0.5 ± 0.53, C: 0 ± 0.43
Corpeleijn, 2007 [59]	103; 45.5%	I: 55.6 ± 0.9, C: 57.8 ± 1.0	I: 29.8 ± 0.5, C: 29.3 ± 0.4	IGT	NA	52	Advice to perform 30 min of moderate intensity physical activity at least 5 times per week	Hypocaloric diet aiming at 5–7% weight loss, carbohydrate intake of at least 55% of total energy intake; total fat of 30 to 35% of total energy intake, with <10% energy intake of saturated fatty acids and cholesterol intake of <33 mg/MJ; protein 10 to 15% of total energy; dietary fiber at least 3 g/MJ	Adiponectin: I: −0.06 ± 0.63, C: −0.03 ± 0.55 leptin: I: −2.37 ± 1.3, C: 0.06 ± 0.93

[a] Values expressed as median (95% confidence interval (CI)), † Units: µg/mL for adiponectin, ng/mL for leptin; T2D—type 2 diabetes, IFG—impaired fasting glucose, IGT—impaired glucose tolerance, VO_{2max}—maximal oxygen consumption, MET—metabolic equivalent, kcal—kilocalories, MJ—mega-joule, BMI—body mass index, I: intervention group, C: control group, 1-RM: 1 repetition maximum.

3.2. Influence of Exercise on Adiponectin and Leptin Levels

The present meta-analysis shows that physical exercise significantly increases adiponectin levels (Figure 1) in prediabetic and diabetic individuals (mean difference (MD): 0.42 µg/mL; 95% CI 0.23, 0.60, $p < 0.00001$), but significant study heterogeneity was found ($I^2 = 82\%$).

As shown in Figure 2, physical exercise significantly reduced leptin levels (MD: −1.89 ng/mL; 95% CI, −2.64, −1.14, $p < 0.00001$); here, as well, high heterogeneity was found ($I^2 = 63\%$).

With regards to effects of different exercise modalities, aerobic exercise significantly increased adiponectin levels (MD: 0.83 µg/mL; 95% CI, 0.23, 1.42, $p = 0.007$, $I^2 = 89\%$), but neither concurrent/resistance exercise nor exercise advice significantly affected adiponectin levels. With regards to leptin levels, both aerobic exercise (MD: −2.55; 95% CI, −3.99, −1.12, $p = 0.0005$, $I^2 = 65\%$) and exercise advice (MD: −2.42; 95% CI, −2.86, −1.98, $p < 0.00001$, $I^2 = 0\%$) led to a significant reduction in serum levels.

