Aus der Medizinischen Klinik und Poliklinik IV

Klinik der Universität München

Direktor: Prof. Dr. med. Martin Reincke

Hormone and cytokine profiles in pathophysiological subtypes of type 2 diabetes mellitus

Dissertation

zum Erwerb des Doktorgrades der Humanbiologie

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität zu München

vorgelegt von

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aus

München

2024

Mit Genehmigung der Medizinischen Fakultät
der Universität München

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1. Abbreviations

BMI	body mass index
GDM	gestation diabetes
hGH	human growth factor
IGF-I	insulin-like growth factor I
MSTN	myostatin gene
OGTT	oral glucose tolerance test
PPSDiab	“Prediction, Prevention, and Subclassification of type 2 diabetes mellitus”
T2DM	type 2 diabetes mellitus

2. Introduction – Type 2 Diabetes Mellitus (T2DM)

2.1 Prevalence and general pathophysiology of T2DM

Type 2 diabetes mellitus (T2DM) is a common disease. In 2021, an estimated 8.5 million people in Germany carried this diagnosis while the number of unreported cases was estimated at an additional 2 million [1, 2]. Furthermore, a projection based on health insurance data, sees a relative increase of diagnosed T2DM cases in Germany of 54% - 77% from 2015 to 2040, which will likely include many young adults [1, 3]. The associated burden on the healthcare system, both financially and in terms of capacity, will be significant. Not surprisingly, similar trends are seen globally, in particular in high and middle income countries [2, 4].

In T2DM, glucose metabolism is disturbed primarily because of insulin resistance in target tissues such as adipose or muscle. For compensation, the pancreatic beta cells initially often overproduce insulin [5]. However, in the course of the disease, beta cell failure develops as a second pathophysiologic component of T2DM in many cases.

The insidious nature of T2DM means that it can develop unnoticed and untreated over a long period of time, all the while causing damage to beta cells and other tissues [5]. A reversible disease initially, it will turn into a continuously progressive condition over time [5]. While lifestyle interventions can be particularly helpful in prediabetic and early diabetic disease stages, antihyperglycemic medications will often be necessary in a later stage [6].
The high prevalence of T2DM, its insidious onset and severe consequences require further efforts to develop early diagnostic and therapeutic tools for this disease.

2.2 Risk factors of T2DM

The development of T2DM usually involves metabolic and/or hormonal changes, as well as changes in the body composition. However, it is difficult to determine what initiates the pathophysiological process in the first place [7]. Factors that promote T2DM are very diverse. Family history is a common initial indicator, as well as age and physical fitness, and in most T2DM cases, features of the metabolic syndrome are present [8, 9]. Patients with an underlying metabolic syndrome usually have ectopic fat accumulation, dyslipidemia, and metabolic inflammation [7, 10]. The study landscape of T2DM still largely covers patients within this “classic phenotype” [11-15]. Increasingly, however, young, lean individuals are being diagnosed with T2DM. These patients especially illustrate the crucial need for better prevention and early detection of T2DM or prediabetes.

2.3 Prevention and early detection of T2DM

T2DM is diagnosed either by means of an oral glucose tolerance test (OGTT) or by determining long-term (HbA1c) and fasting glucose. The most reliable method - the “gold standard” - is the OGTT. It is, however, time-consuming and costly, meaning that HbA1c and fasting glucose are routinely tested first [16]. At the same time, without an OGTT, early stages of T2DM and prediabetes can be missed [17]. These forms of the disease often manifest without symptoms and thus, remain unnoticed for a long time. Conversely, if they were to be detected at an early stage, effective preventive measures could be taken [16].
To this end, health insurance companies have been increasingly providing prevention offers. Nevertheless, these are hardly being taken advantage of by people with increased risk of disease. Lack of general knowledge, ignorance regarding one’s own health risk as well as missing awareness of the possibilities for preventative action still represent frequent obstacles [18].

This underscores the need to identify risk groups more specifically and create better prevention awareness.

2.4 Women post gestational diabetes – a specific risk group for T2DM

One sex-specific risk factor connected to T2DM is gestational diabetes (GDM), which can occur in women during pregnancy. Although this type of diabetes usually resolves post-partum and is therefore only temporary, women post GDM have a substantially higher risk of subsequently developing T2DM than women after a normoglycemic pregnancy [19]. This observation is one of the reasons, why GDM screening became part of the routine medical examination for all pregnant women in Germany in 2011 [20, 21].

For research purposes, women post GDM represent a unique population and as such an important addition to the study landscape. Studying this high-risk population offers the possibility of better understanding the development of T2DM in order to adapt diagnostic possibilities, and to establish further preventive measures.

3. The PPSDiab study cohort

The prospective, monocentric observational study „Prediction, Prevention, and Subclassification of T2DM" (PPSDiab) was started in Munich in 2011 and tries to understand the metabolic specifics of women post GDM and what predisposes them to T2DM later in life. To this end, two groups of women were included in the study in a 2:1 ratio: on the one hand, women diagnosed with GDM in the index pregnancy (the most recent pregnancy), on the other hand women with a normoglycemic index pregnancy [7].

Overall, 304 women participated in the baseline visit, which took place 3-16 months after the index pregnancy. These women were further invited to annual follow-up examinations.

The overall aim is to monitor the metabolic health of these subjects up until 10 years after the index pregnancy. For this purpose, a variety of relevant data is collected. This includes the results of an OGTT, blood parameters, anthropometric data, body composition, information on family medical history, as well as several other examinations, thus, enabling the study of a deeply phenotyped cohort.

As we now know from the recently published 5-year analysis of the study, 6% of women post GDM in this cohort already developed T2DM five years after their index pregnancy [20]. This was less than previously assumed, based on pre-2011 risk-based GDM screening data [22-24]. However, 55% of women post GDM already showed prediabetic features five years after their index pregnancy [20].

4. Aims of this thesis

4.1 Myostatin – connection with muscle and adipose tissue

Decreased physical fitness, obesity, and metabolic syndrome are often interrelated. All of these features are associated with an impaired glucose metabolism, insulin resistance, and “classic” T2DM. Furthermore, they promote a dysregulated cross-talk between different tissue types, such as muscle and adipose tissue [25].

Previously, my research group could show that in our PPSDiab cohort women post GDM had a lower physical fitness than women after a normoglycemic pregnancy. Interestingly, this was independent of the women’s BMI. We deduced from this, that reduced fitness itself was one of the contributing factors for the increased risk of T2DM in women post GDM [26].

Since physical fitness is also related to muscle mass [27, 28], it seemed interesting to further investigate muscle mass factors in the PPSDiab cohort in addition to fitness parameters. One of the factors we selected for our investigation was myostatin. This myokine is a negative regulator of muscle cell proliferation and growth [29]. It is also related to adipose tissue function [30]. These observations make myostatin an interesting candidate within the muscle adipose tissue crosstalk.

With regards to muscle mass, previous literature has shown in both mouse models and human studies, that higher myostatin concentrations are associated with lower muscle mass and vice versa [29, 31-34]. In human studies however, so far mostly extreme phenotypes have been examined, for example cases with a loss-of-function mutation in the myostatin gene (MSTN) [34].

Meanwhile, concerning adipose tissue and insulin signaling, mouse models have shown that diet-induced obesity can be inhibited in MSTNnull mice. These animals additionally showed lower insulin resistance, thus linking, for the first time, myostatin, obesity, and insulin signaling [35, 36]. When recombinant MSTN was administered to MSTNnull mice, insulin resistance was induced [37]. This contrasts with results from human studies, in which associations between myostatin and insulin signaling have revealed to be inconsistent. Some reported about an association of higher myostatin levels in plasma and serum with insulin resistance and also metabolic syndrome, but others did not see any associations [36, 38-40]. However, healthier, lean phenotypes tend to be underrepresented in these studies.

Given that the cohort of the PPSDiab study includes women in a broad range of metabolic states, we could interrogate signaling between muscle and adipose tissue under many different conditions. The specific research question was, if plasma myostatin is associated with specific metabolic changes and with impaired glucose metabolism.

4.2 Leptin, hGH, and IGF-I – prediabetes and screening-diagnosed T2DM

Young and lean people are increasingly being diagnosed with T2DM. In these patients in particular, the prediabetic state is not detected early, due to the fact that there is no initially obvious T2DM risk - unlike the “classic” subtype of T2DM that occurs in the context of the metabolic syndrome.

One major objective of the PPSDiab study is the identification and better understanding of just such less obvious risk phenotypes. Within this project, we described two T2DM subtypes, one associated with metabolic syndrome and the other without [41].

To better delineate these two phenotypes, we now wanted to examine several hormones and molecules from diverse signaling pathways that are associated with glucose metabolism. We were interested in leptin [42, 43], adiponectin [44, 45], FGF21 [46], fetuin-a [7, 47], resistin [11], myostatin [40], hGH [48, 49], IGF-I [50], and the proinsulin/insulin ratio [51].
In the following, the molecules most relevant for this thesis are presented in further detail.

Leptin

Leptin is an adipokine that, as a hormone, is initially involved in the control of appetite and satiety sensations and has an effect on glucose metabolism by increasing insulin sensitivity [52, 53]. Although its primary function is the control of the amount of adipose tissue, it has been shown in the past that elevated leptin levels are also associated with obesity, poorer fitness, and insulin resistance [7, 8, 26]. Furthermore, leptin appears to be related to inflammatory processes in the body as well as being elevated in people with diabetes [54, 55]. These opposing properties have been described in the past as “leptin resistance”, meaning that leptin signaling is saturated or impaired and therefore, its beneficial effect eliminated [42, 43, 56].

hGH

An interesting antagonist of insulin is the growth factor (GH). Hyperinsulinemia, for example, reduces GH secretion, at the same time GH has anti-insulin effects [57, 58]. In humans, lower human growth factor (hGH) levels usually occur in obesity and in conditions related to increased insulin resistance [49, 57]. The extent to which abnormalities exist in lean people with increased risk of prediabetes as well as T2DM, however, remains unknown.

The lowest point of hGH suppression during an OGTT (the hGH nadir) was previously examined in our PPSDiab cohort, revealing that hGH secretion was not only suppressed during the OGTT, but that a lower nadir was in fact correlated with a higher BMI [49].

