DISSERTATION

Role of the IGF system in modulating the risk of metabolic disease progression in patients with high-risk prediabetes under lifestyle intervention / Rolle des IGF-Systems in der Modulation des metabolischen Progressionsrisikos von Patienten mit Hochrisiko-Prädiabetes unter Lebensstilintervention

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List of abbreviations

AKT	Protein kinase B
ALS	Acid-labile subunit
ANOVA	Analysis of variance
BMI	Body mass index
BP	Binding protein
DGE	Deutsche Gesellschaft für Ernährung / German Nutrition Society
DI	Disposition Index
DIfE	Deutsches Institut für Ernährungsforschung / German Institute of Human Nutrition Potsdam-Rehbruecke
DINA-P	Diabetes Nutrition Algorithm – Prediabetes
DZD	German Center for Diabetes Research
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinases
GH	Growth Hormone
HIRI	Hepatic insulin resistance index (Abdul-Ghani)
^{1}H-MRS	Proton Magnetic resonance spectroscopy
HOMA	homeostatic model assessment
IFG	Impaired fasting glucose
IGF	Insulin-like Growth Factor
IGFBP	Insulin-like Growth Factor Binding Protein
IGI	Insulinogenic Index (Seltzer)
IGT	Impaired glucose tolerance
IHL	Intrahepatic lipid content
IQR	Interquartile range

IR	Insulin resistance
IS	Insulin sensitivity
ISC	Insulin secretory capacity
kDA	Kilodaltons
MAPK	Mitogen-activated protein kinase
MR	magnetic resonance
MRI	magnetic resonance imaging
mTOR	mammalian target of rapamycin
NAFLD	Non-alcoholic Fatty Liver Disease
NFG	Normal fasting glucose
NGT	Normal glucose tolerance
oGTT	Oral glucose tolerance test
OptiFiT	Optimal Fibre Trial
PI3K	Phosphoinositide 3-kinase
PLIS	Prediabetes Lifestyle Intervention Study
SD	Standard deviation
T2DM	Type 2 Diabetes mellitus
VAT	Visceral adipose tissue
WHO	World Health Organization
WHR	Waist-hip ratio

Abstract

Objective

Although the prevention of type 2 diabetes (T2DM) by lifestyle approaches is highly effective, some individuals develop T2DM despite significant improvements of anthropometric and metabolic parameters. IGF-1 and its binding proteins 1 and 2 are closely associated with glucose metabolism and beta-cell function. We thus hypothesized that they might determine the capacity for metabolic regeneration, and hence predict the occurrence of T2DM in patients with pre-existing prediabetes.

Research Design and Methods

We conducted a post-hoc analysis on a group of 414 high-risk prediabetic individuals (58% women, aged between 28 to 80 years) featuring a) impaired glucose tolerance and/or b) decreased insulin secretion and/or c) insulin resistance with fatty liver. These individuals were enrolled in one of three randomized lifestyle intervention trials. These trials involved a minimum of one year of intervention and an additional year of follow-up. The one-year interventional period was finished by 345 subjects. The analysis focused on fasting serum levels of IGF-1, IGFBP-1, and IGFBP-2 in relation to the incidence of T2DM, as well as anthropometric and metabolic parameters over a period of two years.

Results

The lifestyle intervention had a significant positive impact on the entire cohort, leading to improvements in several metabolic and anthropometric parameters (body weight, liver fat, insulin sensitivity and secretion). Despite this, 14% (n= 57) of the subjects developed T2DM over the period of two years. Among those, baseline levels of IGF-1 were lower and IGFBP-1 levels were higher compared to subjects without the occurrence of T2DM. This significantly predicted the incidence of T2DM. Moreover, high

baseline levels of IGF-1 and low levels of IGFBP-1 was associated with stronger improvements in both anthropometric and metabolic parameters.

In contrast to baseline levels, an increase of IGFBP-1 was associated with an improvement in glycemic metabolism and characterized the group that did not develop T2DM.

Individuals who developed T2DM, did not demonstrate any improvements in insulin sensitivity, insulin secretion or IGFBP-1 levels, in spite of lifestyle induced metabolic and anthropometric ameliorations.

Conclusions

In the context of prediabetes, low levels of IGF-1 and high levels of IGFBP-1 indicated an advanced impairment of beta-cell function along with hepatic insulin resistance, predicting the incidence of T2DM. These individuals were unable to compensate for this impairment even with lifestyle changes, which suggests that more extensive interventions may be necessary.

Zusammenfassung

Ziele

Lebensstilmaßnahmen sind effektiv zur Prävention von Typ-2-Diabetes (T2DM). Dennoch tritt die Erkrankung bei einigen Personen trotz Verbesserungen der Körperkonstitution und metabolischer Parameter auf. IGF-1 und seine Bindeproteine 1 und 2 (IGFBP-1/-2) sind mit dem Glukosestoffwechsel und der Betazell-Funktion verbunden. Unsere Hypothese: IGF-1, IGFBP-1 und -2 bestimmen die Fähigkeit zur metabolischen Regeneration mit und sind somit prädiktiv für das Auftreten von T2DM bei Personen mit bestehendem Prädiabetes.

Design und Methoden

In einer Kohorte von 414 Personen (58% Frauen, 28 - 80 Jahre) mit Hochrisiko-Prädiabetes (gestörte Glukosetoleranz und/oder eine verminderte Insulinsekretion und/oder Insulinresistenz mit Fettleber) führten wir eine Post-Hoc Analyse durch. Alle hatten an einer von drei randomisierten Lebensstil-Interventionsstudien teilgenommen, jeweils mit mindestens einjähriger Intervention und mindestens einem zusätzlichen Jahr Nachbeobachtung. 345 Personen schlossen die einjährige Interventionszeit ab.
Wir untersuchten den Zusammenhang zwischen Nüchtern-Serumspiegel von IGF-1, IGFBP-1 und IGFBP-2 mit dem Auftreten von T2DM sowie mit anthropometrischen und metabolischen Veränderungen über den Zeitraum von zwei Jahren.

Ergebnisse

Die Intervention hatte eine signifikant positive Auswirkung auf die Gesamtkohorte mit Verbesserung von metabolischen und anthropometrischen Parametern (Körpergewicht, Leberfettgehalt, Insulinsensitivität und -sekretion). Trotzdem entwickelten im Laufe von

zwei Jahren entwickelten 14 % (n= 57) der Probanden einen T2DM.

Bei diesen zeigten sich die Ausgangswerte von IGF-1 niedriger und von IGFBP-1 höher im Vergleich zu Personen ohne Auftreten von T2DM. Dies sagte die Inzidenz von Diabetes signifikant vorher. Darüber hinaus waren hohe Werte für IGF-1 und niedrige für IGFBP-1-Werte zu Beginn der Studie mit stärkeren Verbesserungen sowohl anthropometrischer als auch metabolischer Parameter verbunden.

Konträr zu den Ausgangswerten war ein Anstieg von IGFBP-1 mit einer Verbesserung des Glukosestoffwechsels verbunden und charakterisierte die Gruppe ohne Inzidenz von T2DM.

Trotz der metabolischen und anthropometrischen Verbesserungen durch die Intervention zeigten sich bei Personen, die einen T2DM entwickelten, keine Verbesserungen der Insulinsensitivität und -sekretion oder der IGFBP-1-Spiegel.

Schlussfolgerungen

Bei Prädiabetes deuten niedriges IGF-1 und hohes IGFBP-1 auf eine fortgeschrittene Beeinträchtigung der Betazellfunktion hin, begleitet von Insulinresistenz, und sagen das Auftreten von T2DM vorher. Betroffene mit dieser Konstellation zeigen eine Unempfindlichkeit gegenüber Lebensstilinterventionen zur Kompensation metabolischer Beeinträchtigungen. Hier sind möglicherweise intensivere Lebensstilmaßnahmen erforderlich.

1 Introduction

The study in context

Obesity and its metabolic sequelae are increasing worldwide and are the primary causes of the most prevalent diseases of industrialized countries linked to the metabolic syndrome. Lifestyle approaches aiming to prevent diabetes by moderate weight loss, healthy diet and increased physical activity have proven highly successful due to improvements in insulin sensitivity and insulin secretion (1). However, in these trials some study participants did not show the expected improvements despite significant weight loss and reduction of liver fat (2). These subjects may require more intense programs aiming at lifestyle factors or might profit from early pharmacological interventions. There is a lack of biomarkers for identifying these individuals at early time points.

The Growth Hormone- Insulin-like Growth Factor axis

1.1 Overview

Over the last decades, the Growth Hormone (GH)- Insulin-like Growth Factor 1 (IGF-1) axis has been associated to a wide range of different states of health and disease – both in positive and negative respect – including cardiovascular, malignant, neurodegenerative and metabolic diseases (3-7). IGF-1 and its binding proteins are predominantly regulated by GH, insulin and nutrition-related stimuli (8-11). Altogether, they have a crucial impact on growth and metabolism against the background of nutritional state (12).
As this represents a notably complex interplay, the actual role of the IGF-1-axis in this context is, however, not yet clarified and continuously discussed in a controversial

manner.

In this context, IGF-1 and its binding proteins 1 and 2 have been shown to be closely related to changes in insulin sensitivity (IS), glucose homeostasis and with this, development of type 2 diabetes (3, 13). The exact associations, though, remain still ambiguous.

Deterioration in glucose metabolism, finally resulting in type 2 diabetes (T2DM), is firstly based on decreasing glucose tolerance which progresses with time. Crucial feature for the progression of the metabolic disturbance to T2DM is – next to a decrease in insulin sensitivity – a progressive decline in pancreatic beta-cell function, leading to lower insulin levels. This occurs early within the course of the disease – before the onset of overt diabetes (14). The early decline in beta-cell function was therefore suggested as a useful target in the prevention and therapy of T2DM. However, this might be limited to specific subtypes of (pre-) diabetes (14). Fitting into the picture, in some people, successful weight loss and liver fat reduction through lifestyle intervention – which has proven to be effective for metabolic improvement (15) – are not sufficient to prevent the occurrence of T2DM (2). As the IGF system is so closely involved in glucose and insulin metabolism, the question arises as to what role it plays in the prevention of type 2 diabetes.

1.2 GH

GH is a peptide hormone, formed in and secreted from somatotropic cells in the anterior lobe of the pituitary gland (hypophysis). It is an anabolic hormone, exerting its effects through various mechanisms of actions and in multiple tissues. One of the most important functions is promotion of body growth after birth. Moreover, it acts more specifically on various tissues such as bone, muscle, liver, heart, and has influence on

metabolism and the immune system (16). An important mediator of the anabolic GH action is IGF-1 which is secreted by the liver upon GH-stimulation (16, 17) – on the ground of sufficient energy and protein intake (18). The secretion underlies multiple regulatory mechanisms. Among them, dietary stimuli – especially energy and protein uptake – influence the secretion of GH and even its biological activity (16). Also, negative feedback loops play an important regulatory role. Thus, amongst others, IGF-1 inhibits further GH-secretion (7). Also age influences GH production: during life, the concentration of GH in the blood initially increases steadily, it reaches a maximum in the second decade of life and declines after that continuously with age (5).

1.3 IGF-1

As a growth factor, IGF-1 is – next to growth stimulation – involved in the regulation of cell differentiation and proliferation as well as metabolism. Upon stimulation by GH, IGF-1 is synthesized and released ubiquitously but mainly by the liver, where it also primarily exerts its biological function mediated via both the IGF-1 and insulin receptor (17, 19). Stimulating the PI3K/AKT/mTOR and ERK/MAPK5 pathways, IGF-1 exerts anabolic and glucose lowering functions (20, 21), analogue to insulin with which it shares a strong structural homology (19). With this, IGF-1 is involved in glucose and lipid metabolism and closely linked to insulin sensitivity (7). IGF-1 concentrations in the circulation are influenced by nutrition-related signals, mediated especially through energy or protein intake (22). Thereby, it is characterized as biomarker reflecting the nutritional state (23). Above all, age and genetic background co-determine IGF-1 levels (24).

1.4 IGFBPs

Within the bloodstream, approximately 99% of IGF-1 is bound to specific IGF-binding proteins (IGFBP) of which six are known (25). They bind IGF-1 with the same or higher affinity than its own receptor (19). These binding proteins do not only regulate the transport of IGF-1 but also its bioavailability as well as its biological function: whereas on one hand, they increase the half-life of IGF-1 in the circulation, they may on the other hand inhibit the binding to its receptor by reducing the concentration of free, bioactive IGF-1 (7, 26). In some physiologic circumstances though, they might also facilitate this binding (27). Thus, the IGF-binding proteins exert inhibitory as well as stimulating effects on IGF-1 signalling. Beyond that, some of those binding proteins have discrete functions independent from the IGF-1 pathway, including effects on cell migration and proliferation as well as glucose metabolism (7).

The most abundant IGF-binding protein is IGFBP-3 which binds about 80% of plasmatic IGF-1. However, IGFBP-3 does not seem to crucially affect IGF-1 bioactivity (11). In contrast, especially IGF-binding proteins 1 and 2 have emerged as important metabolic regulators. IGFBP-1 more than IGFBP-2 is supposed to acutely modulate IGF-1 bioavailability and activity (12, 28).

Both binding proteins are produced and secreted by the liver (19), depending, amongst other factors, on nutritional stimuli (29).