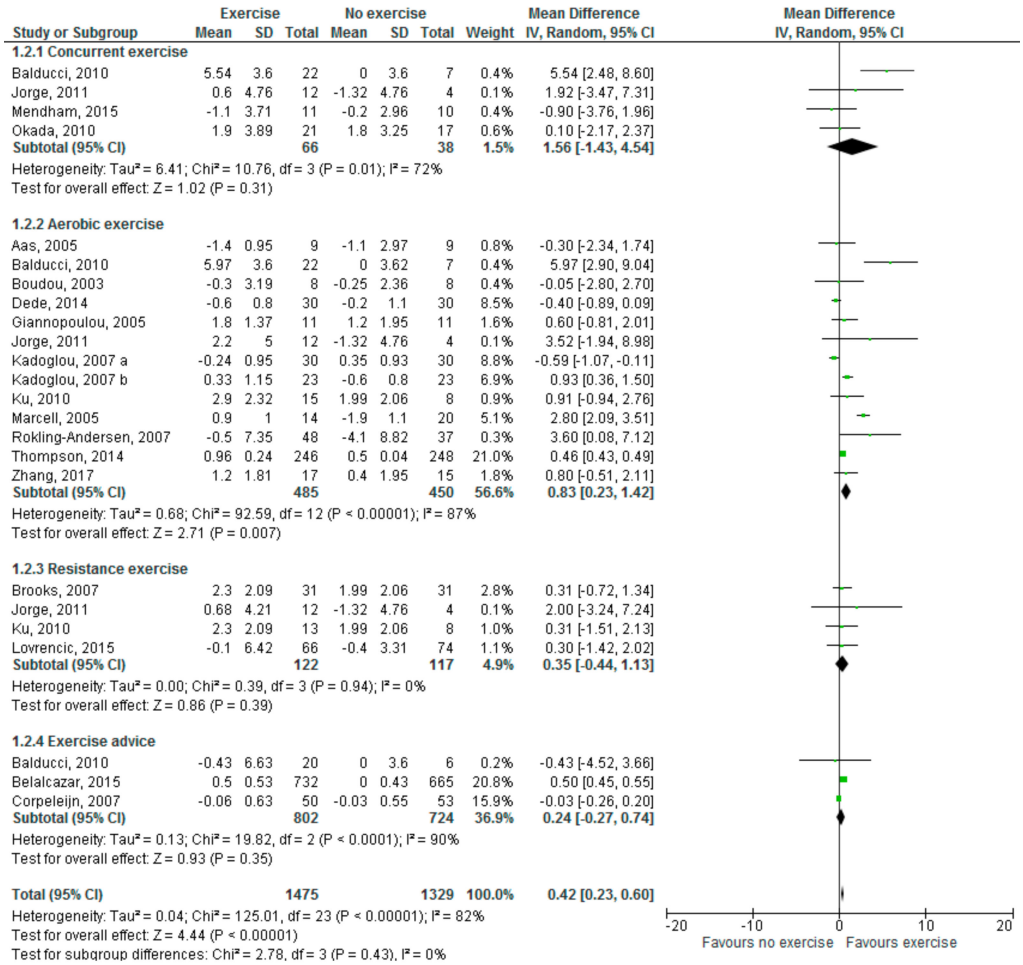

Figure 1. Effects of physical exercise, including different modalities, on adiponectin levels (µg/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 24 effect sizes pooled from 19 trials (two separate effect sizes were pooled for different exercise modalities from Jorge [40] and Ku [51], and three from Balducci [39]). The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups.