IGF-I

Insulin-like growth factor I (IGF-I) is related to insulin resistance, and its synthesis is stimulated by GH. The role of IGF-I in metabolism is not yet fully understood. Though IGF-I values lie in the normal range in insulin resistance, interestingly, these values manifest at both the low and high extremes within this normal range [50].

Different observations regarding IGF-I and obesity have been made in the past. However, the consensus is increasingly that IGF-I is lower in obesity than in normal weight individuals [59-61]. Due to its interaction with GH, IGF-I appears to be of particular interest for our analyses.

5. Conclusion

5.1 Myostatin – association with adipose tissue and inflammatory parameters

Our results suggest a role for myostatin in the altered muscle adipose tissue crosstalk in metabolic syndrome and the linked metabolic inflammation in premenopausal women.
Overall, the crosstalk between myokines and adipokines is complex. For example, the same factor can have both pro- and anti-inflammatory functions. This depends, for instance, on the activation status or the cell maturity of the interacting immune cells [62]. With respect to myostatin, determining causality remains difficult. On the one hand, inflammatory responses in adipose tissue may be promoted by higher myostatin secretion from muscle [63]. On the other hand, inflammation may be triggered by the visceral fat itself [12], in turn activating signaling pathways that regulate myostatin secretion [64]. Either way, chronic inflammation of adipose tissue represents an important link between obesity and both insulin resistance and T2DM [15].

Aerobic exercise has been shown to decrease myostatin levels as well as to improve insulin sensitivity [36]. However, it is conceivable that the increased myostatin levels of obesity and metabolic inflammation impair the effect of exercise interventions on the improvement of glucose metabolism, due to the negative effect of myostatin on muscle mass. Indeed, less muscle mass could lead to the decrease in muscular glucose uptake and this in turn, to impaired insulin sensitivity [65, 66]. However, we demonstrated that, in our study cohort, morphological muscle parameters are less able to provide information on insulin sensitivity than functional muscle parameters [67]. At this point, it is important to emphasize that our cohort represents a comparatively healthy group of individuals lacking particularly extreme phenotypes. A possible explanation for our results may be that the association between insulin sensitivity and myostatin with muscle mass may, in fact, be more relevant within those more extreme phenotypes, which are not represented in our data.

Taken together, as suggested previously by Hittel and colleagues [36], we believe that myostatin could function as a biomarker for metabolic inflammation and may predict individual therapeutic outcomes of exercise interventions. Further mechanistic investigations of human myostatin signaling may also be warranted.

5.2 Leptin, hGH, and IGF-I – differences in distinct risk phenotypes

In previous work, we proposed two human risk phenotypes for T2DM, one related and one unrelated to metabolic syndrome [41]. In one of my first-author publications, we further determined some of the hormonal underpinnings of these two phenotypes. Specifically, we demonstrated as a novel finding that plasma leptin values were elevated not only in the risk phenotype associated with metabolic syndrome, but also the other without, i.e. in the absence of obesity. It remains unclear, whether increased leptin levels contribute to the prevailing insulin resistance in this risk group, or whether insulin resistance leads to hyperleptinemia. Both possibilities have already been described for T2DM associated with metabolic syndrome [42, 56].

We also demonstrated as a novel finding that plasma IGF-I was elevated in the risk phenotype unrelated to metabolic syndrome. Dysfunction of hGH/IGF-I signaling therefore probably is involved in both risk phenotypes for T2DM. In the one unrelated to metabolic syndrome, autonomic oversecretion of IGF-I may occur, suppress hGH secretion and detrimentally affect glucose metabolism [68].

Thus, dysregulation of leptin and hGH/IGF-I signaling is likely contributing to the pathophysiology of both risk phenotypes for T2DM. This information provides starting points for further mechanistic evaluations, in particular of the risk phenotype unrelated to metabolic syndrome, which is still incompletely understood.

6. Outlook

The high prevalence and predicted increase of T2DM globally, as well as the heterogeneous characteristics of this disease highlight the importance of new risk markers, early detection possibilities, and a subtype-specific pathophysiologic understanding. In my thesis, I specifically determined parameters that may be involved in the altered muscle adipose tissue crosstalk in metabolic syndrome on the one hand, and parameters that appear relevant in the T2DM risk phenotype unrelated to metabolic syndrome on the other hand. Further work can extend these observations in a mechanistic direction. Thereby, preventive and therapeutic interventions that are targeted to specific pathophysiologies can be developed.

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8. Published articles

8.1 Contribution to the publications

I contributed to the conceptualization of both publications presented in my thesis and created the original drafts (Kern-Matschilles et al. *Experimental and clinical endocrinology & diabetes* 2021: see section 8.2; Kern-Matschilles et al. *Hormone and metabolic research* 2022: see section 8.3). I further contributed to the formal analysis and visualization (Kern-Matschilles et al. *Experimental and clinical endocrinology & diabetes* 2021 and Kern-Matschilles et al. *Hormone and metabolic research* 2022). During OGTT and cardiopulmonary exercise testing, I collected and prepared blood, and measured anthropometric data (e.g. height, weight, body composition, and vital signs) (Kern-Matschilles et al. *Experimental and clinical endocrinology & diabetes* 2021 and Kern-Matschilles et al. *Hormone and metabolic research* 2022). For the publication in the journal *Experimental and clinical endocrinology & diabetes* I did the analysis of serum myostatin via an ELISA.

8.2 Kern-Matschilles et al. Experimental and clinical endocrinology & diabetes 2021

Title: Association of Serum Myostatin with Body Weight, Visceral Fat Volume, and High Sensitivity C-Reactive Protein But Not With Muscle Mass and Physical Fitness in Premenopausal Women

Authors: Stefanie Kern-Matschilles, Christina Gar, Lorena Wanger, Stefanie J. Haschka, Anne L. Potzel, Nina Hesse, Cornelia Then, Jochen Seissler, Andreas Lechner

Authors Contributions: Conceptualization, S.K.M, C.G., and A.L.; formal analysis, S.K.M. and A.L.; investigation S.K.M, C.G., L.W., S.H., A.L.P., C.T., and N.H.; data curation, C.G., A.L.; writing: original draft, S.K.M; writing: review and editing, all; visualization, S.K.M; supervision, J.S. and A.L.; project administration A.L.; funding acquisition A.L., J.S.; A.L. is the guarantor of this work.

Exp Clin Endocrinol Diabetes, 2021. 130(6): p. 393-399. DOI 10.1055/a-1500-4605

Article published online: 2021-08-18

Original Article Thieme

Association of Serum Myostatin with Body Weight, Visceral Fat Volume, and High Sensitivity C-Reactive Protein But Not With Muscle Mass and Physical Fitness in Premenopausal Women

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Key words
diabetes risk, myokines, adipose tissue, ergospirometry, MRI measurement of muscle mass

received 29.01.2021
revised 15.04.2021
accepted 03.05.2021
published online 18.08.2021

Bibliography
Exp Clin Endocrinol Diabetes 2022; 130: 393-399
DOI 10.1055/a-1500-4605
ISSN 0947-7349

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Supplementary Material is available under http://doi.org/10.1055/a-1500-4605

ABSTRACT

Background The myokine myostatin regulates muscle mass and has been linked to insulin resistance and metabolic syndrome. However, data on its role in humans is still limited. We, therefore, investigated the associations of serum myostatin with muscle mass, physical fitness, and components of the metabolic syndrome in a cohort of premenopausal women.

Methods We undertook a cross-sectional analysis of 233 women from the monocenter study PPSDiab, conducted in Munich, Germany. Participants had recently completed a pregnancy with or without gestational diabetes. Our analysis included medical history, anthropometrics, oral glucose tolerance testing, laboratory chemistry, cardiopulmonary exercise testing, and magnetic resonance imaging (n = 142) of visceral fat volume, left quadriceps muscle mass, and muscle fat content. Serum myostatin was quantified by a competitive enzyme-linked immunosorbent assay.

Results We observed positive correlations of serum myostatin with body mass index (ρ = 0.235; p = 0.0003), body fat percentage (ρ = 0.166; p = 0.011), waist circumference (ρ = 0.206; p = 0.002), intraabdominal fat volume (ρ = 0.182; p = 0.030) and high-sensitivity C-reactive protein (ρ = 0.175; p = 0.008). These correlations were reproduced in linear regression analyses with adjustment for age and time after delivery. We saw no correlations with muscle mass, physical fitness, insulin sensitivity, triglycerides, HDL cholesterol, and blood pressure.

Conclusions Our observation of elevated serum myostatin in women with a higher body fat percentage, visceral obesity, and elevated c-reactive protein suggests that this myokine contributes to the altered muscle-adipose tissue crosstalk in metabolic syndrome. Elevated myostatin may advance this pathophysiologic process and could also impair the efficacy of exercise interventions. Further mechanistic studies, therefore, seem warranted.

Introduction

Myostatin, also known as growth–differentiation factor 8 (GDF8), is a member of the transforming growth factor–β superfamily. Its name–giving function is the negative regulation of muscle cell proliferation [1]. Myostatin is expressed in and secreted from skeletal muscle and, to a lower extent, from adipose tissue [1–3]. Absence or blockage of myostatin increases muscle mass and reduces adipose tissue volume [1, 4]. However, these functions of myostatin have mainly been investigated in mouse models and human subjects with specific genetic mutations or severe concomitant conditions, such as end–stage renal disease [1, 4–7]. Data from healthy human subjects is still inconclusive.

Elevated serum or plasma myostatin has been linked to insulin resistance and metabolic syndrome in some previous studies. However, these have mainly included subjects with severe obesity in groups of less than 30 individuals [8–10]. Brandt et al., on the other hand, examined plasma and muscle myostatin in insulin–resistant individuals with type 2 diabetes in comparison to healthy controls in groups of 76 and 92 individuals, respectively [11]. In their study, plasma myostatin did not differ between the 2 groups. Only muscle myostatin mRNA was elevated with type 2 diabetes.