1.4.1 IGFBP-1

The regulation of IGFBP-1 secretion is mainly exerted by GH, having stimulating effects, and insulin that acutely inhibits IGFBP-1 synthesis (29). Thus, IGFBP-1 serum concentrations reflect acute hepatic insulin exposure due to food intake (10, 30, 31) with a 60% suppression of IGFBP-1 levels within 4 hours after glucose infusion (31). Conditions that cause a decrease in insulin levels (such as fasting) lead to an acute

increase in IGFBP-1 levels in healthy subjects (32) – with observed increases in IGFBP-1 levels by a factor of 3.5 - 12 after an overnight fast (31) – while hyperinsulinemia as in insulin resistance or incipient type 2 diabetes mellitus goes along with decreased IGFBP-1 levels (11). In chronic liver disease, however, where portal insulin concentrations are low, peripheral insulin and IGFBP-1 might be simultaneously higher compared to healthy subjects (32). In a healthy state, IGFBP-1 negatively correlates with IGF-1: an increase in IGFBP-1 levels results in lower concentrations of total and free IGF-1 (7, 26, 30, 32, 33). Additionally, IGFBP-1 levels are closely linked to body composition – being negatively associated with liver fat, visceral fat mass (10) and with this, sensitive towards changes in body weight (data not yet published; registered with clinicaltrials.gov: NCT01631123).

1.4.2 IGFBP-2

IGFBP-2 is regulated by insulin-like growth factors and GH – the latter one suppressing IGFBP-2 synthesis – but also by nutritional factors: protein restriction was seen to lead to a rapid increase in IGFBP-2 levels while a high-protein diet induced a decrease in IGFBP-2 serum levels (Schuler et al., data not yet published).
On IGF-1 action, IGFBP-2 exerts both stimulating and inhibitory effects (10, 34): on one hand it induces the insulin/IGF-1 pathway via AKT-PI3-Kinase (10) on the other hand, it leads to a decrease in free IGF-1 levels, inhibiting its biological action (30). Correlating negatively with fat mass (35), reduced expression of hepatic IGFBP-2 promotes hepatic steatosis (36) and low levels of IGFBP-2 are seen predominantly in patients with metabolic syndrome (37).

1.4.3 IGFBP-3

Among IGF-binding proteins, IGFBP-3 is the most abundant, binding approximately 80% of plasmatic IGF-1. Together with the acid-labile subunit (ALS), IGF-1 and IGFBP-3 form a 150 kDa complex, which maintains and prolongs the half-life of IGF-1 in the blood, and with this, determines the concentration of free and thus biologically active IGF-1 (7, 38, 39). Just as with IGF-1, transcription and consequently synthesis of IGFBP-3 and ALS are stimulated by GH (16).

Although it plays a major role in the transport of IGF-1 within the bloodstream, it does not seem to relevantly affect bioactivity of IGF-1 (11). Next to its central role as an IGF-carrier, IGFBP-3 has also been seen to be independently involved in tumor pathogenesis, associated to suppressing as well as promoting effects (40).

1.2 The Growth hormone axis in health and disease

1.2.1 GH, insulin and IGF-1

The GH-IGF-1 axis, including IGF binding proteins, has a central role in human health and longevity. IGF-1 is predominantly investigated regarding its impact on aging processes (reviewed here (7)): both increased as well as decreased signal transduction have been related to accelerated aging and a subsequently shortened life span as well as a higher risk for metabolic disorders, including type 2 diabetes.

On one hand, the occurrence of inflammatory processes and malignant diseases but also metabolic disorders, including insulin resistance (IR), are attributed to increased signaling of the insulin/ IGF system. Suppression of its activity was seen to prolong lifetime spent in health (41, 42): the genetically induced suppression of the GH/ IGF-signaling seemed to prevent the onset of T2DM as well as the development malignancies in humans and animal models (7). On the other hand, in epidemiological

studies, decreased activity of the IGF system was seen to associate with multiple degenerative processes such as sarcopenia, cognitive decline, cardiovascular diseases and T2DM (43-45).

Indeed, the GH/ insulin/ IGF-pathway is crucial for glycemic metabolism, being mandatory for beta-cell functioning and with this, insulin secretion (46), as well as influencing whole body insulin sensitivity (32). In adults with GH deficiency, insulin sensitivity was improved by supplementation of GH as well as IGF-1 (13, 47). In mice, pancreatic beta-cell function was dependent on intact insulin and IGF-1 receptors (46, 48).

1.2.2 IGFBP-1 and -2

Concerning their metabolic influence, the evidence for IGF-1 binding proteins 1 and 2 is similarly ambiguous. IGFBP-1 is acutely downregulated by increases of insulin and thus by food intake which increases the bioavailability of IGF-1. Hence, IGFBP-1 has been associated with deterioration of glucose metabolism and insulin resistance (12). Conversely, in healthy subjects, IGFBP-1 was seen to correlate positively with insulin sensitivity, postulated as a stronger marker than the well-established Homeostasis Model Assessment (HOMA) index (11). In cohort studies, IGFBP-1 was mostly found to be negatively associated with diabetes risk (reviewed in (13)). Concerning anthropometric parameters that are important metabolic influencing factors, a clearer picture emerges: IGFBP-1 levels correlate negatively with BMI, visceral adipose tissue (VAT) mass and liver fat content (28, 49).

Also IGFBP-2 was described as independent predictor of insulin sensitivity (11). In a cohort study, the epigenetic modification of the IGFBP-2 gene leading to its silencing was related to higher incidence of T2DM (50), marking IGFBP-2 as a protective factor

against T2DM. In subjects with normal glucose tolerance (NGT), low levels of IGFBP-2 were predictive for the onset of T2DM (21, 50).

1.3. Research gaps and questions

The exact physiological mechanisms behind these aforementioned interactions are still not fully elucidated. In order to attain a better understanding of health and disease, especially with regard to metabolism, the comprehension of the IGF-1 axis and its effects is needed (51).

Up to now, most of the data obtained on that subject is derived from epidemiological or preclinical experimental studies. Useful data from human intervention trials is sparse, especially regarding the impact of nutrition on the IGF-axis (5, 7) and regarding the aspect of T2DM prevention.

Prospective trials on prediabetic subjects provide an ideal basis to identify biomarkers predicting prediabetes progression and onset of overt diabetes.

We therefore combined data of three recent randomized controlled studies on prediabetic subjects, conducted in Germany. They all aimed for improvement of metabolism by lifestyle intervention, namely by dietary means. The interventional approach in combination with a long-term follow-up period allow analyzing several metabolic changes in detail. Against this background, the aim of this project was to clarify the role of IGF-1 and its binding proteins 1 and 2 in glucose metabolism: are inter-individual differences in serum levels predictive for the development of diabetes? Which would be the underlying mechanisms? Are serum levels sensitive towards lifestyle intervention and mirror improvement or impairment of metabolic state?

For the analysis, we used data of subjects at high risk of developing T2DM.

Parts of the introduction are modified adapted from (52).

2 Methods

2.1 Cohorts and study designs

The aim of this study was to investigate IGF-1 and its binding proteins 1 and 2 as predictive biomarkers for the development or prevention of T2DM in the presence of high risk prediabetes. For this analysis, we combined data of three different recent lifestyle intervention studies on subjects with prediabetes: the Prediabetes Lifestyle intervention study (PLIS), conducted in Dresden, Germany, the Diabetes Nutrition Algorithm - Prediabetes trial (DiNA-P), conducted in Nuthetal and Berlin, Germany, both still ongoing, as well as the recently completed Optimal Fibre Trial (OptiFIT), accomplished in Berlin, Germany. Within those trials, subjects were allocated to various interventional arms, differing in dietary requirements and/or modes of consultation, all together focusing on prevention of diabetes by specific dietary means.

2.1.1 PLIS

PLIS is a multicenter study, enrolled in 2013 by the Eberhard-Karls University in Tübingen and conducted at six different sites in Germany, being part of the national research association German Center for Diabetes research (DZD).

For this analysis, we included data from 135 subjects that had been enrolled in the study at the university hospital Carl Gustav Carus of the Technical University Dresden, characterized at baseline as *high risk* prediabetic subjects: reduced insulin secretory capacity (ISC) and/or presence of Non-alcoholic fatty liver disease (NAFLD) with reduced insulin sensitivity (cf. Figure 1).

Within the trial, participants underwent a hypo- to isocaloric diet based on low fat intake correspondent to recommendations of the German Nutrition Society (DGE; < 30 kcal% of fat, < 10 kcal% of fatty acids, >15 g/1000 kcal of fiber intake per day; diet scheme 1)

for 12 months. They received dietary one-to-one counselling in either 8 or 16 sessions of equal length, depending on randomized allocation. Randomization was done by a supervisor at the study center in Tübingen, using a randomiser and applying permuted block randomization (block size: 30). Personnel involved in the study was blinded, excluding nutrition consultants and principal investigators.

After the 1-year intervention period, the study was pursued for long-term follow-up. As the study is still ongoing, the analyzed cohort represents a subsample of the final cohort.

A more detailed description of the study design can be found elsewhere(2). The study protocol was accepted by the Ethics Committee at the Technical University of Dresden. The trial was registered at clinicaltrials.gov, reference number NCT01947595.

2.1.2 DiNA-P

The DiNA-P study represents a monocentric trial, aligned to the PLI study described above. Since June 2013, it is conducted at the German Institute for Human Nutrition (DIfE) and the Charité University Hospital Berlin, both partner institutions of the DZD, at their clinical wards in Berlin and Nuthetal, Germany.

As this study is also still running, only a subsample of the final cohort, counting 155 subjects, likewise characterized as *high-risk* prediabetic at screening (cf. PLI study), were included into this analysis.

Within DiNA-P, the participants followed a two-phase one-year dietary intervention: three weeks based on a restrictive and hypocaloric (1200-1500 kcal) regimen, followed by an isocaloric-to-moderate-hypocaloric diet phase up to 12 months – both focusing on decreasing specifically either intake of fat (< 30 kcal% of fat; diet scheme 2) or carbohydrate (1st phase< 40g/day; 2nd phase: <40 kcal%; diet scheme 3). On the lines of the PLI study, individual dietary counselling was given 8 or 16 times per year,

according to allocated interventional arm. Randomization was performed analogously to the PLI study.

Again, the first year of intervention was followed by long-term follow-up as mere observation period, still ongoing.

A more detailed description of the study design be found under clinicaltrials.gov (NCT02609243). The study protocol was accepted by the Ethics Committee at the Charité – University Medicine, Berlin.

2.1.3 OptiFIT

OptiFIT was a trial on subjects with impaired glucose tolerance, conducted between March 2010 and October 2014 at a clinical ward in Berlin, Germany. Here, we used data from 124 participants having taken part in the intervention. Within this 24 months-trial the first year was covered by a modified version of the structured lifestyle program called PREDIAS: all subjects were asked to follow a diet low in fat and relatively high in fiber intake, according to the recommendations of the DGE (fat: <30 kcal%/ day, saturated fat: <10 kcal%/day, fibers:15 g/1000 kcal/day). Dietary consultation consisted of 12 group-based sessions throughout the year. In parallel, participants were randomly assigned to one of two groups with regard to a drinking supplement that they were asked to consume on a daily basis for the whole 2 years: insoluble oat fiber (consisting of 70 weight% cellulose, 25 weight% hemicellulose and 3–5 weight% lignin; Vitacel OF 560-30; Rettenmaier & Söhne, Holzmuehle, Germany; diet scheme 4) versus placebo (maize starch low in fiber, guar gum and isomaltulose; diet scheme 5). Block randomization based on computer algorithm was performed by study personnel without participant contact. Allocation concealment was ensured until the end of intervention. Blinding was achieved by neutral numbering of supplement containers and composition of supplements which were equal in consistency, color, odor and taste.

The precise study design (53) as well as the exact composition of supplements (53, 54) are published elsewhere. The study protocol was accepted by the Ethics Committee at the University of Potsdam; reference number at clinicaltrials.gov: NCT01681173.

2.1.4 Combining the three studies

These three studies have in common to aim on metabolic improvement accompanied by moderate weight loss in an interventional setting based on lifestyle changes.

The respective nutrient and energy intake were registered at baseline and throughout the study via dietary records. The analysis of food records was performed software-based by study personnel. Next to the described nutritional interventions, in all three trials, participants were required to reach a certain level of daily physical activity (PLIS, DiNA-P: according to randomization either 6 h/week with 50% of guided activity or 3 h/week; OptiFIT: 240 min/week), recorded and monitored by accelerometers (PLIS, DiNA-P) or pedometers and questionnaires (OptiFIT).

The trials were conducted respecting Good Clinical Practice according to the Declaration of Helsinki and every participant had given written informed consent before being included into each study. Also, all subjects had undergone a detailed medical evaluation before inclusion. This included taking an anamnesis, physical examination, routine blood examination and urine analysis.

Evidence for the presence of severe chronic metabolic/ cardiovascular/ pulmonary/ gastrointestinal/ autoimmune/ malignant diseases did not emerge in any of the subjects prior to study start. Moreover, according to study protocols, no subject was taking medications that could have seriously affected the outcome variables.

Combining data from those three trials, the ultimate cohort used for the analysis consisted exclusively of subjects presenting prediabetes at baseline and thus enabled us to specifically examine diabetes incidence in a prospective manner.

If, in rare cases, the diagnosis of prediabetes or the allocation to a specific subtype (isolated IFG vs. isolated IGT vs. IFG+ IGT) was not unequivocal due to variations in measurements (e.g. serum vs. capillary), subjects were allocated to the next subcategory according to blood glucose levels.