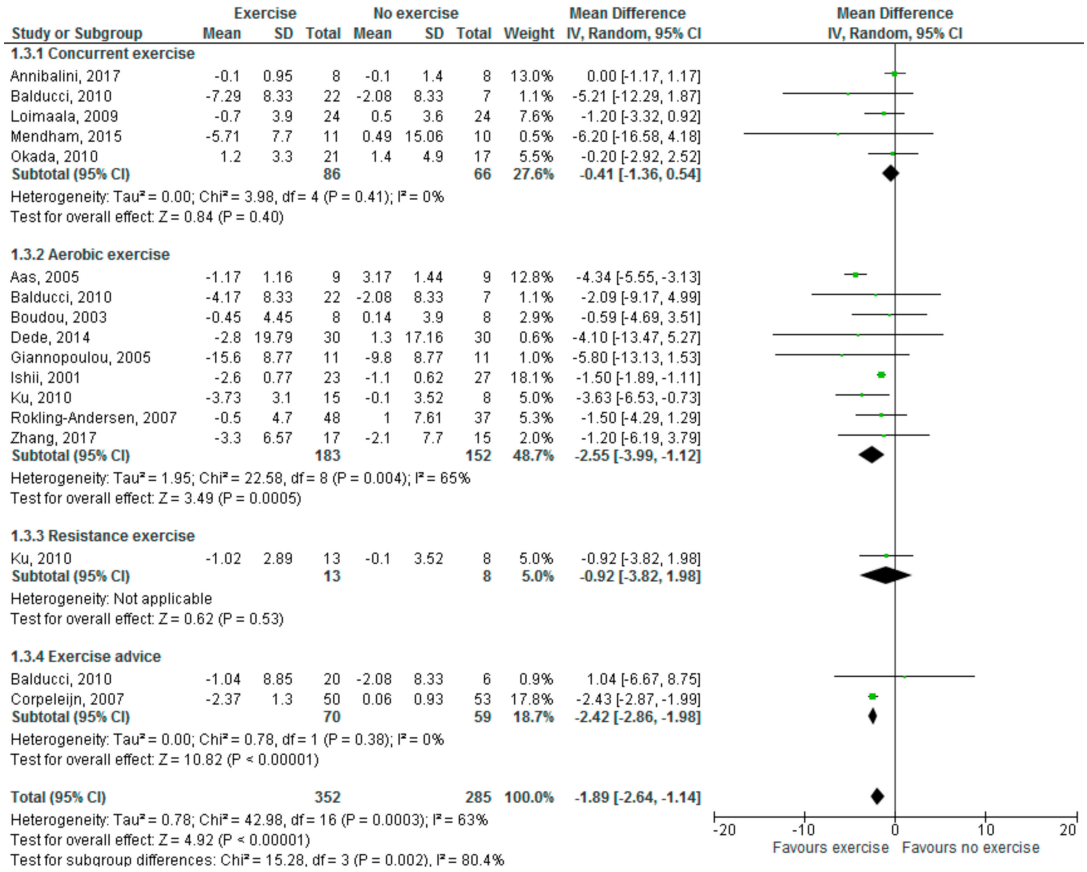

Figure 2. Effects of physical exercise, including different modalities, on leptin levels (ng/mL). Forest plot shows pooled mean differences with 95% confidence intervals (CI) for 17 pooled effect sizes from 14 trials (two separate effect sizes were pooled for different exercise modalities from Ku [51] and three from Balducci [39]). The green colored square represents the point estimate of the effect of the intervention for each trial. The horizontal line joins the upper and lower limits of the 95% CI of the effects. The square area represents the relative weight of the trial in the meta-analysis. The black colored diamond at the bottom represents the pooled mean difference with 95% CI for all study groups.

3.3. Sensitivity Analysis

As described above, each study was removed once from the meta-analysis, and the effects recalculated with the remaining studies. No major changes in the effect size were found, suggesting a robustness of data in the primary analysis. For adiponectin, the minimal effect size was found once Marcell [52] was removed from the meta-analysis (MD: 0.30; 95% CI, 0.14, 0.46, $p = 0.0003$, $I^2 = 74\%$), while removing Thompson [54] from the analysis generated the largest effect size (MD: 0.64; 95% CI, 0.24, 1.04, $p = 0.002$, $I^2 = 82\%$).

Once Aas [44] and Kadoglu [50] were removed from the analysis, there was an increase in effect size, but the effects remained statistically significant (MD: 0.65 µg/mL; 95% CI, 0.21, 1.09, $p = 0.004$, $I^2 = 84\%$).

Removing studies which involved prediabetic subjects [52,53,59] from the analysis did not change the overall effect size of physical exercise on adiponectin levels (MD: 0.37 µg/mL; 95% CI, 0.21, 0.53, $p < 0.00001$, $I^2 = 68\%$). However, removing Corpeleijn [59] from the analysis doubled the effect size of exercise advice, reduced study heterogeneity to 0%, and the effect was statistically significant (MD: 0.50 µg/mL; 95% CI, 0.45, 0.55, $p < 0.00001$, $I^2 = 0\%$). For leptin, removing Aas [44] decreased the effect size the most, but also reduced heterogeneity below 50% (MD: −1.55; 95% CI, −2.20, −0.91, $p < 0.00001$, $I^2 = 44\%$). Once Annibalini [38] was left out from the analysis, the largest effect size was seen for leptin (MD: −2.19; 95% CI, −2.92, −1.46, $p < 0.00001$, $I^2 = 53\%$). Removing the two studies

with high risk of bias [44,48] from the analysis reduced study heterogeneity both for the overall effect of physical exercise (MD: −1.54; 95% CI, −2.45, −0.63, $p = 0.0009$, $I^2 = 38\%$), and in the aerobic exercise subgroup (MD: −2.28; 95% CI, −3.86, −0.69, $p = 0.005$, $I^2 = 0\%$). Limiting the analysis to type 2 diabetics only did not change the overall effects of physical exercise (MD: −1.81; 95% CI, −2.84, −0.79, $p = 0.0005$, $I^2 = 59\%$), but the results in the exercise advice subgroup became non-significant after removing Corpeleijn [59] (MD: −1.04; 95% CI, −6.67, 8.75, $p = 0.79$).