To help clarify the contradicting results of previous studies, we examined serum myostatin in a cohort of premenopausal women after a recent pregnancy with or without gestational diabetes (GDM). A preceding GDM identifies women at high risk for subsequent permanent diabetes. The underlying pathophysiology often includes insulin resistance and metabolic syndrome [12]. Therefore, we were able to cover a wide range of metabolic states and insulin sensitivity within this study cohort. In the same cohort, we had also demonstrated previously that physical fitness was lower in women after GDM than in women after a normoglycemic pregnancy [13].

The specific aims of this cross–sectional analysis were to examine the association of serum myostatin with physical fitness and skeletal muscle mass on the one hand and with obesity, insulin resistance, and other components of metabolic syndrome on the other hand. Insights into these associations could help to understand the role of myostatin in metabolism, specifically in the altered crosstalk between musculature and adipose tissue in metabolic syndrome [10, 14–16].

Materials and Methods

Study design

All data for this cross–sectional analysis were collected at the baseline visit of the Prediction, Prevention, and Subclassification of Type 2 Diabetes (PPSDiab) study. Detailed information on this study has been published previously [17]. In short, 304 women 3–16 months after a completed pregnancy were recruited from the Diabetes Center and the obstetrics department of the Ludwig–Maximilians–University Hospital in Munich, Germany (LMU Klinikum München). Women after GDM and women after a normoglycemic pregnancy were included in a 2:1 ratio. The main exclusion criteria for this study were alcohol or substance abuse, pre–pregnancy diabetes, and chronic diseases requiring systemic medication (except for medication related to hypothyroidism [n=52], mild hypertension [n=4], gastroesophageal reflux [n=2], and a history of pulmonary embolism resulting in Rivaroxaban prophylaxis [n=1]). GDM during the preceding pregnancy was diagnosed by a 75 g oral glucose tolerance test (oGTT) using the cut–off values of the International Diabetes and Pregnancy Study Groups recommendations [18]. The ethics committee of the medical faculty of the LMU approved the PPSDiab Study (ID: 300–11), and written informed consent was obtained from all participants.

Study cohort

At the baseline visit of PPSDiab, all women received an oGTT, anthropometric measurements, and questionnaires. They were also invited to participate in a cardiopulmonary exercise test and, on a separate day, a whole–body magnetic resonance imaging (MRI) test. These tests were not mandatory, and not all women participated due to time constraints or family obligations.

We excluded 16 of the 304 PPSDiab study participants from this analysis: 11 due to missing values, 2 because of newly diagnosed type 1 diabetes, 2 because of hyperthyroidism, and 1 because of an upper respiratory infection at the time of the relevant study visit. Of the remaining 288 women, 233 participated in cardiopulmonary exercise testing and had a valid test result. These women constituted the study cohort for this analysis. MRI measurements were available from 142 of these participants who thus constituted the MRI subcohort of this analysis (▶ **Fig. 1**).

oGTT

A 5–point, 75 g oGTT with measurements of plasma glucose and serum insulin was performed after an overnight fast, as described previously [17]. The insulin sensitivity index (ISI) according to Matsuda and deFronzo was calculated as: [10 000/(SQRT(glucose0' * insulin0' * (glucose0' * 15 + glucose30' * 30 + glucose60' * 30 + glucose90' * 30 + glucose120' * 15)/120 + (insulin0' * 15 + insulin30' * 30 + insulin60' * 30 + insulin90' * 30 + insulin120' * 15)/120))] [19].

In a previous study, we had already validated ISI against hyperinsulinemic–euglycemic clamp data in the PPSDiab cohort [17].

Anthropometrics and physical examination

Bodyweight, body fat content (as a percentage of body weight), and muscle mass were quantified using a bioelectrical impedance analysis (BIA) scale (Tanita BC–418; Tanita Corporation, Tokyo, Japan). Height and waist circumference were measured to the nearest centimeter. The body mass index (BMI) was calculated as weight (kg)/height (m)^2. Blood pressure readings were obtained in a seated position with repeated measurements on both arms, separated by at least 15 min. The average from the "higher" arm was recorded. The mean blood pressure was calculated as (diastolic value ×2 + systolic value)/3.

Biochemical measurements

Plasma glucose (Glucose HK Gen.3, Roche Diagnostics, Mannheim, Germany), serum insulin (CLIA, DiaSorin LIASON systems, Saluggia, Italy), high sensitivity c–reactive protein (hsCRP; wide–range CRP, Siemens Healthcare Diagnostics, Erlangen, Germany), and blood lipids (HDL cholesterol, triglycerides; enzymatic caloric test, Roche Diagnostics, Mannheim, Germany) were quantified in a central laboratory [13].

Myostatin serum levels were measured in duplicate by a competitive ELISA (myostatin ELISA; Immundiagnostik AG, Bensheim,

Germany) from serum samples taken after an overnight fast. The samples had been snap-frozen on dry ice immediately after centrifugation and had been stored at −80 °C until used for the ELISA. The assay measures total myostatin immunoreactivity. Its calibration curve has an optimal range from 0.3 to 83.3 ng/ml, and the inter- and intra-assay coefficients of variability of our measurements were in the reported target ranges (11% and 10%, respectively) [20].

Cardiopulmonary exercise testing

We conducted a cardiopulmonary exercise test until exhaustion on a bicycle ergometer (MasterScreen CPX; CareFusion, Höchberg, Germany) as described previously [13]. The workload was increased by 25 W every 3 min until exhaustion, where the maximum workload (W_{max}) was determined. Peak oxygen uptake (VO_{2peak}) was defined as the maximum oxygen uptake that was reached during workload. For a valid exercise test, a maximal respiratory exchange ratio (RER) of at least 1.05 had to be reached [13].

MRI

All MRI tests were conducted on a 3 Tesla system (Ingenia or Achieva; Philips Health Care, Hamburg, Germany). Intraabdominal visceral fat volume was quantified by a semi-manual segmentation method from a whole-body axial sequence using the 2-point Dixon technique (SliceOmatic image analysis software version 5.0 rev. 7, TomoVision, Magog, Canada). The left quadriceps muscle was chosen for muscle volume quantification [21], and the cross-sectional area at 40% of the femur length was measured [22]. The distance between the femoral head and the lateral condyle defined the femur length. Muscle volume (MV) was estimated by multiplying the cross-sectional area with muscle length and a shape factor [23].

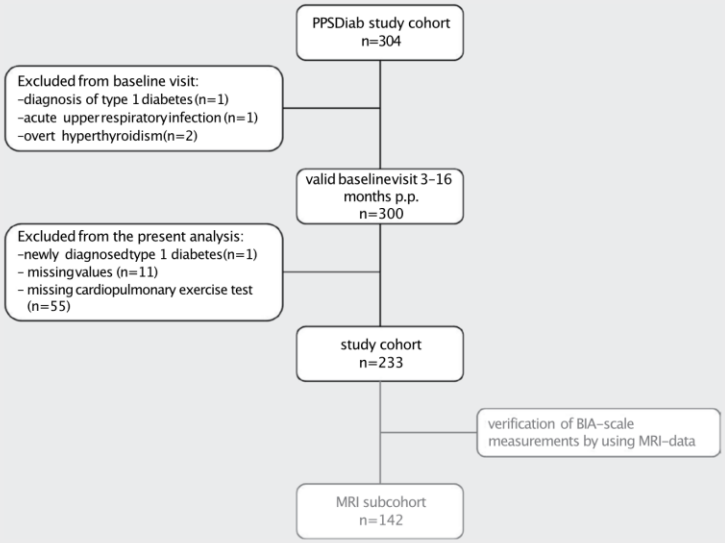
For intramyocellular lipid content, a single-voxel (^{1}H) magnetic resonance spectroscopy (MRS) of the left anterior tibial muscle was conducted by using a point resolved spectroscopy (PRESS) according to Torrianiand colleagues [24]. The spectroscopy was analyzed by using the jMRUI software (version 4.0, jMRUI Consortium, Brno, Czech Republic).

Statistics

Normally distributed, metric variables are presented as mean ± standard deviation, other metric variables as median (first quartile-third quartile). Categorical variables are shown as frequency (percent). Non-parametric Spearman correlation analyses were computed. For linear regression models, serum myostatin was logarithmized as the dependent variable to achieve a near-normal distribution. Three or more groups were compared applying the Kruskal-Wallis-Test followed by the Dwass, Steel, Critchlow-Fligner post-hoc procedure for pairwise comparisons. Comparisons of the MRI subcohort with the group of women who did not participate in this test (Suppl. ▶**Table 1**) were conducted using the Mann-Whitney-U Test for metric variables and the χ^2 or Fisher exact test for categorical variables. A p-value less than 0.05 was considered statistically significant. All analyses were done using SAS University Edition version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The baseline characteristics of the study cohort are listed in ▶**Table 1**. All 233 participants were premenopausal women after a recent pregnancy, which was complicated by GDM in 150 cases (64%). The time since delivery was 9.3 ± 2.8 months. Women with previous GDM had comparable serum myostatin to women after a normoglycemic pregnancy (44.5 ± 11.0 ng/ml vs. 44.6 ± 13.6 ng/ml; p = 0.75). At



▶**Fig. 1** Flow chart describing the study cohorts. MRI: magnetic resonance imaging.

▶**Table 1** Baseline characteristics of the study cohort (n=233).