2.1.5 Final cohort

414 subjects make up the final cohort with available data for fasting levels of IGF-1, IGFBP-1 and IGFBP-2. The cohort is mainly Caucasian, the age range is from 28 to 80 years.

At the time of analysis, the included participants from PLIS and DiNA-P had at least already completed the one-year intervention period (unless they had dropped out), whereas the OptiFIT study was already completed anyway. As the OptiFIT study lasted only 2 years, we decided to exclusively use data covering 2 years of observational period for all three trials.

The project design is depicted in Figure 1.

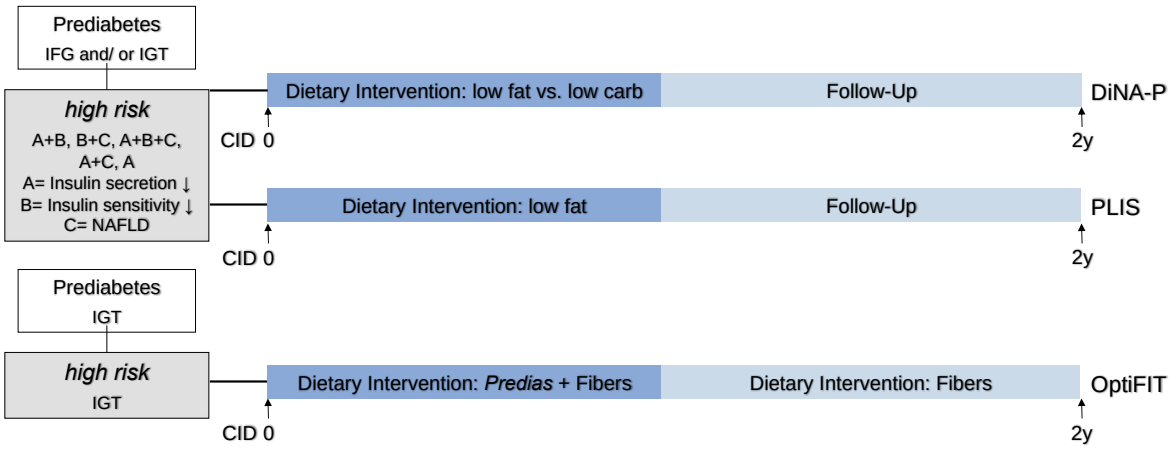

Figure 1 Project design: combined data from three randomized clinical trials

IFG= Impaired Fasting Glucose. IGT= Impaired Glucose Tolerance. NAFLD= Non-alcoholic fatty liver disease. CID= Clinical Investigation Day. 0= Baseline. Carb= Carbohydrate. Y= Year. DiNA-P= Diabetes Nutrition Algorithm-Prediabetes. PLIS= Prediabetes Lifestyle Intervention Study. OptiFIT=

Optimal Fibre Trial. Predias= Prevention of Diabetes Self-management. Own illustration: Nina Marie Tosca Meyer. Created with Microsoft® PowerPoint.

2.2 Data collection

After having been screened and declared suitable by a study physician, participants were included into the study and underwent the baseline visit: here, they obtained medical examination, fasting blood draws, an oral glucose tolerance test, anthropometric measurements as well as magnetic resonance (MR) examination, next to the provision of dietary protocols and activity meters plus a first dietary counselling.

The same procedures were repeated at visits 3 weeks (DiNA-P only), 6 months (PLIS and DiNA-P only), 1 year and 2 years respectively after inclusion into the study.

Within the OptiFIT cohort, only a small subject sample underwent MR examination.

2.2.1 Anthropometrics

Anthropometry included measurements of body weight, height, waist and hip circumference, body fat and muscle mass as well as liver fat content and was performed by study personnel.

Body weight, height and circumferences were measured of participants in light clothing, without shoes. Afterwards, Body-mass index (BMI) was calculated by dividing weight in kg by height in meters squared, waist-to-hip ratio (WHR) by dividing waist by hip circumference, both in cm. Fat and muscle mass were determined by bioelectrical impedance analysis (BIA) as well as magnetic resonance imaging (MRI).

Hepatic fat storage was detected via magnetic resonance spectroscopy (^{1}H-MRS) according to a protocol published elsewhere (55).

MR-examination was only done on suitable volunteers that had given their discrete consent and were eligible.

Contraindications were the presence of metallic implants and claustrophobia or subject's refusal. The evaluation of the scans was done in a blinded way by a radiologist from the Department of Diagnostic and Interventional Radiology at the University Hospital Tübingen Germany.

2.2.2 Glucose metabolism

Data on glucose homeostasis, insulin sensitivity and secretion are based on both fasting blood samples and oGTT derived data, including corresponding indices.

In PLIS and DiNA-P, blood samples were obtained in the fasting state as well as 30, 60, 90 and 120 minutes after a 75 g oral glucose load, respectively. Samples were used for measuring glucose, insulin and c-peptide, after having been stored constantly at -80°C.

In OptiFiT, glucose was determined from capillary blood, insulin measurements were performed for time points 0', 60' and 120'. Here, capillary blood glucose concentrations were measured immediately using the glucose oxidase method.

Fasting as well as 120'-glucose levels were used to determine T2DM.

HOMA-IR (56) and Matsuda-index (57) were calculated as standard surrogate parameters of insulin resistance, indices according to Abdul-Ghani et al. (58) were used for estimation of hepatic insulin resistance (HIRI) and the modified Insulinogenic Index (IGI) according to Seltzer (59) as well as the Disposition Index-2 (DI) (60) were applied to approximate insulin secretion.

These indices are based on different oGTT data. Accordingly, for calculation, we included only subjects with complete data sets for respective required time points.

2.3 Laboratory analyses

Analyses of blood samples were performed in established laboratories.

Glucose and insulin were analyzed via standard methods (for insulin in DINA-P and

OpitFit, the ELISA kit of Mercodia, Uppsala Sweden, was used; insulin in PLIS was measured via chemiluminescent immunoassay; Siemens Healthcare GmbH, Erlangen Germany).

For determination of fasting serum concentrations of IGF-1, IGFBP-1 and IGFBP-2 we used commercially available ELISA assays (Mediagnost®, Reutlingen, Germany), following manufacturer's instructions. These assays had been previously established in our group (61). The following kits were used: for IGF-1 Mediagnost® Cat# E20, RRID:AB_2813791 (mean intra-assay coefficient of variation: 5.81%; mean inter-assay coefficient of variation: 8.57%), for IGFBP-1 Mediagnost® Cat# E01, RRID:AB_2813788 (mean intra-assay coefficient of variation: 6.52%; mean inter-assay coefficient of variation: 6.05%), and for IGFBP-2 Mediagnost® Cat# E05, RRID:AB_2813797 (mean intra- and inter-assay coefficient of variation < 10%). Extreme outliers were double checked and non-physiologic values (n=2) were not included into analysis. Reference values for IGF-1 and IGFBP-1 in a healthy population are published elsewhere (62, 63). Compared to reference values within a healthy population with normal glucose tolerance, here, IGFBP-1 levels appeared to be relatively low. In supplemental tables 1 a-c, we depicted assay-specific reference values for IGF-1, IGFBP-1 and -2, respectively.

Routine laboratory, including liver enzymes, kidney values and lipid profile were analyzed using standard methods.

2.4 Statistical analyses

2.4.1 Outcomes

The primary outcome of the analysis was the incidence of T2DM according to WHO criteria (64) within a 2-years observation period.

Secondary outcomes were changes in markers for overall metabolic health, including in

insulin sensitivity and secretion, body composition and liver fat content in associations with changes in IGF-1, IGFBP-1 and IGFBP-2.

2.4.2 Calculations

Statistical analyses are based on an as-treated analysis. Unless otherwise stated, parametric tests were used, assuming normal distribution as given due to a sufficient number of cases.

Continuous variables are reported as means ± SD, when normally distributed, or as median [IQR], when non-normally distributed.

To analyze changes in IGF-1 and its binding proteins, anthropometric as well as metabolic parameters during the intervention period, we accordingly used data only of subjects with complete follow-up data at 1 year (n= 345).

We used Student's t-tests to assess differences in continuous normally distributed variables, paired t-test for within-group and unpaired t-tests for between-group comparisons. In case of skewed continuous variables, we used Wilcoxon-tests for within-group and Mann-Whitney-U tests for between-group comparisons.

For between group comparisons of categorical variables, we applied Chi-squared tests.

We applied repeated-measures ANOVAS and respective post-hoc tests for within-group-comparisons with adjustments or with > 2 points in time. One-way ANOVA or Welch-Test was used for between-group differences of > 2 groups, depending on homogeneity of variance either followed by Bonferroni or Games-Howell post-hoc tests.

To determine associations between IGF-1, IGFBP-1 and IGFBP-2 and metabolic parameters as well as between their changes, we used correlation analyses (Spearman's rank correlation, adjusting for relevant confounding factors, where reasonable and applicable).

Cox Proportional Hazards models were applied to analyze the association between baseline serum levels of IGF-1, IGFBP-1 and IGFBP-2 and risk of incident T2DM within a 2-years-period. Study entry was defined as day of inclusion into the study, study exit as diagnosis of diabetes or censoring, whichever appeared first. Different models were developed for adjustments of preselected baseline variables, including correlates of IGF-axis and possibly confounding risk factors for diabetes incidence (sex, age, body mass index, fasting glucose levels).

To test assumptions about proportional hazards, we applied graphical methods.

Level of statistical significance was considered to be reached at $p < 0.05$. Analyses were done with SPSS for Windows, Version 25 (SPSS Inc®, Chicago, IL, USA). Statistical figures were created with GraphPad Prism® Version 9.5.1.

The methods described are modified from Meyer NMT et al., 2022 (52), pp. 556-558.

3 Results

3.1. Baseline characteristics

Among the 414 participants in our study, the majority met central criteria for metabolic syndrome to baseline, including abdominal obesity (mean waist-hip ratio for women: 0.88, men: 1.00, (52)) and increased fasting glucose. Of those who underwent magnetic resonance spectroscopy (MRS), 59.9% (52) had liver fat content greater than or equal to 5.56%, indicating a fatty liver. Hence, this cohort represents a metabolic phenotype being per se associated with risk for T2DM. Detailed baseline characteristics are depicted in Table 1 (reprinted from Meyer NMT et al., 2022 (52), page 559.).

Table 1: Baseline characteristics

Parameters	Value	N
Demographic factors		
Women (%)	58.0	240
Age (years)	61.8 ± 9.4	414
Allocated study		
PLIS (%)	32.6	135
DiNA-P (%)	37.4	155
OptiFiT (%)	30.0	124
Anthropometry		
BMI (kg/m²)	31.1 ± 5.6	414
WHR (cm/cm)	0.93 ± 0.09	409
VAT_{-MRI} (l)	5.11 [3.88; 6.69]	266
IHL_{-MRS} (%-abs.)	9.5 ± 8.1	272
Glycemic parameters		

Fasting glucose (mmol/L)	5.7 ± 0.7	414
2-h glucose (mmol/L)	8.3 ± 1.5	414
Fasting insulin (pmol/L)	73.80 [51.00; 106.12]	397
Insulin sensitivity		
HOMA-IR	2.6 [1.7; 3.8]	397
Matsuda Index	2.6 [1.8; 3.6]	275
HIRI	39.1 ± 12.3	280
Insulin secretory capacity		
IGI	12.0 [7.5; 21.2]	280
DI	31.6 [21.6; 44.3]	275
IGF-1 and BPs		
IGF-1 (µg/L)	143.3 ± 54.7	414
IGFBP-1 (µg/L)	2.23 [1.04; 4.26]	414
IGFBP-2 (µg/L)	263.44 [143.17; 417.23]	414

Data are described as mean± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables and as n (%) for categorical variables. PLIS Prediabetes Lifestyle Intervention Study. DiNA-P Diabetes Nutrition Algorithm- Prediabetes. OptiFiT Optimal Fibre Trial. BMI Body mass index. WHR Waist-hip ratio. VAT Visceral adipose tissue. MRI Magnetic resonance imaging. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. HOMA Homeostatic model assessment. IR Insulin Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index (Seltzer). DI Disposition Index-2. IGFBP-1/-2 Insulin-like Growth Factor Binding Protein-1/-2. Table from Meyer NMT et al., 2022 (52), page 559.

Baseline levels of IGF-1 and IGFBPs were compared between groups based on risk factors for T2DM (Table 2, reprinted from Meyer NMT et al., 2022 (52), page 560): IGF-1 levels were inversely associated with age and did not differ between metabolic subgroups based on BMI, IHL or glycemic state. IGFBP-1 and IGFBP-2 were associated with anthropometric markers, with obese individuals as well as those with NAFLD exhibiting lower levels in both IGFBP-1 and -2. IGFBP-1 was also lower in

individuals with isolated impaired fasting glucose (IFG) compared to those with isolated impaired glucose tolerance (IGT).

Compared to non-incident cases, those who developed T2DM during the study had notably lower baseline levels of IGF-1 and higher levels of IGFBP-1, as shown in Figure 2 a+b (reprinted from Meyer NMT et al., 2022 (52), page 561).