3.4. Subgroup Analysis

Subgroup analyses were conducted for intervention duration, dietary co-intervention, and the number of training sessions per week (Table 2). For the number of training sessions per week, Okada [43] and Giannopoulou [47] were excluded from the analysis as they did not specify the exact number of training sessions per week; all exercise advice studies were excluded, as well.

A statistically significant increase in adiponectin levels was found across all subgroups, but study heterogeneity remained high. Interestingly, for intervention duration, studies which lasted ≥12 weeks produced an approximately 5-fold higher increase in adiponectin levels than studies with longer duration (MD: 0.12 vs. 0.49 μg/mL). Interventions that did not include a dietary co-intervention produced a double higher increase in adiponectin levels (MD: 0.99 vs. 0.40 μg/mL). Limiting the number of training sessions to three times per week or less led to approximately 2-fold higher adiponectin levels increase than a higher number of training sessions (MD: 1.70 vs. 0.76). However, statistically significant differences were found only between the subgroups for intervention duration ($p = 0.0009$).

As for leptin levels, a significant reduction was found across all subgroups as well. Intervention duration >12 weeks led to a higher reduction than shorter duration (MD: −2.69 vs. −1.50 ng/mL). Dietary co-intervention tripled the reduction effect on leptin levels (MD: −2.60 vs. −0.87 ng/mL). Exercising three or less times per week reduced leptin more than a higher frequency (MD: −2.27 vs. −1.72 ng/mL). Interestingly, in subgroups with ≤12 weeks treatment duration, no dietary co-intervention, and >3 training sessions per week, there was very low study heterogeneity. Based on the test for subgroup differences, statistically significant differences were found for the intervention duration and dietary co-intervention subgroup analyses.

Table 2. Results of subgroup analysis.

	Adiponectin		Leptin	
	MD (95% CI), *p*-Value, I^2-Value	**Test for Subgroup Differences**	**MD (95% CI), *p*-Value, I^2-Value**	**Test for Subgroup Differences**
		Intervention duration		
≤12 weeks	0.12 (0.02, 0.29), $p = 0.57$, $I^2 = 0\%$	$p = 0.0009$, $I^2 = 85.2\%$	−1.50 (−1.88, −1.12), $p < 0.00001$, $I^2 = 0\%$	$p = 0.05$, $I^2 = 74.7\%$
>12 weeks	0.49 (0.29, 0.68), $p < 0.00001$, $I^2 = 88\%$		−2.69 (−3.79, −1.58), $p < 0.00001$, $I^2 = 46\%$	
		Dietary co-intervention		
Yes	0.40 (0.28, 0.51), $p < 0.00001$, $I^2 = 85\%$	$p = 0.11$, $I^2 = 60.2\%$	−2.60 (−3.72, −1.47), $p < 0.00001$, $I^2 = 92\%$	$p = 0.01$, $I^2 = 83.6\%$
No	0.99 (0.27, 1.71), $p = 0.007$, $I^2 = 82\%$		−0.87 (−1.65, −0.09), $p = 0.03$, $I^2 = 0\%$	
		Training sessions/week		
≤3 times/week	1.70 (0.29, 3.12), $p = 0.02$, $I^2 = 76\%$	$p = 0.24$, $I^2 = 28.1\%$	−2.27 (−4.49, −0.05), $p = 0.05$, $I^2 = 75\%$	$p = 0.68$, $I^2 = 0\%$

3.5. Publication Bias

Visually inspecting the funnel plots both for adiponectin (Figure 3A) and leptin (Figure 3B) revealed a moderate asymmetry, such that it cannot be excluded that a publication bias, such as not publishing indecisive data, could have affected the results of the present meta-analysis.