Age [years]	35.9±4.1	
Time since delivery [months]	9.3±2.8	
Glucose tolerance status	NGT	178 (76%)
	IFG	21 (9%)
	IGT	19 (8%)
	IFG+IGT	10 (5%)
	T2D	5 (2%)
GDM in preceding pregnancy	yes	150 (64%)
	no	83 (36%)
Breast feading status	full	4 (2%)
	partial	86 (37%)
	no	143 (61%)
BMI [kg/m^2]	23.3 (21.2 –26.9)	
Waist circumference [cm]	78 (73 –86)	
Body muscle mass [kg]	44.2±4.6	
Body fat content [%]	31.6±7.8	
Systolic blood pressure [mmHg]	117.2±11.4	
Diastolic blood pressure [mmHg]	73.4±9.1	
Mean blood pressure [mmHg]	88.0±9.4	
Triglycerides [mg/dl]	67 (63 –72)	
HDL cholesterol [mg/dl]	62 (53 –90)	
ISI (missing n=1)	5.4 (3.6 –7.5)	
hsCRP [mg/dl]	0.05 (0.01 –0.14)	
W_{max} [W]	130±27	
VO_{2peak} [ml/min]	1856±367	
Myostatin [ng/ml]	44.5±11.9	
additional variables in the MRI subcohort (n=142)		
Intraabdominal fat volume [dm^3]	1.63 (1.06 –2.79)	
Quadriceps muscle volume [dm^3]	1.31 (1.16 –1.43)	
IMCL [%] (missing n=27)	0.93 (0.61 –1.44)	

NGT=normal glucose tolerance; IFG=impaired fasting glucose; IGT=impaired glucose tolerance; T2D=type 2 diabetes; GDM=gestational diabetes; BMI=body mass index; ISI=insulin sensitivity index; hsCRP=high sensitivity C-reactive protein; W_{max} =maximum workload; VO_{2peak} =peak oxygen uptake; IMCL=intramyocellular lipid content (Musculus tibialis anterior).

▶**Table 2** Correlations of selected parameters with serum myostatin level (n =233).

	ρ	p-value
BMI	**0.235**	**<0.001**
Body fat content	0.166	**0.011**
Waist circumference	0.206	**0.002**
Intraabdominal fat volume (n=142)	0.182	**0.030**
Body muscle mass	0.095	0.148
Quadriceps muscle volume (n=142)	−0.132	0.118
IMCL (n=115)	−0.031	0.745
W_{max}	−0.108	0.101
VO_{2peak}	0.021	0.752
ISI	−0.082	0.213
Triglycerides	0.0864	0.189
HDL cholesterol	−0.053	0.419
Mean blood pressure	0.068	0.300
hsCRP	0.175	**0.008**

BMI=body mass index; IMCL=intramyocellular lipid content (M. tibialis anterior); W_{max} =maximum workload; VO_{2peak} =peak oxygen uptake; ISI=Insulin sensitivity index; hsCRP=high sensitivity C-reactive protein; ρ=Spearman correlation coefficient; significant p-values are marked in bold.

the time of the study visit, 178 women (76%) were normoglycemic, 50 women (22%) had prediabetes, and 5 women (2%) had incident type 2 diabetes diagnosed by the study oGTT. Serum myostatin of women with prediabetes or diabetes did not differ from that of normoglycemic women (44.0 ±10.8 ng/ml vs. 44.7 ±12.3 ng/ml; p =0.78). Furthermore, breastfeeding status (full and partial vs. no) was not associated with changes in serum myostatin (44.6 ±11.4 ng/ml vs. 44.6 ±12.3 ng/ml; p =0.81). The women in the MRI subcohort had baseline characteristics comparable to the women who did not participate in this test (Suppl. ▶**Table 1**).

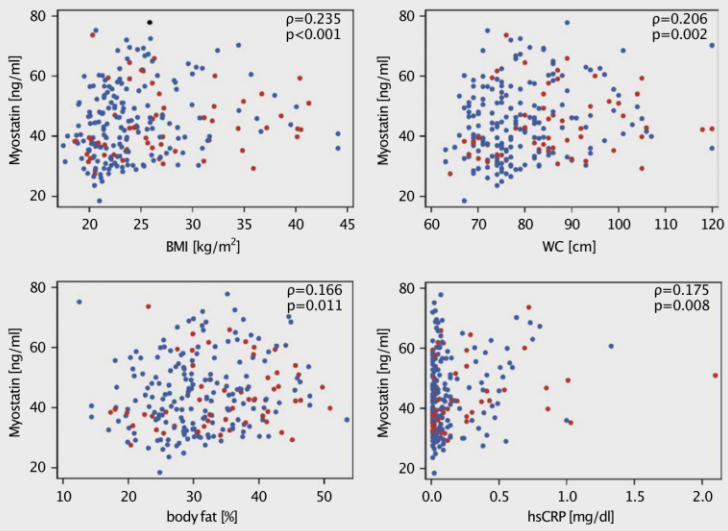
In the whole study cohort, serum myostatin correlated positively with BMI, waist circumference, body fat percentage, intraabdominal fat volume, and hsCRP. No correlations were observed between serum myostatin and body muscle mass, musculus quadriceps volume, VO_{2peak}, W_{max}, ISI, triglycerides, HDL cholesterol, and mean blood pressure (▶**Table 2**; ▶**Fig. 2**). In linear regression analyses adjusted for age and time since delivery, the associations of serum myostatin with BMI, body fat content, waist circumference, intraabdominal fat volume, and hsCRP remained significant (▶**Table 3**). ▶**Fig. 3** illustrates the serum myostatin concentration for waist circumference and hsCRP in combination. For this analysis, we grouped the study cohort according to the medians of these 2 parameters. Within the resulting 4 groups, only the one with both parameters below their median differed significantly from the one with both parameters above their median.

Discussion

In a cohort of premenopausal women, serum myostatin was associated with higher BMI and body fat percentage, visceral obesity (waist circumference and intraabdominal fat volume), and elevated hsCRP. However, it was not associated with physical fitness, muscle mass, insulin resistance, dyslipidemia, and hypertension.

Myostatin, visceral obesity, and metabolic inflammation

Our results are in line with previous human studies that examined severely obese individuals [9, 16]. However, they also extend previous work by demonstrating that less extreme amounts of body fat are similarly associated with higher serum myostatin. Furthermore, our findings suggest a connection of circulating myostatin specifically with visceral obesity and metabolic inflammation, exemplified by hsCRP in our study. These 2 components appeared to have additive effects. Due to the cross-sectional, observational design of our analysis, we cannot elucidate the physiology underlying these associations. Higher myostatin secretion from the musculature may support adipose tissue inflammation, as suggested by Dong et al. based on mouse studies [25]. Alternatively, visceral

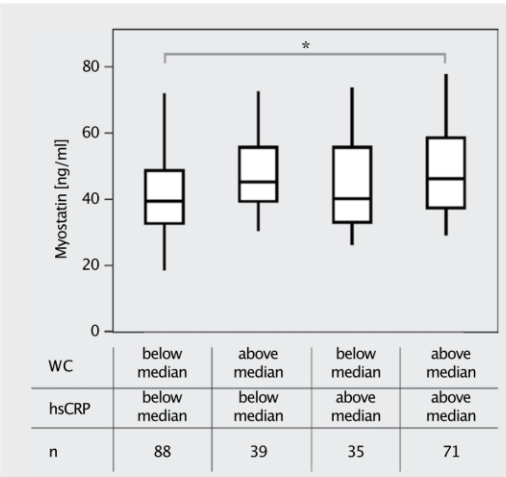


▶**Fig. 2** Scatter plots of the significant correlations of clinical parameters and serum myostatin in the whole study cohort (see also ▶**Table 2**). Blue dots indicate normoglycemia, red dots prediabetes (IFG and/or IGT), or type 2 diabetes.

▶**Table 3** Linear regression analyses with logarithmized serum myostatin as the dependent variable; each analysis adjusted for age and time since delivery (n=233).

	Standardized Beta-Coefficient	p-value
BMI	0.181	**0.006**
Body fat content	0.155	**0.018**
WC	0.200	**0.002**
hsCRP	0.199	**0.002**
Intraabdominal fat volume (n=142)	0.169	**0.048**

BMI=body mass index; WC=waist circumference; hsCRP=high sensitivity C-reactive protein; significant p-values are marked in bold.



▶**Fig. 3** Serum myostatin levels stratified by waist circumference and high-sensitivity C-reactive protein (hsCRP) below vs. above the respective median of the study cohort; Kruskal-Wallis-Testwith p=0.01; * indicates the only pairwise significance in the Dwass, Steel, Critchlow-Fligner post-hoc procedure.

adiposity could drive inflammation [26] and also a rise in serum myostatin, maybe via adipokines like follistatin [27]. This hypothesis is supported by data from Carvalho et al. who compared metabolically "healthy" versus "unhealthy" obese individuals. In their study, serum myostatin was only elevated in the metabolically "unhealthy" group [8].

Myostatin and insulin resistance

Despite serum myostatin correlating with some components of metabolic syndrome in our study, we did not observe a significant association with insulin resistance. Such an association has been demonstrated in previous analyses but only in severely obese individuals (BMI >40 kg/m² or ~ 35 kg/m²) [9, 28], which were underrepresented in our study cohort (3% and 7%, respectively).

Myostatin, muscle mass, and physical fitness

The link between a loss of function mutation in the myostatin gene or short-term pharmacologic use of myostatin antagonists and increased muscle mass has been demonstrated in mice and humans

[4, 5, 29–31]. Furthermore, serum myostatin is increased in sarcopenia associated with end-stage renal disease [6, 7]. Our study included a whole-body estimate of muscle mass from bioimpedance and direct quantification of left quadriceps muscle volume. However, for both parameters, we did not observe a correlation with serum myostatin. Similarly, we detected no correlation between serum myostatin and physical fitness and muscle fat content. These results are consistent with previous work [11, 32]. However, Carvalho et al., who also saw no correlation of myostatin with physical fitness, observed a negative correlation with muscle mass [8].

Our findings may indicate that circulating myostatin is not relevant as a regulator of adult muscle mass in premenopausal women. Alternatively, the serum level of the myokine may not accurately reflect its physiologically relevant concentration since myostatin circulates mainly in inactive states and only gets activated at its target sites [33]. In that case, tissue measurements would be needed to make an accurate assessment.

Strengths and limitations

The main strength of this work is its study cohort, which includes individuals with a broad range of metabolic states but is otherwise very homogeneous. Additionally, the sample size exceeded that of most previous studies and serum myostatin was quantified by a well-established assay. The most important limitation of this study is the potential difference between the measured serum myostatin concentration and its physiologically relevant concentration at its target sites. This phenomenon may obscure important links but is difficult to resolve in human cohort studies. An additional limitation is the cross-sectional nature of this analysis, which precludes the inference of cause-effect relationships. Finally, we did not estimate insulin sensitivity with the gold-standard technique of a hyperinsulinemic clamp. However, we had previously validated the oGTT-derived ISI with clamp data in the same cohort [17].