Table 2: Baseline concentrations of IGF-1 and IGFBPs between different groups according to demographic, metabolic and prognostic characteristics

Groups	N	IGF-1 (µg/L)	P	IGFBP-1 (µg/L)	p	IGFBP-2 (µg/L)	p
Sex							
Female	240	141.1 ± 56.5		2.28 [1.08; 4.34]		263.4 [149.7; 407.0]	
			0.355		0.764		0.884
Male	174	146.2 ± 52.1		2.08 [1.03; 4.15]		263.2 [141.3; 422.6]	
Age[a]							
< 65 years	236	150.3 ± 54.9		1.76 [0.89; 4.43]		261.1 [133.0; 370.8]	
			0.002		0.063		0.079
≥ 65 years	178	133.9 ± 53.1		2.50 [1.28; 4.19]		281.67 [156.5; 461.4]	
Obesity status							
Non-obese	198	145.5 ± 52.7		2.72 [1.25; 4.94]		281,21 [164.72; 461.03]	
			0.417		**0.007**		**0.008**
Obese	216	141.2 ± 56.4		1.64 [0.93; 3.85]		252.68 [123.10; 365.24]	
NAFLD status							
No NAFLD	109	144.1 ± 59.2		2.69 [1.35; 5.38]		330.84 [200.78; 471.21]	
			0.410		**<0.001**		**0.011**
NAFLD	163	138.7 ± 48.7		1.44 [0.80; 3.10]		261.29 [156.06; 414.65]	
Glycemic status							

IFG+NGT	126	145.8 ± 54.7		1.73 [0.91; 3.69]		310.01 [161.97; 467.06]	
NFG+IGT	146	148.9 ± 60.9	0.067[c]	2.81 [1.25; 5.57]	**0.005**[b, c]	249.53 [127.72; 364.88]	**0.015**
IFG+IGT	142	135.2 ± 46.7		2.07 [1.11; 4.20]		263.30 [145.77; 402.15]	
Incidence of type 2 diabetes							
No	357	146.6 ± 55.2		2.09 [0.97; 4.07]		262.31 [150.87; 399.42]	
Yes	57	122.5 ± 46.7	**0.002**	3.32 [1.62; 5.87]	**0.002**	284.82 [125.40; 477.81]	0.463

Data are described as mean± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables and as n (%) for categorical variables. Between-group comparisons for IGF-1: t-tests. Between-group comparisons for IGFBP-1, IGFBP-2: MWU tests. [a]Selection according to Levine et al.(22). [b]Welch-Test. [c]Post-hoc tests (Games-Howell) for IGFBP-1: IFG+NGT vs. NFG+ IGT: p= 0.005; IFG+NGT vs. IFG+IGT: p= 0.232; NFG+IGT vs. IFG+IGT: p= 0.168. NAFLD Non-alcoholic Fatty Liver disease. IFG Impaired Fasting Glucose. NGT Normal Glucose tolerance. NFG Normal Fasting Glucose. IGT Impaired Glucose Tolerance. Table from Meyer NMT et al., 2022 (52), page 560.

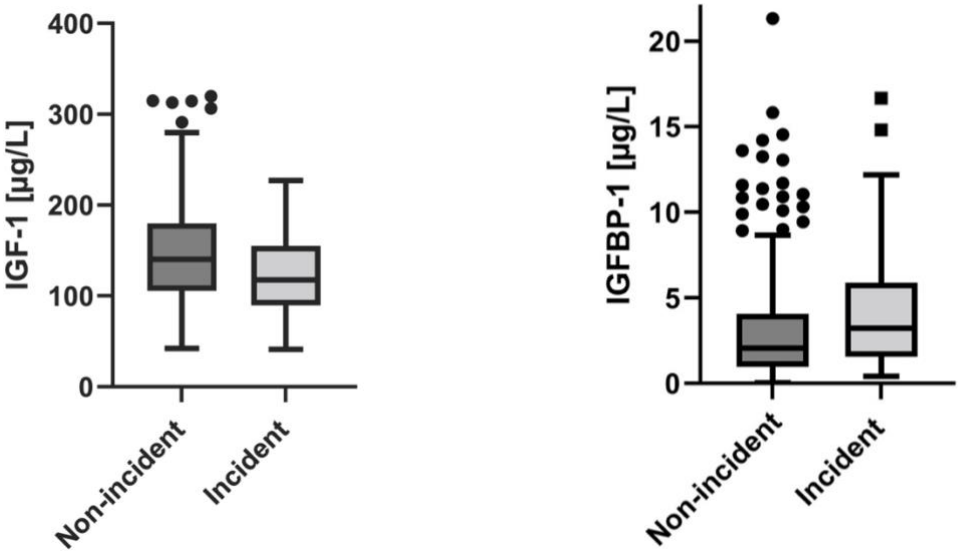

Figure 2 a + b Baseline values of IGF-1 and IGFBP-1 in non-incident and incident cases

a. IGF-1

Boxplots of serum levels of IGF-1 in µg/L to baseline in non-incident (n= 357) and incident cases (n= 57). P for between-group difference= 0.002 (t-test). Figure from Meyer NMT et al., 2022 (52), page 561.

b. IGFBP-1

Boxplots of serum levels of IGFBP-1 in µg/L to baseline in non-incident (n= 357) and incident cases (n=57). P for between-group difference= 0.002 (MWU test). Figure from Meyer NMT et al., 2022 (52), page 561.

3.2 Baseline correlations

To assess metabolic implications of the IGF-components at study onset, we analyzed correlations of baseline variables adjusted for age, sex and study cohort (Table 3, reprinted from Meyer NMT et al., 2022 (52), supplemental material). This revealed that IGF-1 levels were negatively correlated with waist-hip ratio, visceral adipose tissue mass, and fasting glucose levels. Similarly, both IGFBP-1 and IGFBP-2 were negatively associated with markers of diabetes risk such as BMI, waist-hip ratio, visceral adipose tissue, and intrahepatic lipid content.

High IGFBP-1 levels went along with lower hepatic and whole-body insulin resistance (HIRI, HOMA-IR) and consecutively higher insulin sensitivity (Matsuda index). On the other hand, high IGFBP-1 was associated with lower insulin secretory capacity (IGI). Subsuming, IGFBP-1 was linked to lower fasting insulin and lower insulin resistance on one side and with impaired glucose tolerance and insulin secretion on the other side. Thus, subjects with high IGFBP-1 at baseline are identified as a cohort with reduced insulin reserve or impaired beta-cell function against the background of high-risk prediabetes.

Importantly, the abovementioned significant correlations persisted even after adjusting for baseline BMI. This highlights the relation of IGF-components to specific phenotypes.

Table 3: Correlation structure of baseline variables, adjusted for age, sex and study cohort

Baseline Variables	IGF-1 (µg/L)	IGFBP-1 (µg/L)	IGFBP-2 (µg/L)
IGF-1 (µg/L)		-0.081	-0.094
IGFBP-1 (µg/L)	-0.081		**0.165****
IGFBP-2 (µg/L)	-0.094	**0.165****	
BMI (kg/m²)	-0.056	**-0.166****	**-0.139****
WHR (cm/cm)	**-0.118***	-0.082	-0.069
VAT_{-MRI} (l)	**-0.163****	**-0.327****	**-0.182****
IHL_{-MRS} (%-abs.)	-0.035	**-0.283****	**-0.186****
Fasting glucose (mmol/L)	**-0.116***	0.007	0.053
2-h glucose (mmol/L)	-0.029	0.051	-0.068
Fasting insulin (pmol/L)	-0.050	**-0.216****	-0.050
HOMA-IR	-0.069	**-0.215****	-0.035
Matsuda Index	-0.004	**0.327****	**0.152***
HIRI	-0.047	**-0.326****	-0.104
IGI	0.056	**-0.360****	-0.065
DI	0.062	-0.114	0.032

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). IGFBP-1/-2: Insulin-like Growth Factor Binding Protein-1/-2. BMI Body mass index. WHR Waist-hip ratio. VAT Visceral adipose tissue. MRI Magnetic resonance imaging. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. HOMA Homeostatic model assessment. IR Insulin

Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index. DI Disposition Index 2 (Seltzer). Table from Meyer NMT et al., 2022 (52), supplemental material.

3.3 Responses to lifestyle intervention

3.3.1 Overall responses

The lifestyle interventions allowed highly significant improvements in anthropometrics and glucose metabolism. Thus, the intervention led to reductions in BMI, VAT and IHL in the 354 individuals, moreover to improvements in insulin sensitivity and secretion. Among the analyzed parameters, IGI was the only one that did not show a significant amelioration (as shown in Table 4, reprinted from Meyer NMT et al., 2022 (52), page 561).

Table 4: Parameters' values at baseline and after 1 year of intervention

Characteristics	Baseline	1 year	N	p
IGF-1 and IGFBP-1				
IGF-1 (µg/L)	141.8 ± 53.7	143.0 ± 52.1	345	0.623
IGFBP-1 (µg/L)	2.13 [1.04;4.07]	2.24 [1.28; 4.32]	345	**0.025**
Anthropometry				
BMI (kg/m²)	30.9 ± 5.4	29.9 ± 5.2	342	**<0.001**
WHR (cm/cm)	0.933 ± 0.091	0.925 ± 0.088	332	**0.037**
VAT_{-MRI} (l)	5.6 ± 2.4	5.1 ± 2.2	197	**<0.001**
IHL_{-MRS} (%-abs.)	7.48 [3.09; 15.50]	4.00 [1.63; 8.67]	202	**<0.001**

Glycemic parameters				
Fasting glucose (mmol/L)	5.7 ± 0.7	5.5 ± 0.8	344	**<0.001**
2-h glucose (mmol/L)	8.2 ± 1.6	7.4 ± 2.0	344	**<0.001**
Fasting insulin (pmol/L)	73.44 [51.65; 105.47]	68.00 [46.39; 99.00]	335	**<0.001**
Insulin sensitivity				
HOMA-IR	2.6 [1.7; 3.8]	2.3 [1.6; 3.5]	334	**<0.001**
Matsuda Index	2.6 [1.8; 3.5]	3.2 [2.2; 4.7]	234	**<0.001**
HIRI	37.15 [30.57; 44.33]	33.97 [28.85; 40.65]	241	**<0.001**
Insulin secretory capacity				
IGI	11.7 [7.5; 20.8]	11.9 [7.6; 18.4]	241	0.969
DI	30.9 [21.6; 43.6]	36.7 [22.8; 64.2]	234	**<0.001**

Data are described as mean ± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables. Between-group comparisons for normally distributed variables: paired t-test / for non-normally distributed variables: Wilcoxon-test. IGFBP-1: Insulin-like Growth Factor Binding Protein-1. BMI Body mass index. WHR Waist-hip ratio. VAT Visceral adipose tissue. MRI Magnetic resonance imaging. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. HOMA Homeostatic model assessment. IR Insulin Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index. DI: Disposition Index 2 (Seltzer). Table from Meyer NMT et al., 2022 (52), page 561.

3.3.2. Responses of the IGF-axis

These improvements were, however, not reflected by major changes of the IGF-axis: IGF-1 or the binding proteins did not show any remarkable changes, except for a small decrease of IGFBP-1 from 3.3 to 3.2 µg/l, despite their significant correlations with

anthropometric and metabolic parameters (52). This might firstly suggest that the levels of these hormones are primarily constitutional or inherited and thus, eventually not changeable by only moderate lifestyle changes but require more drastic interventions.

But, to further analyze this surprising finding, we tested whether higher or lower baseline levels of IGF-1 and IGFBPs might associate with responses to lifestyle interventions and thus, compared changes in subjects with levels above or below the 50th percentiles (tables 5 and 6). This unveiled highly significant changes of IGF-1 during the study, which differed significantly between groups: subjects with baseline IGF-1 levels above the median experienced a decrease in IGF-1 levels (from 183.5 µg/L to 168.7 µg/L, $p< 0.001$). Subjects with baseline levels below the median showed an increase (from 99.9 µg/L to 117.2 µg/L, $p< 0.001$). This led to a significant difference in the change of IGF-1 between these two groups ($p< 0.001$). This between-group-difference was still present after adjusting for sex and age ($p= 0.025$) as well as change in BMI ($p< 0.001$), the latter however reduced the difference between the groups.

In terms of the metabolic changes induced by the intervention, individuals in the higher percentile group of IGF-1 exhibited a more favorable development compared to those with lower IGF-1 levels at baseline (refer to Tables 5 and 6): specifically, although both groups showed significant reductions in visceral fat mass and IHL, these reductions were significantly greater in the upper percentile group. In terms of glucose metabolism, both groups exhibited significant improvements in fasting and 2-hour glucose levels, but only the upper percentile group showed improvements in fasting insulin and HOMA-IR. Changes in fasting insulin and Matsuda index were even significantly different between both percentile groups.

The same trend was observed for baseline IGFBP-1 percentile groups: individuals with levels below the median showed a significant increase in IGFBP-1 levels (from 1.1 to

1.8 µg/L, p< 0.001), while those with levels above the median experienced a significant decrease (from 5.5 to 4.5 µg/L, p= 0.045). Furthermore, individuals with lower baseline IGFBP-1 concentrations exhibited a significant increase in IGF-1 levels (p= 0.003), while those with higher levels showed a slight decrease (p= 0.087).

Both percentile groups for IGFBP-1 showed overall metabolic improvements upon intervention, in terms of BMI, visceral fat mass, IHL, fasting and 2h-glucose levels, as well as fasting insulin levels, insulin sensitivity and secretion. However, except for fasting glucose levels, each of these improvements were more pronounced in the group with lower baseline IGFBP-1 levels. Significantly greater improvements were observed only for IHL (Tables 5 and 6).