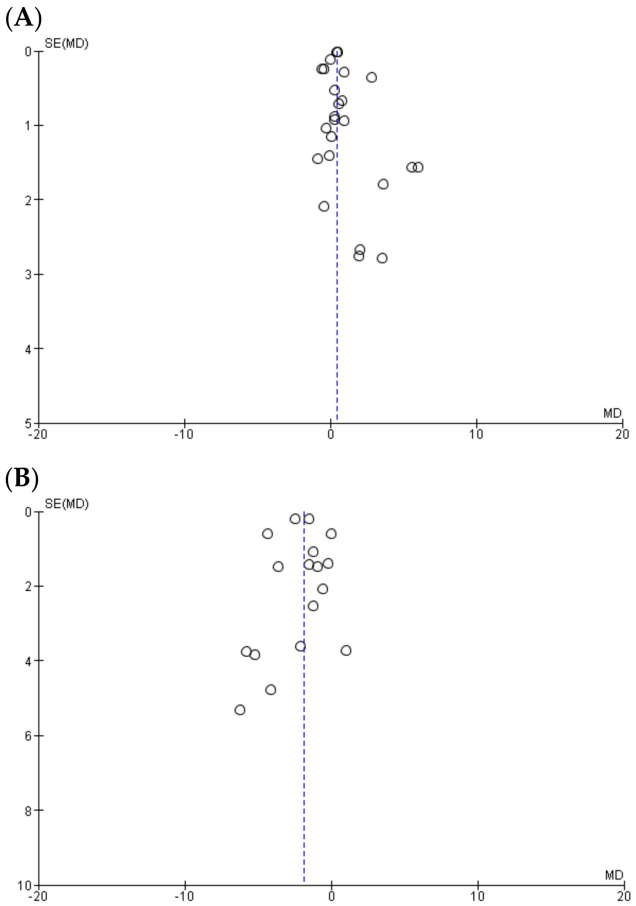

Figure 3. Funnel plot showing study precision against the mean difference effect estimate with 95% confidence interval for (**A**) adiponectin and (**B**) leptin. SE—standard error, MD—mean difference.

4. Discussion

Our meta-analysis shows that physical exercise and, specifically, aerobic exercise, increased adiponectin and reduced leptin levels in prediabetic and diabetic individuals.

As global diabetes rates continue to increase and the underlying conditions, such as obesity and prediabetes, are on the rise, it is of utmost importance to identify strategies for their successful management. Although numerous pharmacotherapy options are available for type 2 diabetes, lifestyle interventions always form an integral part of a diabetes management plan. A preponderance of evidence demonstrates the benefits of physical exercise for a whole set of criteria relevant for prediabetes and diabetes, including better immediate glucose clearance from the blood and long-term improvements of blood sugar and HbA(1c) levels, favorable body composition changes, increased aerobic capacity, better cardiovascular outcomes, and overall reduced morbidity and mortality [1,7,10,11,16].

Adipokines might represent a possible explanation when it comes to the mechanisms mediating the beneficial effects of physical exercise on impaired glucose metabolism.

Hypoadiponectinemia has been associated with impaired glucose regulation, inflammation, obesity, atherosclerosis, and type 2 diabetes [60]. Increasing adiponectin levels has been associated with a lower risk for developing diabetes across populations in a dose–response relationship [61]. In diabetic

individuals, enhancing adiponectin levels has emerged as a promising strategy due to its beneficial clinical effects, including anti-inflammatory [62], insulin-mimicking, and insulin-sensitizing [62] properties. Physical exercise exerts increasing effects on adiponectin comparable to the one of some anti-diabetic drugs [63]. Our meta-analysis shows that physical exercise, in general, and aerobic exercise, significantly increase adiponectin levels in prediabetic and diabetic adults. These results are in line with previous meta-analysis done in overweight and obese individuals [64] and, also, with systematic reviews [36]. Another meta-analysis from 2014 [65] did not find significant changes in adiponectin levels in response to physical exercise in diabetic individuals; however, this meta-analysis included much less studies than ours, it did not include prediabetic individuals and, also, considered interventions which included drug co-treatment. The robustness of the data was demonstrated in the sensitivity analysis, where it was demonstrated that the results were not dependent on any single study included. Interestingly, exercise advice also led to a significant increase in adiponectin levels once the analysis was constrained to diabetic individuals only. However, high inter-study heterogeneity was generally found. In the subgroup analysis, intervention duration was the only characteristic with significant differences between the subgroups and might, therefore, explain, in part, the heterogeneity. Notably, high study heterogeneity for adiponectin levels was found in previous meta-analysis as well [65].