Conclusion

We observed elevated serum myostatin in association with higher body weight and body fat percentage, visceral obesity, and elevated hsCRP. These results suggest a role of myostatin in the altered muscle adipose tissue crosstalk in metabolic syndrome and related conditions in premenopausal women. Moreover, elevated serum myostatin could impair the efficacy of exercise interventions. Therefore, further mechanistic investigations of this myokine in the context of human metabolism seem warranted.

Funding

This work was funded by LMU Klinikum and Helmholtz Zentrum München, Germany, and the German Center for Diabetes Research.

Author Contributions

Conceptualization, S.K.M, C.G., and A.L.; formal analysis, S.K.M. and A.L.; investigation S.K.M, C.G., L.W., S.H., A.L.P., C.T., and N.H.; data curation, C.G., A.L.; writing: original draft, S.K.M; writing: review and editing, all; visualization, S.K.M; supervision, J.S. and A.L.; project administration A.L.; funding acquisition A.L., J.S.; A.L. is the guarantor of this work. This work was not published previously.

Data Availability

Data from the PPSDiab study are available from the corresponding author upon reasonable request.

Acknowledgments

We thank all participants in the PPSDiab Study, who made this work possible. We also thank Vanessa Sacco, Mandy Meisel, Irina Benz, Barbara Rauch, and Friederike Banning for their contribution to conducting the PPSDiab Study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary Material

Supplemental Table 1: Baseline characteristics of the MRI subcohort and the women who did not participate in the MRI test

		MRI subcohort (n=142)	MRI non-participants (n=91)	p-value
Age [years]		35.7 ±4.1	36.3 ±4.0	0.416
Time since delivery [months]		9.3 ±2.8	9.3 ±2.8	0.911
Glucose tolerance status	NGT	107 (75 %)	71 (78%)	0.839
	IFG	14 (10%)	7 (8%)	
	IGT	12 (8%)	7 (8%)	
	IFG+IGT	5 (4%)	5 (5%)	
	T2D	4 (3%)	1 (1%)	
GDM in preceding pregnancy	yes	93 (65%)	57 (63%)	0.657
	no	49 (35%)	33 (36%)	
BMI [kg/m^2]		23.6 (21.3 –27.1)	23.2 (21.1 –26.5)	0.556
Waist circumference [cm]		78 (72 – 87)	77 (73 – 85)	0.876
Body muscle mass [kg]		44.3 ±4.2	44.1 ±5.1	0.382
Body fat content [%]		31.9 ±8.1	31.1 ±7.4	0.503
Systolic blood pressure [mmHg]		116.2 ±10.8	118.6 ±12.2	0.159
Diastolic blood pressure [mmHg]		72.9 ±9.0	74.2 ±9.3	0.330
Mean blood pressure [mmHg]		87.3 ±9.1	89.0 ±9.8	0.241
Triglycerides [mg/dl]		69 (54 – 93)	63 (50 – 85)	0.128
HDL cholesterol [mg/dl]		63 (52 – 73)	61 (56 – 70)	0.590
ISI		5.2 (3.5 –7.5)	5.8 (3.8 –7.6)	0.387
hsCRP [mg/dl]		0.05 (0.02 –0.12)	0.05 (0.01 –0.2)	0.936
Myostatin [ng/ml]		45.1 ±12.4	43.6 ±11.3	0.385

NGT =normal glucose tolerance; IFG =impaired fasting glucose; IGT =impaired glucose tolerance; T2D=type 2 diabetes; GDM =gestational diabetes; BMI =body mass index; WC =waist circumference; BP =blood pressure; ISI =Insulin sensitivity index; hsCRP=high sensitivity C-reactive protein; Mann-Whitney-U test for metric and $\chi 2$ or Fisher exact test for categorial variables

8.3 Kern-Matschilles et al. Hormone and metabolic research 2022

Title: Altered Circulating Leptin, hGH, and IGF-I in Prediabetes and Screening-Diagnosed T2DM Unrelated to Metabolic Syndrome in Women Post Gestational Diabetes

Authors: Stefanie Kern-Matschilles, Christina Gar, Katharina Schilbach, Stefanie Julia Haschka, Barbara Rauch, Cornelia Then, Jochen Seissler, Martin Bidlingmaier, Andreas Lechner

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Horm Metab Res, 2022. 54(9): p. 613-619. DOI 10.1055/a-1850-5392

Accepted Manuscript online: 2022-05-12 Article published online: 2022-07-12

Original Article: Endocrine Care

Thieme

Altered Circulating Leptin, hGH, and IGF-Iin Prediabetes and Screening-DiagnosedT2DM Unrelated to Metabolic Syndrome in Women Post Gestational Diabetes

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Key words
insulin resistance, lean risk phenotype, subclassification, insulin secretion

received 10.02.2022
accepted after revision 12.05.2022
published online 12.07.2022

Bibliography
Horm Metab Res 2022; 54: 613-619
DOI 10.1055/a-1850-5392
ISSN 0018-5043

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ABSTRACT

Recently, we proposed two pathophysiologic subtypes of type 2 diabetes mellitus (T2DM), one related and one unrelated to metabolic syndrome. To begin to understand the pathophysiology of the subtype unrelated to metabolic syndrome, we now measured selected hormones and signaling molecules in affected individuals. In this cross-sectionalanalysis, we examined 138 women out of the monocenter, post gestational diabetes study PPSDiab. Of these women, 73 had prediabetes or screening-diagnosed T2DM, 40 related to metabolic syndrome and 33 unrelated. The remaining 65 women were normoglycemic controls. Our analysis included medical history, anthropometrics, oral glucose tolerance testing, laboratory chemistry, and cardiopulmonary exercise testing. In addition, plasma proinsulin/insulin ratio, growth hormone (hGH) nadir during oral glucose tolerance testing, Insulin-likeGrowth Factor I (IGF-I), Leptin, Resistin, Adiponectin, Fetuin-a, FGF21, and myostatin were measured. Compared to controls, women with prediabetes or screening-diagnosedT2DM unrelated to metabolic syndrome depicted higher plasma Leptin [10.47(6.6-14.57) vs. 5.52(3.15-10.02); p <0.0001] and IGF-I [193.01(171.00-213.30) vs. 167.97(138.77-200.64); p =0.0008], as well as a lower hGH nadir [0.07(0.05-0.15) vs. 0.14(0.08-0.22; p <0.0001]. These differences were independent of body adiposity. Women with prediabetesor T2DMrelated to metabolic syndrome, in comparison to controls, displayed elevated Leptin, Fetuin-a, and FGF21, as well as reduced Adiponectin and hGH nadir. Based on our study, altered Leptin and hGH/IGF-Isignaling could potentially contribute to the pathophysiology of prediabetes and T2DM unrelated to metabolic syndrome. Further mechanistic investigations of these signaling pathways in the context of lean T2DM are necessary to test causal relationships.

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous disease. To advance its subclassification, we recently proposed two pathophysiologic subtypes of T2DM, one related and one unrelated to metabolic syndrome. We based this proposition on two studies. First, the monocenter, post gestational diabetes study PPSDiab, where we mainly examined prediabetic women. And second, the White-

hall II Study, where we analyzed all incident cases of T2DM [1]. In both studies, the T2DM subtype related to metabolic syndrome exhibited, as expected, ectopic fat disposition, metabolic inflammation, and dyslipidemia. Insulin resistance in this subtype was severe. The T2DM subtype unrelated to metabolic syndrome, on the other hand, displayed no ectopic fat, no metabolic inflammation, and no dyslipidemia. In this subtype insulin resistance fell in the middle between healthy control subjects and T2D patients related to metabolic syndrome. Simultaneously, insulin secretion was low. This suggested inadequate adaption of insulin secretion to the underlying insulin resistance in this subtype. Other than glucose metabolism, the only differences of this subtype to healthy, lean controls in the PPSDiab study were higher fasting non-esterified fatty acids (NEFA) and a lower physical fitness. Further details of the T2DM pathophysiology unrelated to metabolic syndrome are still unknown.

The objective of the present analysis was to foster pathophysiologic understanding of T2DM unrelated to metabolic syndrome by examining several hormones and signaling molecules in the same PPSDiab cohort as in our previous publication [1]. All these hormones and signaling molecules have established connections to T2DM in the literature. Specifically, we now determined the proinsulin to insulin ratio, for which elevated levels were suggested to indicate early beta cell failure [2]. We also examined human growth hormone release (hGH) by analyzing the hGH nadir during oral glucose tolerance testing (OGTT) [3]. This nadir can be affected by hyperinsulinemia and is significantly increased in conditions related to increased insulin resistance, like acromegaly [3, 4]. Additionally, we measured Insulin-like Growth Factor I (IGF-I), which may be involved in the development of insulin resistance [5]. Of the hepatokines, we analyzed fibroblast growth factor 21 (FGF21) and Fetuin-a. FGF21 gained attention in 2005 when positive effects on lipid and glucose metabolism in obese diabetic mice were discovered [6]. Fetuin-a, on the other hand, was suggested as a marker for T2DM development [7, 8]. Among the adipokines, we measured Leptin, Resistin, and Adiponectin. Leptin is mainly involved in the regulation of satiety and correlates with fat storage [9]. However, high plasma Leptin also associates with insulin resistance, conceivably due to resistance to Leptin itself [10, 11]. Resistin was found in rodent studies to be proinflammatory, upregulated in obesity, and involved in insulin resistance [12]. Adiponectin has antidiabetic properties, as it is involved in the lowering of blood glucose, weight loss, and fatty-acid oxidation in muscle [13, 14]. Finally, since it is known that low physical fitness is another risk factor for T2DM development [15], we also tested the myokine myostatin. This negative regulator of muscle mass might link reduced physical fitness to glucose metabolism [16]. Our research hypothesis was that alterations in any of these signaling pathways could suggest potential pathophysiologic processes warranting further exploration.

Subjects and Methods

Study design and cohort

The study cohort for this analysis is the same PPSDiab cohort as in the previous publication of Rottenkolber and colleagues [1] and has been described there in detail. In brief, the cohort consists of women consecutively recruited 3–16 months after a pregnancy, either with GDM or with normoglycemia. Recruitment took place between 2011 and 2016 in Munich, Germany. All data shown here is from the baseline visit of the study. PPSDiab was approved by the ethics committee of the Medical Faculty of the Ludwig Maximilians University in Munich, Germany. All participants provided written informed consent.