Table 5: Changes of metabolic parameters over time in association with IGF-1 / IGFBP-1 baseline levels in percentiles (n=345)

	IGF-1 < 134.2 µg/L		IGF-1 ≥ 134.2 µg/L			IGFBP-1 < 2.13 µg/L		IGFBP-1 ≥ 2.13 µg/L		
Parameters	**Delta**	**N**	**Delta**	**N**	**p**	**Delta**	**N**	**Delta**	**N**	**p**
Δ IGF-1	17.3 ± 31.1[##]	172	-14.8 ± 51.7[##]	173	**<0.001[1]**	9.0 ± 39.6[##]	172	-6.5 ± 49.7	173	**0.001**
Δ IGFBP-1	-0.18 ± 3.03	172	-0.12 ± 4.55[#1]	173	0.886	0.74 ± 1.37[##]	172	-1.04 ± 5.14[##]	173	**<0.001[1]**
Anthropometry										
Δ Body Mass Index (kg/m²)	-0.86 ± 1.70[##]	171	-1.17 ± 1.8[##]	171	0.105	-1.10 ± 1.61[##]	171	-0.93 ± 1.88[##]	171	0.365
Δ Waist-to-hip ratio (cm/cm)	-0.02 ± 0.08[#]	166	-0.00 ± 0.06	166	0.093	-0.01 ± 0.06[#1]	165	-0.01 ± 0.09	167	0.929
Δ Body fat content$_{\text{-BIA}}$ (%)	-1.1 ± 3.4[##]	145	-1.0 ± 4.1[##]	147	0.773	-1.2 ± 3.5[##]	149	-1.0 ± 4.0[##]	143	0.550
Δ Visceral fat mass$_{\text{-MRI}}$ (l)	-0.37 ± 0.86[##]	111	-0.61 ± 0.86[##]	86	**0.034[1]**	-0.53 ± 0.83[##]	106	-0.42 ± 0.92[##]	91	0.168[1]
Δ Intrahepatic Lipid Content$_{\text{-MRS}}$ (%-abs.)	-2.72 ± 6.20[##]	113	-4.47 ± 5.87[##]	89	**0.011[1]**	-4.08 ± 6.61[##]	110	-2.79 ± 5.39[##]	92	**0.049[1]**

Glycemic parameters										
Δ Fasting glucose (mmol/L)	-0.20 ± 0.60[##]	172	-0.17 ± 0.56[##]	172	0.641	-0.17 ± 0.53[##]	172	-0.21 ± 0.63[##]	172	0.443
Δ 2-h glucose (mmol/L)	-0.74 ± 2.06[##]	172	-0.80 ± 1.73[##]	172	0.789	-0.83 ± 1.78[##]	172	-0.71 ± 2.02[##]	172	0.549
Δ Fasting insulin (pmol/L)	0.53 ± 101.00	170	-10.78 ± 39.59[##]	165	**0.031**[1]	-8.14 ± 41.86[#]	165	-2.02 ± 100.33[#1]	170	0.642[1]
Insulin sensitivity										
Δ HOMA-IR	-0.1 ± 3.7	170	-0.5 ± 1.5[##]	164	0.086[1]	-0.4 ± 1.6[##]	165	-0.2 ± 3.7[##1]	169	0.703[1]
Δ Matsuda Index	0.5 ± 1.7[##]	122	0.9 ± 2.4[##]	112	**0.019**[1]	0.8 ± 1.6[##]	128	0.5 ± 2.5[#]	106	0.484[1]
Δ HIRI	-2.4 ± 8.6[##]	127	-3.6 ± 8.6[##]	114	0.232[1]	-3.3 ± 8.2[##]	133	-2.7 ± 9.1[##]	108	0.785[1]
Insulin secretory capacity										
Δ IGI	2.4 ± 27.0	127	-2.7 ± 16.1	114	0.118[1]	0.7 ± 27.5	133	-1.0 ± 14.8	108	0.375[1]
Δ DI	17.0 ± 66.2[##]	122	9.4 ± 43.2[#]	112	0.679[1]	15.2 ± 69.7[#]	128	11.1 ± 34.3[##]	106	0.786[1]

Values are presented as mean ± standard deviation. Unless otherwise stated. independent-sample t-tests were applied for between-group-differences. For calculation of oGTT-based indices, only subjects with complete data sets for respective required timepoints were analyzed. [1]non-parametric test. [#]change was significant within the group on a 0.05 level. [##]change was significant within the group on a 0.01 level. Own illustration: Nina Marie Tosca Meyer.

Table 6: Comparisons of absolute changes in metabolic parameters over time in association with IGF-1 / IGFBP-1 baseline levels in percentiles (n=345)

a) Medians of IGF-1

	IGF-1 < 134.2 µg/L						**IGF-1 ≥ 134.2 µg/L**						
	Baseline		**1 year**				**Baseline**		**1 year**				
Parameter	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**N**	**P within group diff.**	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**N**	**P within group diff.**	**P between group diff.***
IGF-1	101.2	[84.4; 120.5]	114.4	[89.9; 141.1]	172	**<0.001**	172.31	[152.7; 197.5]	164.0	[137.4; 196.1]	173	**0.001**	**<0.001** [1]
IGFBP-1	2.2	[1.2; 4.4]	2.5	[1.3; 4.5]	172	0.460	2.1	[0.9; 3.7]	1.9	[1.2; 4.0]	173	**0.015**	0.886

BMI (kg/m²)	30.8	5.2	29.9	5.1	171	**<0.001**	31.1	5.6	29.9	5.4	171	**<0.001**	0.105
WHR (cm/cm)	0.94	0.1	0.92	0.1	166	**0.022**	0.93	0.1	0.93	0.1	166	0.718	0.093
Body fat content$_{\text{-BIA}}$ (%)	35.2	8.7	34.0	9.2	145	**<0.001**	34.0	8.6	33.0	9.2	147	**0.003**	0.773
VAT$_{\text{-MRI}}$ (l)	5.1	[3.9; 6.9]	4.7	[3.7; 6.0]	111	**<0.001**	5.2	[4.0; 6.7]	4.7	[3.4; 6.4]	86	**<0.001**	**0.034**[1]
IHL -MRS (%-abs.)	7.0	[3; 14.9]	5.3	[2.4; 9.0]	113	**<0.001**	8.0	[3.4; 17.0]	3.2	[1.0; 7.3]	89	**<0.001**	**0.011**[1]
Fasting glucose (mmol/L)	5.8	0.7	5.6	0.8	172	**<0.001**	5.7	0.7	5.5	0.7	172	**<0.001**	0.641
2-h glucose (mmol/L)	8.3	1.5	7.6	1.9	172	**<0.001**	8.1	1.6	7.3	2.0	172	**<0.001**	0.789
Fasting insulin (pmol/L)	79.7	[56.1; 108.2]	77.8	[55.0; 111.2]	170	0.239	65.8	[49.6; 98.4]	59.6	[44.0; 88.7]	165	**<0.001**	**0.031**[1]
HOMA-IR	3.0	[2.0; 3.9]	2.7	[1.8; 3.9]	170	0.051	2.4	[1.6; 3.7]	2.0	[1.4; 3.0]	164	**<0.001**	0.086[1]

Matsuda Index	2.5	[1.8; 3.3]	2.9	[2.0; 4.3]	122	**<0.001**	2.8	[1.9; 3.6]	3.6	[2.5; 5.0]	112	**<0.001**	**0.019[1]**
HIRI	37.5	[30.8; 45.3]	34.26	[29.8; 42.0]	127	**0.003**	36.7	[30.0; 42.6]	33.5	[27.2; 39.3]	114	**<0.001**	0.232[1]
IGI	11.7	[7.5; 21.4]	12.4	[7.9; 20.0]	127	0.273	11.6	[7.5; 19.2]	11.4	[7.4; 16.8]	114	0.232	0.118[1]
DI	28.2	[19.5; 43.6]	34.3	[21.4; 63.1]	122	**<0.001**	33.6	[22.9; 44.5]	38.7	[25.0; 68.0]	112	**0.001**	0.679[1]

b) Medians of IGFBP-1

	IGFBP-1 < 2.13 µg/L						**IGFBP-1 ≥ 2.13 µg/L**						
	Baseline		**1 year**				**Baseline**		**1 year**				
Parameter	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**N**	**P within group diff.**	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**N**	**P within group diff.**	**P between group diff.***
IGF-1	141.5	48.5	150.5	52.5	172	**0.003**	142.1	58.5	135.6	50.6	173	0.087	**0.001**

IGFBP-1	1.04	[0.70; 1.46]	1.47	[0.85; 2.19]	172	**<0.001**	4.07	[2.84; 6.8]	3.89	[2.30; 5.57]	173	**0.045**	**<0.001** [1]
BMI (kg/m²)	31.8	5.0	30.7	4.8	171	**<0.001**	30.0	5.7	29.1	5.6	171	**<0.001**	0.365
WHR (cm/cm)	0.94	0.08	0.93	0.08	165	0.070	0.93	0.10	0.92	0.09	167	0.192	0.929
Body fat content$_{\text{-BIA}}$ (%)	35.5	8.1	34.3	8.8	149	**<0.001**	33.6	9.1	32.7	9.6	143	**0.005**	0.550
VAT$_{\text{-MRI}}$ (l)	5.8	[4.4; 7.3]	5.0	[4.1; 6.8]	106	**<0.001**	4.5	[3.4; 5.8]	4.3	[3.1; 5.7]	91	**<0.001**	0.168[1]
IHL -MRS (%-abs.)	9.9	[5.4; 17.5]	5.50	[2.5; 10.8]	**110**	**<0.001**	4.3	[1.4; 9.4]	2.5	[0.7; 6.8]	92	**<0.001**	**0.049**[1]
Fasting glucose (mmol/L)	5.8	0.6	5.6	0.7	172	**<0.001**	5.7	0.7	5.5	0.8	172	**<0.001**	0.443
2-h glucose (mmol/L)	8.2	1.5	7.3	2.0	172	**<0.001**	8.3	1.6	7.6	2.0	172	**<0.001**	0.549
Fasting insulin (pmol/L)	82.0	[59.3; 115.3]	73.9	[55.0; 112.0]	165	**0.002**	64.2	[43.4; 98.0]	62.4	[44.1; 86.0]	170	**0.019**	0.642[1]

HOMA-IR	3.0	[2.1; 4.1]	2.7	[1.8; 4.0]	165	**<0.001**	2.3	[1.5; 3.4]	2.0	[1.3; 3.1]	169	**0.003**	0.703[1]
Matsuda Index	2.4	[1.7; 3.2]	2.8	[2.0; 4.1]	128	**<0.001**	2.9	[2.2; 4.6]	3.7	[2.4; 5.6]	106	**0.002**	0.484[1]
HIRI	38.3	[32.8; 45.5]	35.9	[31.3; 42.3]	133	**<0.001**	34.9	[28.0; 40.9]	31.2	[25.8; 38.6]	108	**0.003**	0.785[1]
IGI	13.7	[8.9; 24.2]	15.1	[8.9; 20.1]	133	0.560	8.5	[5.7; 15.7]	9.8	[6.0; 15.1]	108	0.434	0.375[1]
DI	32.9	[32.9; 32.9]	38.2	[22.6; 65.5]	128	**<0.001**	28.4	[19.5; 39.2]	33.8	[23.1; 63.4]	106	**<0.001**	0.786[1]

Data are described as mean ± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables. Within-group comparisons were calculated via paired t-tests normally distributed variables and Wilcoxon tests for non- normally distributed variables. Within-group differences were calculated using Mixed ANOVA: * median-group/ parameter interaction. For calculation of oGTT-based indices, only subjects with complete data sets for respective required timepoints were analyzed. IGF-1 Insulin-like Growth Factor 1. IGFBP-1 Insulin-like Growth Factor Binding Protein-1. BMI Body mass index. WHR Waist-hip ratio. VAT Visceral adipose tissue. MRI Magnetic resonance imaging. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. HOMA Homeostatic model assessment. IR Insulin Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index. DI Disposition Index 2 (Seltzer). Own illustration: Nina Marie Tosca Meyer.

3.4 Incidence of Type 2 Diabetes

Within the 2-year follow-up period, 57 (14%) individuals had developed T2DM (52). Notably, 28 of them had already developed T2DM after only 1 year, thus within the intervention period (52). On the other hand, 23 of the 57 individuals even showed improved glucose metabolism after 1 year of intervention, and 6 even had normal glucose tolerance (NGT) (see table 7, reprinted from Meyer NMT et al., 2022 (52), supplemental material).