Leptin is an "adipostat" regulating body fat mass, whose concentration changes with changing fat stores under physiological conditions, with the ultimate goal of maintaining stable body energy stores [66]. However, in type 2 diabetes, leptin levels are generally higher independently of body fat mass [67]. This hyperleptinemia is regarded as a marker of leptin resistance, a condition where tissues do not respond normally to leptin [68]. Leptin resistance in diabetes further aggravates the disarrangements in glucose metabolism [69] and is a significant factor in the development of diastolic function and heart failure [70]. Reducing leptin levels, inflammation, and oxidative stress, are suggested to improve overall leptin sensitivity [71]. Physical exercise is known to reduce oxidative stress and inflammation [72]. In our meta-analysis, we show that physical exercise, especially aerobic exercise, significantly reduces leptin levels. The data were robust, as no significant changes of effect size were found in the sensitivity analysis. However, after leaving out studies with a high risk of bias, study heterogeneity was reduced to below 50%. Exercise advice also led to a significant reduction in leptin levels, but the effect disappeared once the analysis was constrained to only type 2 diabetic individuals, which might imply that exercise advice is able to reduce leptin levels primarily in prediabetic individuals. In general, our results are in accordance with previous work, which also reported significant leptin reduction following physical exercise interventions [64,65]. The subgroup analysis showed that intervention duration and presence of dietary co-intervention are variables with statistically significant differences between subgroups. Leptin levels are highly sensitive to energy balance, such that negative energy balance through caloric deficit leads to a reduction in circulating leptin [73]. It is, therefore, plausible that the addition of dietary co-intervention potentiates the reducing effects of exercise on leptin through creating a larger negative energy balance, and the effects are greater with the duration of the negative energy state.

Interestingly, the present meta-analysis revealed that aerobic exercise, but not other exercise modalities, lead to significant increase in adiponectin levels and a reduction in leptin levels. This was also found in previous meta-analyses [64,65], and might be explained through greater negative energy balance induced by aerobic exercise as compared to other exercise modalities [74], but also an overall greater effect of aerobic exercise on body weight and fat mass [75].

However, the present study has several limitations. The risk of bias could not be assessed across many of the preset criteria. In addition, high study heterogeneity was found, and could not be fully explained in the subgroup analyses. Furthermore, the population set analyzed was heterogeneous in terms of age distribution, BMI, and clinical condition. Also, the design of physical exercise interventions differed, e.g., in terms of session duration and intensity. For aerobic exercise, we could not make a differentiation between potentially different effects of interval vs. continuous exercise. Some studies

also had very small study groups, which tends to produce more extreme effects. Publication bias could also not be excluded.

In conclusion, the present systematic review and meta-analysis shows that exercise represents a viable strategy to increase adiponectin and reduce leptin levels in prediabetic and diabetic individuals. However, a cautious interpretation is warranted.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3271/6/4/97/s1, Figure S1: Flow diagram of study selection, Figure S2: Risk of bias assessment. Search strategy for PubMed: ("training" OR "exercise" OR "physical activity") and ("diabetes" OR "prediabetes" OR "insulin resistance" OR "impaired glucose tolerance" OR "impaired fasting glucose" OR "leptin" OR "adiponectin" OR "adipokines") and ("randomized controlled trial" OR "randomized" OR "clinical trials as topic" OR "placebo" OR "randomly" OR "trial") not ("animals" NOT "humans").

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