Groups in this analysis

Three groups of women were included in the present analysis.

Control (n =65)
- normoglycemic in the study oral glucose tolerance test (OGTT)
- 0 points for metabolic syndrome according to the NCEP ATP3 score [17]
- that is, waist circumference <88cm, hdl cholesterol ≥50mg/dl, Triglycerides <150mg/dl, blood pressure <130/85mmHg, fasting plasma glucose <5.6mmol/l; all without medication
- normoglycemic during preceding pregnancy

Pre0 (n =33)
- impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM in the study OGTT according to the criteria of the American Diabetes Association [18].
- 0 points for metabolic syndrome except for the presence of hyperglycemia
- GDM during the preceding pregnancy

Pre≥1 (n=40)
- impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM in the study OGTT
- At least one additional point for metabolic syndrome beyond the presence of hyperglycemia
- GDM during the preceding pregnancy

OGTT

After an overnight fast, we conducted a 5-point, 75 g OGTT with measurements of plasma glucose and serum insulin. The insulin sensitivity index (ISI) was calculated from the OGTT according to Matsuda and DeFronzo [19]. The rise of serum insulin from 0′ to 30′ in the OGTT (Δins 30′), as an approximation of early insulin release [7], was calculated as: $\Delta ins\ 30' = insulin\ 30' - insulin\ 0'$

The disposition index (DI) was calculated according to Kahn et al. as [20]: $DI = ISI * \Delta ins\ 30'$

The hGH nadir was defined as the lowest of the 4 hGH values after the ingestion of glucose [3].

Biochemical measurements

Plasma glucose (Glucose HK Gen.3, Roche Diagnostics, Mannheim, Germany), serum insulin (CLIA, DiaSorin LIASON systems, Saluggia, Italy), and blood lipids (high density lipoprotein (HDL) cholesterol and Triglycerides; enzymatic caloric test, Roche Diagnostics, Mannheim, Germany) were quantified in a central laboratory [15]. Further, serum proinsulin (ELISA; Mercodia Immunoassays & Services, Sweden), plasma Fetuin-a (ELISA, BioVendor, Heidelberg,

Germany), plasma Adiponectin (RIA, Merck Millipore, Darmstadt, Germany), plasma Leptin (ELISA "Dual Range", Merck Millipore, Darmstadt, Germany), plasma Resistin (Quantikine ELISA; R & D Systems, Wiesbaden-Nordenstadt, Germany), serum myostatin (ELISA; Immundiagnostik AG, Bensheim, Germany), serum FGF21 (ELISA; R & D Systems, Minneapolis, Minnesota), serum hGH (CLIA, IDS-iSYS, Immunodiagnostic Systems, Boldon, UK), and serum IGF-I (CLIA, IDS-iSYS, Immunodiagnostic Systems, UK) were determined. Serum and plasma samples were taken in the OGTT context. Immediately after blood collection the samples were centrifuged, snap-frozen on dry-ice and stored at -80 °C until further processing.

Anthropometric measurements

A bioimpedance scale (Tanita BC-418; Tanita Corporation) was utilized to determine the body mass index (BMI), body fat, and muscle mass. Waist circumference was measured to the nearest 1 cm. Blood pressure was obtained twice in a seated position.

Cardiopulmonary exercise testing

For the evaluation of the physical fitness (maximum workload (max. load) and peak oxygen uptake per kilogram body weight (VO_2 peak/body weight), a cardiopulmonary exercise test was conducted until exhaustion, as described in detail previously [15]. Briefly, workload was increased by 25 W every three minutes until exhaustion, when maximum workload (max.load) and peak oxygen uptake (VO_2 peak) were determined [15]. Not all women participated in this test due to time constraints or family obligations (missing n = 26).

Statistics

Metric, near-normally distributed variables are given as mean ± standard deviation, non-normally distributed variables as median (first quartile-third quartile) and categorical variables as frequency (%). For group comparisons, we applied the Kruskal-Wallis test followed by the Dwass, Steel, Critchlow-Fligner post hoc procedure. In addition, logistic regression models were computed. For correlations, non-parametric Spearman coefficients were calculated. A p-value <0.05 was considered statistically significant. All statistical calculations were performed using SAS statistical software package, version 9.4 (SAS Institute, Cary, NC, USA). Figs. were created using Tableau 2020.3 (Tableau Software, Seattle, WA, USA).

Results

First, we compared the 3 groups of women in this study (▶**Table 1**). The clinical parameters in the top part of the table have been reported previously [1]. They are shown again here to illustrate the distinctions between the 3 groups. The novel data on hormones and signaling molecules is listed in the bottom part of the table. All values are fasting, except for the hGH nadir derived from the OGTT. In Pre0 compared to Control, Leptin and IGF-I were higher [Leptin 10.47 (6.6-14.57) vs. 5.52 (3.15-10.02); p <0.0001], [IGF-I 193.01 (171.00-213.30) vs. 167.97 (138.77-200.64); p = 0.0008], whereas hGH nadir was lower [0.07 (0.05-0.15) vs. 0.14 (0.08-0.22); p <0.0001]. In Pre ≥ 1 compared to Control, Leptin, FGF21, and Fetuin-a were higher whereas Adiponectin and hGH nadir were lower (▶**Table 1**). In Pre ≥ 1 compared to Pre0, Leptin was higher [18.98 (12.54-25.99) vs. 10.47 (6.6-14.57)], whereas Adiponectin and IGF-I were lower [Adiponectin 7.45 (5.56-9.13) vs. 12.84 (9.35-13.86)], [IGF-I 148.10 (126.91-186.28) vs. 193.01 (171.00-213.30)]. The proinsulin/insulin ratio, Resistin and Myostatin did not differ between the 3 groups.

Next, we examined correlations with clinical parameters for the 6 hormones and signaling molecules displaying group differences in our comparison. In unadjusted analyses in the whole study cohort (▶**Fig. 1a**), Adiponectin, Fetuin-a, FGF21, hGH nadir, IGF-I, and Leptin displayed multiple correlations to clinical parameters. After adjustment for body fat (▶ **Fig. 1b**), most of these correlations were no longer significant. The exceptions were the positive correlation of Adiponectin with HDL cholesterol (ρ = 0.337, p = 0.0003), the positive correlation of hGH nadir with ISI (ρ = 0.230, p = 0.015), the positive correlations of IGF-I with fasting glucose (ρ = 0.269, p = 0.004), fasting insulin (ρ = 0.251, p = 0.008), and HOMA-IR (ρ = 0.275, p = 0.003), as well as the negative correlations of IGF-I with ISI (ρ = -0.217, p = 0.022) and waist circumference (ρ = -0.189, p = 0.047). Additionally, the positive correlations of Leptin with fasting glucose (ρ = 0.283, p = 0.003), fasting insulin (ρ = 0.524, p <0.0001), HOMA-IR (ρ = 0.548, p <0.0001), Triglycerides (ρ = 0.247, p = 0.009), BMI (ρ = 0.332, p = 0.0004), and waist circumference (ρ = 0.223, p = 0.019), as well as the negative correlations of Leptin with max. load (ρ = -0.287, p = 0.002), VO_2 peak (ρ = -0-220, p = 0.020), ISI (ρ = -0-523, p <0.0001), and DI (ρ = -0.225, p = 0.018) remained significant.

Additionally, we analyzed correlations with clinical parameters for the 3 hormones displaying differences between Controland Pre0 only in these two groups. In unadjusted analyses in Controland Pre0 combined (▶**Fig. 2a**), hGH nadir, IGF-I, and Leptin displayed multiple correlations to clinical parameters. After adjustment for body fat (▶**Fig. 2b**), the positive correlations of hGH nadir with ISI (ρ = 0.319, p = 0.003) and DI (ρ = 0.281, p = 0.010), the negative correlations of hGH nadir with fasting glucose (ρ = -0 to 311, p = 0-004), fasting insulin (ρ = -0 to 240, p = 0.030), and HOMA-IR (ρ = -0.268, p = 0.014), the positive correlations of IGF-I with fasting glucose (ρ = 0.323, p = 0.003), fasting insulin (ρ = 0.375, p = 0.0005), HOMA-IR (ρ = 0.386, p = 0.0003), and Triglycerides (ρ = 0.221, p = 0.045), as well as the negative correlation of IGF-I with ISI (ρ = -0.397, p = 0.0002) remained significant. Significance was also maintained for the positive correlations of Leptin with fasting glucose (ρ = 0.363, p = 0.0007), fasting insulin (ρ = 0.565, p <0.0001), HOMA-IR (ρ = 0.595, p <0.0001), Triglycerides (ρ = 0.228, p = 0.038), and BMI (ρ = 0.353, p = 0.001), as well as for the negative correlations of Leptin with max. load (ρ = -0.388, p = 0.0003), VO_2 peak (ρ = -0.230, p = 0.006), ISI ρ = 0.562, p <0.0001), and DI (ρ = -0.280, p = 0.010).

Finally, we tested the independent capacity of hGH nadir, IGF-I, and Leptin to discriminate between Controland Pre0 in logistic regression models adjusted for age and body fat, age and BMI, as well as age and waist circumference (▶**Table 2**). In these models, all 3 hormones displayed independent discriminatory capacity.