Table 7: Characteristics of subjects with incident diabetes after 2 years (n= 57), baseline and 12-month data

Characteristics	Baseline	1 year	N	p
Demographic factors				
Women (n; %)	27 (47.4)	n.a.	n.a.	n.a.
Age (years)	63.5 ± 7.8	n.a.	57	n.a.
IGF-1 and IGFBP-1				
IGF-1 (µg/L)	122.5 ± 46.7	132.6 ± 49.9	57	**0.025**
IGFBP-1 (µg/L)	3.32 [1.62; 5.87]	2.67 [1.33; 5.12]	57	0.269
Anthropometry				
BMI (kg/m²)	30.94 ± 5.47	30.26 ± 5.51	57	**0.001**
WHR (cm/cm)	0.96 ± 0.09	0.94 ± 0.09	56	0.179
VAT_{-MRI} (l)	5.7 ± 2.1	5.3 ± 2.0	35	**0.005**
IHL_{-MRS} (%-abs.)	7.37 [3.07; 15.87]	5.09 [1.96; 10.05]	35	**0.001**
Glycemic parameters				
Fasting glucose (mmol/L)	6.1 [5.8; 6.5]	6.0 [5.4; 7.0]	57	0.518
2-h glucose (mmol/L)	8.8 ± 1.6	9.3 ± 2.4	57	0.108
Fasting insulin (pmol/L)	79.9 [49.0; 98.0]	72.4 [51.0; 97.0]	54	0.084
Insulin sensitivity				

HOMA-IR	3.0 [1.9; 3.8]	2.7 [1.8; 4.0]	54	0.140
Matsuda Index	2.5 [1.8; 3.0]	2.5 [2.0; 3.9]	38	0.357
HIRI	37.1 [29.9; 41.7]	36.3 [30.6; 40.1]	40	0.436
Insulin secretory capacity				
IGI	8.6 [5.7; 13.2]	9.3 [6.1; 13.4]	40	0.582
DI	22.1 [15.3; 28.3]	24.0 [13.8; 31.1]	38	0.249
Glycemic status				
NFG+NGT	0 (0)	6 (10.5)	n.a.	
IFG+NGT (n; %)	16 (28.1)	6 (10.5)	n.a.	
NFG+IGT (n; %)	9 (15.8)	4 (7.0)	n.a.	**0.005**
IFG+IGT (n; %)	32 (56.1)	13 (22.8)	n.a.	
T2DM	0 (0)	28 (49.1)	n.a.	

Data are described as mean ± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables and as n (%) for categorical variables. Within-group comparisons of normally distributed parameters: t-tests/ of non-normally distributed parameters: Wilcoxon-test / of ordinal parameters: Marginal homogeneity test. IGFBP-1 Insulin-like Growth Factor Binding Protein-1. BMI Body mass index. WHR Waist-hip ratio. VAT Visceral adipose tissue. MRI Magnetic resonance imaging. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. HOMA Homeostatic model assessment. IR Insulin Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index. DI Disposition Index 2 (Seltzer). IFG Impaired Fasting Glucose. NGT Normal Glucose tolerance. NFG Normal Fasting Glucose. IGT Impaired Glucose Tolerance. Table from Meyer NMT et al., 2022 (52), supplemental material.

3.4.1 Characteristics of incident cases

Those who developed diabetes during the study were characterized by significantly lower baseline levels of IGF-1 ($p= 0.002$) and higher levels of IGFBP-1 ($p= 0.013$) (52). Moreover, these individuals exhibited significantly higher fasting ($p< 0.001$) and 2-hour

glucose (p= 0.003) values as well as a lower insulin secretion capacity (IGI: p= 0.002, DI: p= 0.007) compared to those who did not develop diabetes (52).

Also, the individuals who developed diabetes during the study showed a distinct response to the intervention compared to non-incident cases. Specifically, incident cases did not experience improvements in 2-hour glucose levels or insulin sensitivity and – in contrast to non-incident cases – they did not show an increase in insulin secretion (DI; please refer to table 8, reprinted from Meyer NMT et al., 2022 (52), page 562).

Table 8: Comparison of changes between incident and non-incident cases

Delta variables baseline − 1 year	**Non-incident (N=288)**		**Incident (N=57)**		
	Value	N	Value	N	p
Δ BMI (kg/m²)	-0.78 [-2.09; 0.15]$^{\#\#}$	285	-0.58 [-1.38; 0.26]$^{\#\#\#}$	57	0.197
Δ WHR (cm/cm)	-0.01 ± 0.07$^{\#\#1}$	276	-0.02 ± 0.09	56	0.378
Δ VAT_{-MRI} (l)	-0.31 [-0.95; 0.13]$^{\#\#}$	162	-0.15 [-1.05; 0.09]$^{\#}$	35	0.524
Δ IHL_{-MRS} (%-abs.)	-3.7 ± 6.4$^{\#\#}$	167	-2.5 ± 4.2$^{\#\#}$	35	0.297
Δ Fasting glucose (mmol/L)	-0.16 ± 0.63$^{\#\#}$	271	-0.18 ± 0.82	50	0.880
Δ 2-h glucose (mmol/L)	-0.9 ± 1.9$^{\#\#}$	271	0.2 ± 2.8	50	**0.007**
Δ Fasting insulin (pmol/L)	-6.0 [-23.61; 11.0]$^{\#\#}$	281	-6.96 [-21.67; 9.91]	54	0.963
Δ HOMA-IR	-0.27 [-0.88; 0.35]$^{\#\#}$	280	-0.02 [-0.75; 0.34]	54	0.541
Δ Matsuda Index	0.7 ± 2.1$^{\#\#}$	196	0.4 ± 2.1	38	**0.026**^{1}
Δ HIRI	-3.4 ± 8.9$^{\#\#}$	201	-0.8 ± 6.4	40	0.077

Δ IGI	0.26 [-4.32; 3.73]	201	0.26 [-2.24; 2.46]	40	0.686
Δ DI	6.82 [-6.14; 26.81] ##	196	1.41 [-3.66; 7.28]	38	0.117
Δ IGF-1	-0.6 ± 47.4	288	10.2 ± 33.4#	57	0.103
Δ IGFBP-1	0.29 [-0.49; 1.42]##	288	-0.10 [-2.37; 1.46]	57	0.114
Δ IGFBP-2	18.1 ± 192.3#1	286	-18.5 ± 165.4	56	0.185

Data are described as mean± standard deviation for normally distributed variables, median [IQR] for non-normally distributed continuous variables. Unless otherwise stated, parametric tests were used for normally distributed variables, non-parametric tests for non-normally distributed variables. [1]non-parametric test. [#]Within-group comparison: significance at the 0.05 level (2-tailed). [##]Within-group comparison: significance at the 0.01 level (2-tailed). VAT Visceral adipose tissue. IHL Intrahepatic lipid content. MRS Magnetic resonance spectroscopy. Abs absolute. IR Insulin Resistance. HIRI Hepatic insulin resistance index (Abdul-Ghani). IGI Insulinogenic Index (Seltzer). DI Disposition Index 2. IGFBP-1/-2 Insulin-like Growth Factor Binding Protein-1/-2. Table from Meyer NMT et al., 2022 (52), page 562.

3.4.1 Diabetes incidence in relation to the IGF-axis

Regarding IGF-1 levels in relation to diabetes incidence, regression models revealed that individuals with lower baseline IGF-1 levels had a significantly higher risk of incident diabetes (HR 0.991/µg/L; 95% CI 0.985-0.997, p= 0.003; see table 9, model 1, reprinted from Meyer NMT et al., 2022 (52), supplemental material) – even after adjusting for age and sex but also after additional adjustments for baseline BMI (model 2), fasting glucose levels (model 3), and IGFBPs 1 and 2 (model 4) (52). This translates in a 20% risk reduction for subjects with high IGF-1 levels with an absolute difference of 24 µg/L (52).

With regard to IGFBP-1, by contrast, individuals with higher baseline values had a significantly higher risk of developing diabetes (HR 1.061/µg/L; 95% CI 1.021-1.102, p=

0.002) – again, when adjusted for age and sex but also for baseline BMI (table 10, zmodel 2.1), fasting glucose (model 3.1), IGF-1 and IGFBP-2 (model 4.1) (52). This translates in a 9 % risk increase for subjects with high IGFBP-1 levels with an absolute difference of 1.4 µg/L (52).

Last, consider IGFBP-2 levels, these did not seem to have a significant impact on the risk of developing type 2 diabetes (52).

It is noteworthy that, albeit to be expected, fasting glucose was found to be an even stronger predictor of diabetes incidence than both IGF-1 and IGFBP-1 (52).

No violations of the proportional hazard assumption of covariates were detected, which indicates that the results reflect independent associations of IGF-1 and IGFBP-1 with the incidence of T2DM.

Table 9: Multivariate Cox Regression models: IGF-1 and Diabetes incidence within 2 years

Model	Covariates	HR	95% CI		P value
			Lower	Upper	
1	IGF-1 (µg/L)	0.991	0.985	0.997	**0.003**
	Age (years)	1.004	0.973	1.037	0.786
2	IGF-1 (µg/L)	0.991	0.985	0.997	**0.003**
	Age (years)	1.005	0.972	1.038	0.786
	BMI (kg/m²)	1.001	0.953	1.052	0.969
3	IGF-1 (µg/L)	0.991	0.985	0.997	**0.005**
	Age (years)	0.998	0.964	1.032	0.895
	BMI (kg/m²)	1.001	0.954	1.049	0.979
	Fasting glucose (mmol/L)	2.939	1.747	4.944	**<0.001**
4	IGF-1 (µg/L)	0.992	0.986	0.998	**0.010**
	Age (years)	0.995	0.962	1.030	0.788
	BMI (kg/m²)	1.011	0.965	1.060	0.639
	Fasting glucose (mmol/L)	3.096	1.848	5.189	**<0.001**
	IGFBP-1 (µg/L)	1.083	1.037	1.131	**<0.001**
	IGFBP-2 (µg/L)	1.000	0.999	1.002	0.635

5	IGF-1 (µg/L)	0.992	0.986	0.998	**0.011**
	Age (years)	1.005	.973	1.037	0.779
	∆ IGF-1 (µg/L)	1.002	.994	1.009	0.663
6	IGF-1 (µg/L)	0.992	0.986	0.998	**0.015**
	Age (years)	1.008	0.976	1.042	0.617
	∆ IGF-1 (µg/L)	1.002	0.995	1.009	0.587
	∆ BMI (kg/m²)	1.103	0.932	1.304	0.254
	∆ IGFBP-1 (µg/L)	0.962	0.916	1.011	0.124

Sex is included in each model as strata variable. Table from Meyer NMT et al., 2022 (52), supplemental material.

Table 10: Multivariate Cox Regression models: IGFBP-1 and Diabetes incidence within 2 years

Model	Covariates	HR	95% CI		P value
			Lower	Upper	
1.1	IGFBP-1 (µg/L)	1.061	1.021	1.102	**0.002**
	Age (years)	1.014	0.982	1.046	0.397
2.1	IGFBP-1 (µg/L)	1.061	1.021	1.102	**0.002**
	Age (years)	1.015	0.983	1.048	0.366
	BMI (kg/m²)	1.009	0.961	1059	0.724
3.1	IGFBP-1 (µg/L)	1.087	1.043	1.133	**<0.001**
	Age (years)	1.006	0.973	1.040	0.713
	BMI (kg/m²)	1.013	0.968	1.060	0.589
	Fasting glucose (mmol/L)	3.331	1.989	5580	**<0.001**
4.1	IGFBP-1 (µg/L)	1.083	1.037	1.131	<**0.001**
	Age (years)	0.995	0.962	1.030	0.788
	BMI (kg/m²)	1.011	0.965	1.060	0.639
	Fasting glucose (mmol/L)	3.096	1.848	5.189	**<0.001**
	IGF-1 (µg/L)	0.992	0.986	0.998	**0.010**
	IGFBP-2 (µg/L)	1.000	0.999	1002	0.635
5.1	IGFBP-1 (µg/L)	1.110	1.022	1.206	**0.013**
	Age (years)	1.007	0.975	1.040	0.663
	∆ IGFBP-1 (µg/L)	1.053	0.971	1.143	0.210
6.1	IGFBP-1 (µg/L)	1.112	1.025	1.208	**0.011**
	Age (years)	1.009	0.976	1.043	0.598
	∆ IGFBP-1 (µg/L)	1.061	0.977	1.153	0.160
	∆ BMI (kg/m²)	1.122	0.948	1.329	0.181
	∆ IGF-1 (µg/L)	1.006	1.000	1.012	0.052

Sex is included in each model as strata variable. Table from Meyer NMT et al., 2022 (52), supplemental material.

3.4.2. Diabetes incidence in relation to IGF-1, IGFBP-1 and their changes

Cox regression analyses, applied on the cohort with available follow-up data (n= 345), adjusted for age and sex, affirmed IGF-1 as negative predictor of type 2 diabetes incidence (HR/µg/L 0.991; 95% CI 0.986-0.997, p= 0.003) (52). Importantly, even after adjusting for changes in serum levels of IGF-1 over time, which did not have a significant effect on type 2 diabetes incidence, the impact of IGF-1 remained significant (Table 9, model 5). Also, additional adjusting for changes in BMI and IGFBP-1 levels within 1 year did not attenuate the significant influence of IGF-1 (model 6) (52). This reflects the independent impact of IGF-1 on diabetes incidence.

In this smaller cohort of 345 subjects, also IGFBP-1 could be confirmed as positive predictor of type 2 diabetes (HR 1.059; 95% CI 1.020-1.101, p= 0.003) (52). This association remained significant even after adjusting for 1-year changes in IGFBP-1 levels (Table 10, model 5.1, reprinted from (52), supplemental material) and additionally for changes in BMI and IGF-1 (model 6.1) (52).