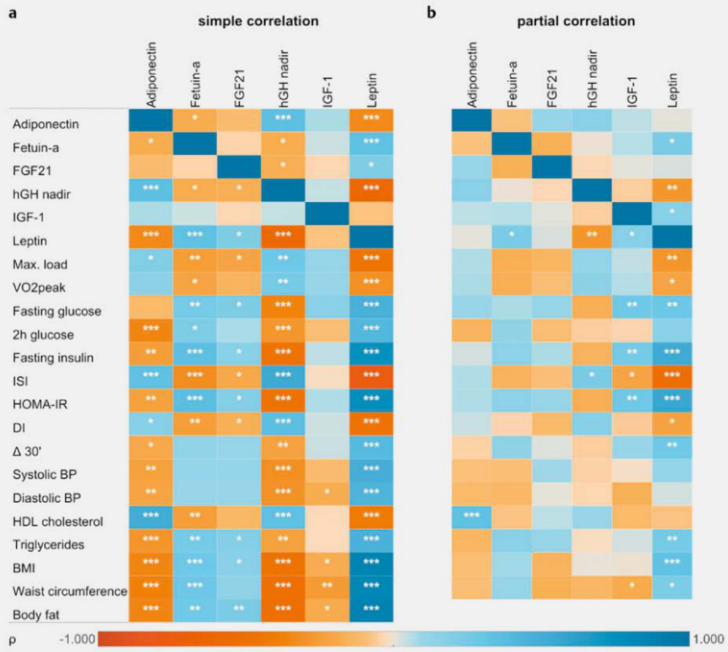
Discussion

The main findings of this study were that the Pre0 group, representing prediabetes and screening-diagnosed T2DM unrelated to metabolic syndrome, displayed higher IGF-I and Leptin, as well as lower

Table 1 Comparison of the 3 study groups.

Group	Control	Pre0	Pre≥1	p-value	Significant post-hocgroup comparisons
n	65	33	40		
Age [years]	35.5±4.1	36.2±3.9	35.7±5.2	0.70	-
Time since delivery [month]	9.5±2.6	10.2±3.2	8.8±3.0	0.21	-
Glucose tolerance status					
NGT	65 (100%)	-	-	-	-
IFG		19 (57.6%)	12 (30.0%)		
IGT		9 (27.3%)	14 (35.0%)		
IFG+IGT		4 (12.1%)	8 (20.0%)		
T2DM		1 (3.0%)	6 (15.0%)		
Systolic BP [mmHg]	110.57±8	113.18±8.06	127.13±12.69	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
Diastolic BP [mmHg]	68.09±6.81	70.03±6.27	80.15±9.95	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
BMI [kg/m²]	22.3±2.4	22.8±2.8	32.5±6.3	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
Waist circumference [cm]	74.4±5.4	76.6±6.5	94.6±11.2	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
Body fat [%]	27.87±5.76	29.17±6.95	40.54±6.71	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
Fasting PG [mmol/l]	4.9 (4.6-5.1)	5.7 (5.4-5.8)	5.7 (5.4-5.9)	<0.0001	Control vs. Pre0, Control vs. Pre ≥ 1
2h PG [mmol/l]	4.9 (4.4-5.9)	6.8 (5.8-8.3)	8.3 (7.1-10.1)	<0.0001	all
Fasting insulin [µU/ml]	5.3 (4-6.6)	8.1 (6.3-12.9)	12.55 (9-17.95)	<0.0001	all
Fasting proinsulin [pmol/l]	3.49 (2.81-4.61)	5.47 (3.52-8.31)	9.41 (5.38-13.51)	<0.0001	all
ISI	7.57 (5.72-9.25)	4.12 (2.98-5.12)	2.6 (1.95-3.61)	<0.0001	all
HOMA-IR	1.1 (0.81-1.44)	2.05 (1.42-3.38	3.06 (2.4-4.52)	<0.0001	all
DI	301.47 (241.4-363.09)	153.37 (131.43-203.83)	158.41 (100.25-205.41)	<0.0001	Control vs. Pre0, Control vs. Pre ≥ 1
Δins 30′	37.5 (29.3-53.8)	36.5 (29.4-67.9)	57.75 (35.35-82)	0.015	Control vs. Pre ≥ 1
HbA1c [mmol/mol]	33±4	36±4	38±6	<0.0001	Control vs. Pre0, Control vs. Pre ≥ 1
TG [mg/dl]	59 (50-72)	68 (57-77)	111 (79.5-172)	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1,
HDL-C [mg/dl]	67.02±13.58	61.85±13.02	51.55±11.65	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
hormonal contraception [n %]	8 (12.31)	3 (9.09)	9 (22.5)	0.213	-
Physical fitness: max. load [W] (missing n=26)	144.0±27.2	113.8±24.0	118.1±23.1	<0.0001	Control vs. Pre0, Control vs. Pre ≥ 1
Physical fitness: VO_2 peak/body weight [ml/min*kg] (missing n=26)	1976.1±404.9	1678.5±309.8	1769.9±304.2	0.001	Control vs. Pre0, Control vs. Pre ≥ 1
Hormones and signaling molecules					
Leptin [ng/ml]	5.52 (3.15-10.02)	10.47 (6.6-14.57)	18.98 (12.54-25.99)	<0.0001	all
Adiponectin [µg/ml]	11.63 (9.78-14.8)	12.84 (9.35-13.86)	7.45 (5.56-9.13)	<0.0001	Control vs. Pre ≥ 1, Pre0 vs. Pre ≥ 1
FGF21 [pg/ml] (missing n=1)	205.21 (126.46-355.99)	262.78 (158.81-329.65)	301.92 (193.26-472.73)	0.039	Control vs. Pre ≥ 1
Fetuin-a [µg/ml]	227.17±25.27	243.06±36.42	259.65±48.64	0.0004	Control vs. Pre ≥ 1
Resistin [ng/ml]	7.06±2.86	6.65±2.18	8.46±4.57	0.287	-
Myostatin [ng/ml] (missing n=1)	43.23±12.7	44.04±11.75	43.84±10.04	0.834	-
hGH nadir [µg/l]	0.14 (0.08-0.22)	0.07 (0.05-0.15)	0.07 (0.04-0.12)	<0.0001	Control vs. Pre0, Control vs. Pre ≥ 1
IGF-I [ng/ml]	167.97 (138.77-200.64)	193.01 (171.00-213.30)	148.10 (126.91-186.28)	0.0008	Control vs. Pre0, Pre0 vs. Pre ≥ 1
Proinsulin/insulin-ratio	0.76±0.34	0.84±0.89	0.8±0.33	0.375	-

Near-normallydistributed values are given as mean ±SD, non-normallydistributed values as median (Q1-Q3) and categorical variables as frequency (%). p-Valuefor group comparison with Kruskal-Wallis test; pairwise significance then determined with the Dwass, Steel, Critchlow-Fligner post hoc procedure. NGT: Normal glucose tolerance; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; T2DM: Type 2 diabetes mellitus; BP: Blood pressure; PG: Plasma glucose; ISI: Insulin sensitivity index; DI: Disposition indexcalculated from OGTT; Δins 30′: Rise of serum insulin during the first 30 minutes of the OGTT; TG: Triglycerides; HDL-C: HDL-cholesterol; Max. load: Maximal workload during cardiopulmonary exercise testing; VO_2 peak: Peak oxygen uptake during cardiopulmonary exercise testing.



▶**Fig. 1** Heatmap of Spearman correlations of Adiponectin, Fetuin-a, FGF21, hGH nadir, IGF-Iand Leptin with clinical parameters in the whole study cohort. **a**: Simple, unadjusted correlations (n=138); **b**: Partial correlations adjusted for body fat (n=112). p-Values:*<0.05, **<0.01, ***<0.001. Max. load: Maximal workload during cardiopulmonary exercise testing; VO_2 peak: Peak oxygen uptake in cardiopulmonary exercise testing; ISI: Insulin sensitivity index; HOMA-IR: Homeostatic model assessment of insulin resistance; DI: Disposition index; IR30: First phase insulin release; BP: Blood pressure.

hGH nadir than the Controlgroup. This occurred independently of measures of body adiposity. The Pre ≥ 1group, representing prediabetes and T2DM related to metabolic syndrome, on the other hand, featured the expected higher levels of Leptin, FGF21, and Fetuin-a, as well as lower levels of Adiponectin and hGH nadir.

Prediabetes and T2DM related to metabolic syndrome

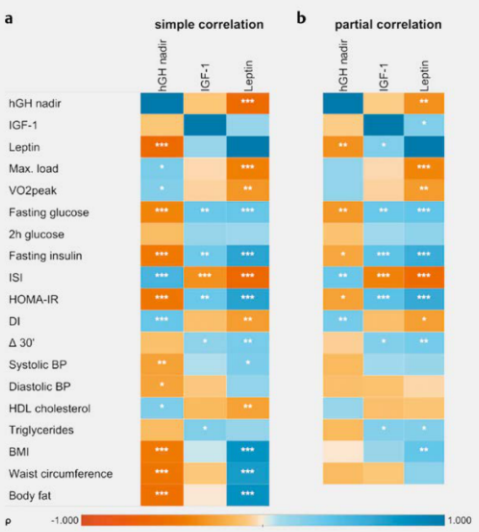
The pattern of hormones and signaling molecules in the Pre ≥ 1group mirrorsthe classic pathophysiology of prediabetesand T2DM in overweight and obese individuals. In conjunction with increased adiposity, ectopic fat deposition, and dyslipidemia, Leptin, FGF21, and Fetuin-aare elevated, whereas Adiponectin is reduced [11, 21–24]. Furthermore, the hGH nadir in the OGTT is lower and a trend towards lower levels of IGF-Ican be observed. These findings are replicated in correlation analyses (▶**Fig. 1**) and correspond to previous work demonstrating a reduced activity of the growth hormone axisin individuals with obesity and metabolic syndrome [3, 25, 26].

Prediabetes and T2DM unrelated to metabolic syndrome

First of all, the pattern of hormones and signaling molecules in the Pre0group supports our proposition that another pathophysiologic processthan in metabolic syndrome-relatedT2DM is at play here. This is exemplified by the unaltered levels of FGF21, Fetuin-a, and Adiponectin in Pre0in conjunction with the absence of hepatic steatosis we have shown previously [1]. Additionally, our findings suggest two hormone axesthat may contribute to insulin resistance and pathologic glucose metabolism in the T2DM subtype unrelated to metabolic syndrome. These are Leptin and hGH/IGF-Isignaling.