Strikingly though, an increase in IGFBP-1 levels was significantly correlated with improvements in both insulin sensitivity and secretion (Matsuda index: ρ= 0.188, p= 0.004; Disposition Index: ρ= 0.129, p= 0.049, (52)) which suggests a general improvement in metabolism. Fitting to this, changes in IGFBP-1 – as opposed to baseline levels – showed a negative association with diabetes incidence, i.e. participants who did not develop diabetes showed an increase in IGFBP-1 levels (table 8, (52)). This association was significant in a logistic regression (Delta IGFBP-1 in percent: OR: 0.995, p= 0.015) and did not lose significance by adjustment for Delta BMI

and Delta IGF-1 in percent within 1 year (OR: 0.996, p= 0.023).

The same opposed relationship was given for IGF-1: while low baseline levels were associated with the incidence of T2DM, hose who developed T2DM showed an increase in IGF-1 levels over time (table 8). This might, however, only be due to the fact that subjects with low levels to baseline showed a relative stronger increase in IGF-1 compared to those with high levels. In a logistic regression, this association did not prove to be significant (Delta IGF-1 in percent: OR: 1.007, p= 0.123).

IGF-1 and IGFBP-1 showed an opposite relationship to each other: the inverse association of IGF-1 baseline levels on diabetes incidence was found to be only significant within the cohort with high IGFBP-1 (above the median; adjusted for age and sex, HR 0.987, 95% CI 0.980-0.995, p_{IGF-1}= 0.001) but not with low levels to baseline (below the median; p_{IGF-1}= 0.578). The positive association between IGFBP-1 baseline levels and diabetes incidence was significant in the cohort with IGF-1 baseline levels below (HR 1.165, 95% CI 1.084-1.252, p< 0.001) but not above the median (p= 0.976).

4 Discussion

4.1 Short summary of results

With this study, we worked out the association between incidence of T2DM and the IGF-axis, a topic that has scarcely been analyzed on an interventional basis. To our knowledge, it is the first analysis of such kind within the scope of an interventional prospective setting on both sexes.

We found that the IGF system determines the individual response to a lifestyle intervention with regard to diabetes prevention: in the setting of prediabetes, higher levels of IGF-1 turned protective towards the development of T2DM, whereas IGF binding protein 1 levels were positively associated with diabetes incidence. Thus, despite anthropometric and metabolic improvements through lifestyle intervention (reduction of BMI, IHL, VAT and improvement in insulin sensitivity), prediabetic subjects with low IGF-1 and high IGFBP-1 levels to baseline developed T2DM. This was accompanied by the inability to improve insulin secretory capacity in these subjects. On the other hand, an increase of IGFBP-1 levels over time, although at first sight possibly paradoxical, was associated with intervention-induced metabolic improvements and present in the group who did not develop T2DM. In our study, IGFBP-2 was not of major impact regarding the incidence of T2DM (52).

4.2 Interpretation of results and embedding the results into the current state of research

It is well established that the IGF-1 system is highly heritable and correlates with anthropometric and metabolic parameters beyond inheritance (65, 66). The new observations are that responses of the IGF-1 and of IGFBP-1 to lifestyle interventions depend on baseline expression levels. Moreover, the baseline levels additionally

correlate with the ability to respond to lifestyle changes and thereby determine the success of lifestyle interventions (52).

4.2.1 IGF-1

Baseline levels of IGF-1 vary widely between individuals primarily due to inheritance (67, 68) and to parameters of glucose and insulin metabolism (65). Although calorie and primarily protein restriction reduce IGF-1 (69), previous studies did not observe significant changes of IGF-1 upon lifestyle interventions and weight loss for 1 or 6 years (70) or reported a decrease of IGF-1 (71).

Unexpectedly, upon moderate weight loss we observed highly significant increases of IGF-1 in people with low levels at baseline while IGF-1 decreased in participants with initially high levels (52). Due to the wide spread of baseline levels, the absolute values were still lower in the lower percentile group and higher in the higher percentile group after the intervention possibly due to the strong inheritance which was estimated at 63% in twin studies (68). Higher levels IGF-1 were associated with reduced risk of type 2 diabetes in cross-sectional (12) and prospective, epidemiological studies (12, 21) but also with increased risk in a Mendelian Randomization study (72).

Our data show that higher levels of IGF-1 predispose to significantly greater improvements of intrahepatic lipids and of visceral fat mass, markers which are strongly associated with the metabolic syndrome, insulin resistance and diabetes risk, despite similar weight loss. In addition, fasting insulin decreased only in subjects with higher IGF-1 to baseline, indicating that the group with low levels was unable to improve insulin sensitivity despite weight loss and significant reductions of VAT and IHL. This remained unaffected by the changes in IGF-1 and IGFBP-1 observed during the intervention.

IGF-1 thus co-determines the capacity for metabolic compensation in this high-risk group (52).

4.2.2 IGFBP-1

Regarding IGFBP-1, other studies found higher levels to be associated with better insulin sensitivity and insulin secretion while low levels were prospectively associated with T2DM and IGT (73, 74). Animal experiments, involving the overexpression or administration of IGFBP-1, have shown conflicting effects depending on the specific promoters and mouse strains used. Translating these findings to humans is challenging (73).

IGFBP-1 is acutely and chronically inhibited by portal insulin levels and therefore low levels closely reflect hepatic fat content and hepatic insulin resistance (75). As insulin resistance plays a significant role in the development of T2DM, it is well explainable that cohort studies have identified low levels of IGFBP-1 as a predictive marker for this condition (74). We observed similar inverse associations of IGFBP-1 with IHL, VAT, hepatic and whole-body insulin resistance as described in literature (10).

Thus, at first sight, individuals with low IGFBP-1 represent a group with unfavorable metabolic prerequisites. It was therefore somewhat unexpected that in our study, individuals with low IGFBP-1 baseline levels showed greater improvements in anthropometric and metabolic parameters upon lifestyle intervention, when compared to the group with high baseline levels - despite similar reductions of body weight between groups (52). Although one may argue that greater improvements were due to greater initial metabolic impairments, higher IGFBP-1 also labelled a group with reduced capacity for improvement.

Thus, the prediabetic group in our study differs from the high-risk groups identified in cross-sectional epidemiological or prospective observational studies regarding IGFBP-1: Several studies identified low IGFBP-1 to be predictive towards development of diabetes. However, most of the cohort studies that discussed the inverse relationship

between IGFBP-1 and the risk of diabetes focused on individuals who were young to middle-aged and had normal glucose tolerance (NGT). In these studies, low IGFBP-1 levels indicated the presence of chronic hyperinsulinemia, as present in early stages of prediabetes. One of these studies from a Swedish group reported an increase of IGFBP-1 in prediabetic subjects as they approached overt type 2 diabetes (76, 77). This appears to relate to the progression of hepatic insulin resistance on one hand, which reduces the suppression of the hepatokine IGFBP-1 relative to circulating insulin levels (23). On the other hand, the progressive beta-cell dysfunction reflected by impaired glucose tolerance appears to contribute to this phenotype. Notably, study participants with higher IGFBP-1 at follow-up showed significantly less reductions of 2h-glucose values and only one quarter of the reduction of fasting insulin compared to the lower 50th percentile (77). This fits to our data according to which higher IGFBP-1 labels the group which is unable to improve beta cell function upon reductions of body weight, visceral and hepatic fat content. Accordingly, high IGFBP-1 identifies individuals with prediabetes who are unresponsive to standard lifestyle interventions.

Furthermore, other studies found a decrease in IGFBP-1 levels when prevention of diabetes was successful (52). This might indicate improved beta-cell function and insulin sensitivity due to lifestyle intervention, as these improvements result in enhanced hepatic insulin exposure (78, 79). In line with this, individuals in our study who developed diabetes (incident cases) did not exhibit any changes in IGFBP-1 levels and did not demonstrate improvements in insulin sensitivity, nor in insulin secretion, despite significant reductions in BMI, IHL and VAT (52).

On the other hand, subjects who did not develop diabetes in our group, showed not only lower levels to baseline but also an increase of IGFBP-1 over time which might reflect

the decrease in hyperinsulinemia in these subjects due to improved insulin sensitivity (52).

4.2.3 Interplay IGF-1 and IGFBP-1

4.2.3.1 Insulin sensitivity

Mechanistically, these observations may relate to the antagonism of IGF-1 activity by IGFBP-1. When insulin resistance is just beginning to develop, higher insulin levels lead to the downregulation of IGFBP-1. This down-regulation acts as a compensatory mechanism, increasing the availability of bioactive IGF-1, which in turn promotes insulin sensitivity. Given this background, individuals with lower levels of IGFBP-1 might have a greater likelihood of developing diabetes compared to those with higher IGFBP-1 levels in the same group. But as the transition to diabetes occurs, particularly in advanced prediabetes, IGFBP-1 levels gradually rise due to escalating hepatic insulin resistance and declining insulin secretory capacity (5). The latter, based on disturbed beta-cell function, relevantly contributes to the onset of diabetes, irrespective of insulin resistance (14, 80).

4.2.3.2 Insulin secretion

Against this background, the antagonism between IGF-1 activity by IGFBP-1 further comes into play, being particularly pronounced in the interstitial and pericellular environment (10, 23, 73). IGF-1 was shown to cooperate with insulin in maintaining beta-cell function in adult animals while its developmental function for beta-cells was negligible (46, 48). The selective deletion of beta-cell IGF-receptors primarily led to impaired glucose sensing rather than loss of beta-cell mass in mice (46). This appears to translate to humans as suggested by the protective effects of higher IGF-1 and lower IGFBP-1 leading to increased biologically active IGF-1.

Taken together, in our prediabetic cohort at high risk, elevated IGFBP-1 levels at baseline may indicate impairment of beta-cells along with hepatic insulin resistance. In fact, individuals with higher baseline IGFBP-1 levels exhibited a significantly diminished insulin secretion compared to those with lower levels (52).

4.2.3.3 Anthropometrics

In addition, higher activity of the IGF system appears to support loss of ectopic fat stores as shown by the greater reductions of visceral and hepatic fat in the group with higher IGF-1 and lower IGFBP-1 levels at baseline.

Moreover, higher baseline levels of IGFBP-1 were associated with favorable, risk-reducing anthropometric variables such as lower body fat content, BMI, intrahepatic liver fat and higher insulin sensitivity. As bioactivity of IGF-1 is regulated through interaction with IGFBP-1, our data overall suggest that a higher biological activity of IGF-1 exerts a protective role in our cohort with moderately advanced age that overweighs the metabolically favorable influence of IGFBP-1.

Fittingly, individuals who developed diabetes showed a tendency towards increased hepatic insulin resistance and decreased insulin secretory capacity compared to those who did not develop diabetes. As reductions in BMI, VAT and IHL were similar between groups, they displayed resistance to lifestyle interventions (52).

4.2.4 IGFBP-2

Although IGFBP-2 was characterized as independent predictor of insulin sensitivity (11), we could not confirm a predictive role of IGFBP-2 on diabetes incidence within our cohort (52).

4.4 Strengths and weaknesses of the study

This study has certain limitations that should be acknowledged. Firstly, it is a post-hoc analysis, which inherently carries known disadvantages. Secondly, the data were pooled from three different trials, and as a result, we were unable to thoroughly analyze the influence of nutrition on the IGF-axis due to variations in the dietary approaches across the studies. However, this pooling of data from multiple trials allowed for a reasonable sample size, enabling us to conduct a robust analysis. Additionally, adjusting for study group did not diminish the significance of our findings, although this is not shown in the data presented. Therefore, the reliability of our findings is reinforced by their consistency across trials and the presence of significant associations despite the relatively short observation period.

Although degradation processes of long-term stored samples cannot be completely ruled out, we took precautions to minimize such effects by avoiding repeated freeze/thaw cycles during the analysis of parameters. It is important to note that due to inherent inter-individual variations, concentrations of IGF-1 and IGFBPs are associated with large standard deviations. Additionally, IGFBP-1 exists in different phospho-isoforms, with its most prevalent form in circulation being highly phosphorylated. In vitro studies have demonstrated that the phosphorylation status of IGFBP-1 plays a role in modulating IGF actions, with the phosphorylated form showing much higher affinity for IGF-1 compared to the non-phosphorylated form (81). This disparity in affinity based on phosphorylation status may also impact the detection of IGFBP-1, potentially leading to the measurement of predominantly unphosphorylated IGFBP-1 in this study (82).

Pooling data from the interventional trials, the use of hormonal medication was not consistently recorded. Thus, we did not adjust for e.g. hormone replacement therapy which might influence IGF-1 and IGFBP-1 levels (7). Likewise, the influence of

menopausal or smoking status was not investigated. However, in a large cohort study, hormone replacement therapy did not reveal to have an effect on the association between IGF-1 levels and diabetes risk (83) and in another cross-sectional study, the proportion of current or former smoker status did not differ between tertiles of IGF-1 levels (12). Of course, also other factors that were not considered in this study might have confounded the results – especially given the complex interplay of factors that regulation of glucose homeostasis is based on. The described association between low IGF-1 levels with a higher diabetes incidence for example, might be biased by the fact that subjects with low IGF-1 levels might present a unfavorable nutritional state as well as might be rather physically inactive (84). One strong limitation is that we did not measure free IGF-1 levels to directly assess its effect on glucose metabolism. Neither did we measure IGFBP-1 pre- and post- glucose load. As high triacylglycerol levels are an important metabolic risk factor that might influence the reported findings, they would have been of interest also in our study.

The effect for IGF-1 and IGFBP-1 revealed by Cox regression analyses turned out to be significant but small. This is probably owed to the use of T2DM as a hard endpoint, the relatively small cohort size and short follow-up period.

Due to differences in the interventional approach between the three studies, the influence of nutrition on the IGF-axis could not be analyzed in detail.

On the other hand, this study also boasts notable strengths. In contrast to the majority of existing interventional studies examining the IGF-1 system in glucose metabolism, we employed a robust primary endpoint, namely the incidence of type 2 diabetes according to WHO criteria. Moreover, the prospective interventional design of our study allowed us to estimate behaviorally induced changes in the IGF system and demonstrate the

impact of lifestyle intervention on the associations between biomarkers and the incidence of diabetes. This aspect remains relatively underexplored in the current body of literature (85).

4.5 Implications for practice and/or future research

Maddux et al. proposed that IGFBP-1 might serve as a marker for insulin sensitivity in basal state and as progression parameter in interventional studies aiming on metabolic improvement (86). Clemmons et al. claimed IGFBP-1 to be a marker for insulin secretion in subjects with normal and impaired glucose tolerance (10). Here, we expand the significance of IGFBP-1 as metabolic biomarker as reflecting both insulin resistance and insulin secretion pattern in different states of impaired glucose metabolism. These findings might facilitate the identification of persons at high risk of developing type 2 diabetes. But as our study was done in a prediabetic high risk group, it may not be translatable to people without metabolic impairments (52) .

Thus, future studies focusing on the use of IGF-1 or IGFBPs as biomarkers should attach importance to a precise baseline characterization of metabolic states as relations and effects might differ between cohorts with different metabolic prerequisites.

Also, the detailed influence of nutrition on the IGF-axis needs to be assessed.
Here, of special interest would be if a concerted increase in IGF-1 levels, e.g. via high-protein diet, is favorable concerning glycemic metabolism (52).

5 Conclusions

In conclusion, our study revealed that besides fasting glucose, individuals with prediabetes that present low IGF-1 and high IGFBP-1 levels are more likely to develop T2DM. This constellation indicates a lack of capacity to metabolically respond to a lifestyle intervention, although anthropometric changes are similar to individuals who do not develop T2DM. Especially elevated levels of IGFBP-1 may indicate advanced impairment of insulin secretion, a crucial factor in diabetes development, in a cohort of older individuals with high-risk prediabetes. Taken together, we put forth the proposition that the restoration of beta-cell function following metabolic improvements is contingent upon the biological activity of the IGF-1 system.

These findings have the potential to aid in identifying individuals at a heightened risk of T2DM and of non-response to lifestyle interventions. These subjects might profit from more intense intervention strategies which should be analysed in further studies, also with a focus on possible target-oriented nutritional interventions (52).

Supplemental material

Supplemental Tables 11 a-c: Various reference values of IGF-1, IGFBP-1 and IGFBP-2

a) IGF-1 reference values according to manufacturer (Mediagnost®)

Serum levels of IGF-I in healthy subjects at various ages

	Percentile													
Age	**0.1**	1	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**95**	**99**
20-30 y.	72	92	115	130	150	167	182	198	215	235	261	302	340	425
30-40 y.	68	87	109	123	142	158	173	188	204	223	248	287	324	404
40-50 y.	64	82	103	116	135	150	164	178	194	212	235	272	310	385
50-60 y.	60	77	97	110	127	142	155	169	184	201	224	260	292	369
60-70 y.	55	72	91	103	120	134	147	161	176	193	215	251	282	362
70-80 y.	25	35	47	55	67	78	88	98	110	124	142	173	207	276
>80 y.	21	30	40	47	58	67	76	85	95	108	125	153	184	245

Serum concentrations are given in ng/ml.

“Reference values have been evaluated by Prof Blum by a radioimmunoassay identical to Mediagnost IGF-R20. Thus, the age and sex specific reference values published in Diagnostics of Endocrine Function in Children and Adolescents (Edited by Prof Ranke. ISBN- 3-335-00496-5) can be applied to all Mediagnost IGF-I assays.” [1]

Reprinted from Manual for Enzyme Immunoassay for Quantitative Determination of Human Insulin-like Growth Factor I (IGF-I) (IGFBP-blocked), Mediagnost®, Reutlingen, Germany; p. 35; 17.07.2018; Version 15 and from (52), supplemental material.

b) IGFBP-1 reference values according to manufacturer (Mediagnost®)

Expectation values in sera of healthy adults (measured values in ng/ml)

Gender	No. of Samples	Average value	Median	Min. – Max.:
female	33	4.79	4.24	0.23 – 16.07
male	36	5.22	2.71	0.42 – 17.94
total	69	5.01	2.77	0.23 – 17.94

Reprinted from Manual for Enzyme Immunoassay for Quantitative Determination of human Insulin-like Growth Factor Binding Protein 1 (IGFBP-1), Mediagnost®, Reutlingen, Germany; p. 27; 13.07.2017 Version 9. and from (52), supplemental material.

c) IGFBP-2 reference value according to manufacturer (Mediagnost®), age-dependent

Age-dependent normal range of serum IGFBP-2

Age (years)	5. percentile (ng/ml)	50. percentile (ng/ml)	95. percentile(ng/ml)
19	84	232	500
25	99	280	580
35	110	381	686
45	130	403	702
55	140	410	715
65	151	418	727
75	153	427	740
80	156	430	744

"IGFBP-2 serum levels (in ng/ml) of > 400 healthy individuals. The normal range is given by the 5., 50. and 95. percentile for age classes."

Reprinted from Manual for Enzyme Immunoassay for Quantitative Determination of human Insulin-like Growth Factor Binding Protein-2 (IGFBP-2), Mediagnost®, Reutlingen, Germany; p. 29; E05 d/e 221211 Version 3 and from and from (52), supplemental material.

Based on: Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA (2000), Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. Hormone Research 54: 60-68 (87).

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Statutory Declaration

“I, Nina Marie Tosca Meyer, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Role of the IGF system in modulating the risk of metabolic dis-ease progression in patients with high-risk prediabetes under lifestyle intervention / Rolle des IGF-Systems in der Modulation des metabolischen Progressionsrisikos von Patienten mit Hochrisiko-Prädiabetes unter Lebensstilintervention“ independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of your own contribution to the publications

Nina Marie Tosca Meyer contributed the following to the below listed publications:

Publication 1: NMT Meyer, S Kabisch, U Dambeck, C Honsek, M Kemper, C Gerbracht, AM Arafat, AL Birkenfeld, PEH Schwarz, J Machann, MA Osterhoff, MO Weickert, AFH Pfeiffer. Low IGF-1 and high IGFBP-1 predict diabetes onset in prediabetic patients. Eur J Endocrinol. 2022 Sep 19.

1. Data acquisition

Within the scope of my work at the German Institute of Human Nutrition (DIfE), I was involved in the collection of data used in my study: as study physician, I jointly conducted the Prediabetes Lifestyle Intervention Study (PLIS) as well as Diabetes-Nutrition-Algorithms - Prediabetes (DiNA-P) at the two clinical study sites of the DIfE in Berlin and Nuthetal. Thereby, I executed medical visits- including medial interviews and physical examination- evaluated examination results (laboratory assessments, metabolic tests, anthropometry, neurological tests) and supervised the metabolic tests that were carried out in the course of the respective studies (especially the oral glucose tolerance test, oGTT). Furthermore, I coordinated and monitored the determination of the central parameters IGF-1, IGFBP-1, IGFBP-2.
Moreover, together with coworkes, I maintained study files of the participants.

2. Data input / maintenance/ verification

Next to data collection, I was, together with others, significantly involved in this process by transferring primary data from analogue records/ documents or digital sources to a central electronic database (SPSS®). At the same time, I acted as contact person for questions regarding data entry.
At regular intervals, both I and coworkers checked the data entered by ourselves or others (dual control principle) in order to guarantee the correctness of the data as far as possible. I corrected or completed incorrect or deficient records or entries. I structured and arranged large-volume primary data sets (MRI data). I also closed existing data gaps – if possible – by review of primary sources.
In addition, I created variables in the electronic database myself that were relevant for the analysis of the data.

3. Statistical evaluation of the data

Prior to the evaluation of the data, I independently selected parameters that were relevant for my study. This was done in consultation with my supervisors, Prof. Dr. med. A. Pfeiffer and Dr. med. S. Kabisch. I carried out the subsequent statistical analyses independently. For this, I used the statistical software SPSS®, version 25 for Windows from IBM (Chicago, IL, USA).
Firstly, I checked the congruence and plausibility of the data. I independently decided on the handling of outlier values as well as on the necessity/ utility of data transformation according to rational criteria. This

was also conducted in close consultation with my above-mentioned supervisors.
The actual statistical evaluation included various statistical tests depending on the actual research question. I decided on the application of the corresponding tests myself, again in consultation with both supervisors and statisticians.

4. Literature research

During the entire work process, I conducted an independent literature search in order to obtain the most comprehensive and up-to-date knowledge about my research area. For this purpose, I obtained literature from relevant databases (especially PubMed®).

5. Placing results in the scientific context and drawing conclusions

After the data analysis and parallel to the literature research, I independently evaluated the significance of obtained results: this included interpretation of the data and drawing of conclusions which I finally placed in the scientific context. This was done in close cooperation with my supervisors.

6. Writing the article and creating tables

After all, I wrote the manuscript independently from beginning to end. This was done in close cooperation with my supervisors, who were involved in the final design through correction, constructive criticism and suggestions for changes. I was personally responsible for the creation and design of all tables and figures included in the publication. These based on the self-compiled data set and on my own statistical evaluations. The tables and figures were also examined and evaluated by my supervisors.
Several corrections, revisions and updates of the used data set as well as statistical and written elaboration were necessary until the final maturity of the now available original article.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

Printing copy(s) of the publication(s)

Meyer NMT, Kabisch S, Dambeck U, Honsek C, Kemper M, Gerbracht C, Arafat AM, Birkenfeld AL, Schwarz PEH, Machann J, Osterhoff MA, Weickert MO, Pfeiffer AFH. Low IGF1 and high IGFBP1 predict diabetes onset in prediabetic patients. Eur J Endocrinol. 2022 Sep 19;187(4):555-565. doi: 10.1530/EJE-22-0034. PMID: 36005859.

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publication list

Publication List Nina Marie Tosca Meyer

Original Articles

J Zhang, SM Schäfer, S Kabisch, M Csanalosi, B Schuppelius, M Kemper, M Markova, NMT Meyer, O Pivovarova-Ramich; F Keyhani-Nejad, S Rohn, AFH Pfeiffer.
Implication of sugar, protein and incretins in excessive glucagon secretion in type 2 diabetes after mixed meals.
Clinical Nutrition. 2023 Feb; doi: 10.1016/J.CLNU.2023.02.011.
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Effect of unsaturated fat and protein intake on liver fat in people at risk of unhealthy aging: 1-year results of a randomized controlled trial.
The American Journal of Clinical Nutrition. 2023 Jan; 10.1016/j.ajcnut.2023.01.010.
Journal: The American Journal of Clinical Nutrition
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NMT Meyer, S Kabisch, U Dambeck, C Honsek, M Kemper, C Gerbracht, AM Arafat, AL Birkenfeld, PEH Schwarz, J Machann, MA Osterhoff, MO Weickert, AFH Pfeiffer:
Low IGF-1 and high IGFBP-1 predict diabetes onset in prediabetic patients.
Eur J Endocrinol. 2022 Sep 19;187(4):555-565. doi: 10.1530/EJE-22-0034. Print 2022 Oct 1.
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S Kabisch, NMT Meyer, C Honsek, M Kemper, C Gerbracht, AM Arafat, U Dambeck, MA Osterhoff, MO Weickert, AFH Pfeiffer:
Predicting Factors for Metabolic Non-Response to a Complex Lifestyle Intervention-A Replication Analysis to a Randomized-Controlled Trial.
Nutrients. 2022 Nov 9;14(22):4721. doi: 10.3390/nu14224721.

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S Kabisch, C Honsek, M Kemper, C Gerbracht, NMT Meyer, AM Arafat, AL Birkenfeld, J Machann, U Dambeck, MA Osterhoff, MO Weickert, AFH Pfeiffer:
Effects of Insoluble Cereal Fibre on Body Fat Distribution in the Optimal Fibre Trial.
Mol Nutr Food Res. 2021 Jun;65(12):e2000991. doi: 10.1002/mnfr.202000991. Epub 2021 May 16.
Journal: Molecular Nutrition and Food Research
Impact Factor: 6.575

S Kabisch†, NMT Meyer†, C Honsek, C Gerbracht, U Dambeck, M Kemper, MA Osterhoff, AL Birkenfeld, AM Arafat, MO Weickert, AFH Pfeiffer:
Obesity Does Not Modulate the Glycometabolic Benefit of Insoluble Cereal Fibre in Subjects with Prediabetes-A Stratified Post Hoc Analysis of the Optimal Fibre Trial (OptiFiT).
Nutrients. 2019 Nov 11;11(11). pii: E2726. doi: 10.3390/nu11112726.
†These authors contributed equally to this publication
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S Kabisch†, NMT Meyer†, C Honsek, C Gerbracht, U Dambeck, M Kemper, MA Osterhoff, AL Birkenfeld, AM Arafat, MF Hjorth, MO Weickert, AFH Pfeiffer:
Fasting glucose state determines metabolic response to supplementation with insoluble cereal fibre: A secondary analysis of the optimal fibre trial (OptiFiT).
Nutrients. 2019 Oct 6;11(10). pii: E2385. doi: 10.3390/nu11102385
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