Elevated plasma Leptin in Pre0occurs independently of body fat content and other markers of body adiposity but correlates with reduced physical fitness (▶**Fig. 2**b; **Table**▶**2**). These findings are in agreement with a previous publication by Wang et al., who saw increased Leptin levels in a lean T2DM group compared to healthy controls [27]. In Pre0, Leptin did not reach the level of Pre ≥ 1but was still twice as high as in Control.This could suggest central Leptin resistance also in this lean risk group, which, in turn, could contribute to this group's insulin resistance. Alternatively, insulin-resistant adipose tissue could cause hyperleptinemia. Both options have also been suggested for metabolic syndrome-related T2DM [11, 28] and further studies are necessaryto sort through these alternatives in the lean subtype. Additionally, the influence of changes in physical fitness should be evaluated.

hGH/IGF-Isignaling in Pre0seems to be altered in a different way than in Pre ≥ 1.Whereas hGH nadir is reduced to a similar extent in both groups, IGF-Iis elevated in Pre0but as mentioned above, de-



▶**Fig. 2** Heatmap of Spearman correlations of hGH nadir, IGF-I and Leptin with clinical parameters in Controland Pre0, combined. **a**: Simple, unadjusted correlations (n=98); **b**: Partial correlations adjusted for body fat (n=84). p-Values:*<0.05, **<0.01, ***<0.001. Max. load: Maximal workload during cardiopulmonary exercise testing; VO_2 peak: Peak oxygen uptake in cardiopulmonary exercise testing; ISI: Insulin sensitivity index; HOMA-IR: Homeostatic model assessment of insulin resistance; DI: Disposition index; IR30: First phase insulin release; BP: Blood pressure.

▶**Table 2** Logistic regression analyses for the discrimination between Pre0 and Control(n=98).

Independent variable	OR (95% CI)	p-Value	ROC-AUC
Models adjusted for age, time since delivery, and body fat			
Leptin	4.82 (1.76–13.19)	0.002	0.719
hGH nadir	0.31 (0.14–0.69)	0.004	0.703
IGF-I	1.02 (1.01–1.03)	0.006	0.709
Models adjusted for age, time since delivery, and waist circumference			
Leptin	2.73 (1.19–6.24)	0.018	0.703
hGH nadir	0.35 (0.16–0.75)	0.007	0.703
IGF-I	1.02 (1.01–1.03)	0.006	0.704
Models adjusted for age, time since delivery and BMI			
Leptin	4.10 (1.61–10.41)	0.003	0.731
hGH nadir	0.32 (0.15–0.68)	0.003	0.703
IGF-I	1.02 (1.01–1.03)	0.008	0.699

picts a trend towards a reduction versus Controlin Pre ≥ 1. In correlation analyses only in the study participants without signs of metabolic syndrome (▶**Fig. 2**), IGF-Iexhibits positive associations with fasting insulin, fasting glucose, and insulin resistance (low ISI, high HOMA-IR). Therefore, taken together, a generally lower activity of the hGH/IGF-I axis can be assumed for Pre ≥ 1, but some form of oversecretion of IGF-I may occur in Pre0. In that case, hGH secretion might be suppressed in this group by a negative feedback loop [29]. A recent Mendelian randomization study, which found evidence for a causal role of elevated IGF-I in the development of T2DM, could support this model [30]. This analysis in the UK Biobank was based on 416 SNPs which associated with IGF-I levels [30, 31]. Additionally, our observations regarding IGF-I in the two pathophysiologic subtypes of T2DM may help explain results of Friedrich et al., who showed a U-shaped association of IGF-I and insulin resistance within the normal range of IGF-I [5]. Unlike us, Cao and colleagues found lower IGF-I levels in lean individuals with T2DM (BMI 22.37 ± 1.71), but this group, despite being lean, displayed other features of metabolic syndrome, namely elevated triglycerides and reduced HDL cholesterol [32]. Therefore, this previous study does not contradict our observations.

Strengths and limitations

This study relies on a well-characterized cohort with a low number of confounding factors. However, it remains unclear to what extent our results can be generalized because we only examined premenopausal women and most of the women in the case groups were still in the prediabetic stage. Furthermore, the measurements of hormones and signaling molecules reported here only offer first hints at the pathways potentially involved in prediabetes and T2DM unrelated to metabolic syndrome, but not yet mechanistic explanations. No cause-effect relationships can be inferred from this cross-sectional analysis. Additional, more detailed studies are therefore required.

Conclusions

We have demonstrated here that dysregulation of Leptin and hGH/IGF-I signaling occurs in prediabetes and screening-diagnosed T2DM unrelated to metabolic syndrome. These signaling pathways could therefore potentially contribute to the yet unknown pathophysiology of this disease subtype. At least, they warrant further exploration in this context.

Acknowledgements

Acknowledgments We thank all participants in the PPSDiab Study, who made the study possible. We also thank Vanessa Sacco, Mandy Meisel, Irina Benz, and Friederike Banning for their contribution to conducting the study. Funding: This work was funded by LMU Klinikum, Helmholtz Zentrum München, the German Center for Diabetes Research, and by a grant from the Friedrich Baur Stiftung to C.G. Author Contributions: Conceptualization S.K.M., C.G., and A.L.; Formal Analysis, S.K.M., C.G., and A.L.; Investigation S.K.M., C.G., K.S., S.H, B.R., and C.T.; Data Curation, C.G. and A.L.; Writing -Original Draft, S.K.M.; Writing - Review & Editing, all; Visualization, S.K.M. and C.G.; Supervision, A.L.; Project Administration, A.L.; Funding Acquisition A.L., J.S., and C.G.; A.L. is the guarantor of this work. This work was not published previously. Conflict of in-

terest: The authors declare no conflicts of interest. Data availability: Data from the PPSDiab study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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9. Summary

9.1 Summary of this thesis

Type 2 diabetes mellitus (T2DM) is a common, heterogeneous disease with a worldwide increase in prevalence. As a result, this disease represents an enormous burden on both the individuals affected by it and the healthcare system. T2DM continuously progresses over time, making the identification of individual risk as important as an early diagnosis in order for preventive actions to be effective. However, it is the early forms of T2DM in particular, which remain unnoticed over a long period of time, one reason for this being that the affected often include young people, who do not fit the “classic” phenotype.

The overall objective of my thesis is to shed light on the underlying pathophysiology of T2DM, as well as its risk phenotypes. To this end, we conducted a variety of studies on a T2DM risk cohort, selected specifically for our large-scale project “Prediction, Prevention and Subclassification of T2DM” (PPSDiab). This cohort included postpartum women diagnosed with gestational diabetes (GDM) during the index pregnancy (i.e., their most recent pregnancy) and women with normoglycemic index pregnancy in a 2:1 ratio. Given that women post GDM are at increased risk for subsequent T2DM, we aim to contribute to a better understanding of the onset and development of this condition by examining various blood parameters in this cohort.

In T2DM a dysregulated muscle-adipose tissue crosstalk occurs. Thus, in our first analysis, we investigated myostatin, a parameter related to muscle and adipose tissue. Within our study cohort we found associations of myostatin with adipose tissue parameters such as visceral fat, as well as inflammation parameters - both factors of the metabolic syndrome, which make up part of the pathophysiology of the “classic” T2DM risk phenotype. These results indicate the involvement of myostatin in the impaired muscle-adipose tissue crosstalk that occurs in metabolic syndrome.

The second analysis examined parameters for their suitability in differentiating two predefined risk groups: one group associated with metabolic syndrome and one without. Our hypothesis regarding different pathophysiologies underlying these risk subtypes is supported by the results from examinations of the selected hormones and signaling molecules. Results are reported specifically for the parameters leptin, hGH, and IGF-I. These suggest that the dysregulation of leptin and hGH/IGF-I signaling contributes to both risk phenotypes. In the phenotype not related to metabolic syndrome the glucose metabolism may be affected by an autonomic oversecretion of IGF-I, that may lead to the suppression of hGH secretion. Our findings offer starting points for further mechanistic evaluations, particularly for the pathophysiology of the risk phenotype unrelated to metabolic syndrome, that is still relatively unknown.

My thesis underscores the heterogeneity in the development of T2DM. Furthermore, it gives insight into those alterations in the metabolic syndrome in premenopausal women, as well as identifies parameters that appear relevant in the T2DM risk phenotype not associated with met-

abolic syndrome. Further mechanistic investigations may aid in the development of preventive and therapeutic interventions targeting specific phenotypes.

9.2 Zusammenfassung dieser Arbeit

Typ 2 Diabetes mellitus (T2DM) ist eine heterogene Zivilisationskrankheit mit weltweit ansteigender Prävalenz. Damit stellt diese Erkrankung nicht nur eine enorme Belastung für die Betroffenen dar, sondern auch für das Gesundheitssystem.
Da die Manifestation eines T2DM prozesshaft erfolgt, lohnt es sich, möglichst frühe Stadien zu erkennen, um rechtzeitig präventiv tätig werden zu können. Allerdings werden gerade die frühen Formen des T2DM häufig zu spät diagnostiziert. Dies liegt zum Teil darin begründet, dass auch junge Menschen, die nicht dem „klassischen“ Phänotyp entsprechen, betroffen sein können.

Mit meiner Arbeit möchte ich dazu beitragen, die vielfältigen Pathophysiologien des T2DM sowie einige Risiko-Phänotypen besser zu verstehen.
Hierzu untersuchten wir eine besondere T2DM Risiko Kohorte, nämlich unsere Prädiktion, Prävention und Subklassifikation von T2DM (PPSDiab) Kohorte. Diese umfasst Frauen post partum, die in der Schwangerschaft vor Studieneinschluss (Indexschwangerschaft) einen Gestationsdiabetes (GDM) hatten und welche, die in der Indexschwangerschaft normoglykäm waren (Verhältnis 2:1).
Nachdem Frauen post GDM ein erhöhtes Risiko für einen späteren T2DM haben, kann die Beobachtung einer solchen Risikokohorte einen wesentlichen Beitrag zum besseren Verständnis der Entstehung und Entwicklung dieser Erkrankung leisten. Dazu untersuchten wir im Rahmen dieser Arbeit diverse Blutparameter in dieser Kohorte.

Bei T2DM findet sich eine Dysbalance des Muskel-Fettgewebs-Crosstalks; daher bestimmten wir in unserer ersten Analyse Myostatin, einen Parameter, der in Zusammenhang mit Muskel- und Fettgewebe steht. In unserer Studienkohorte konnten wir Assoziationen mit Fettgewebsparametern wie beispielsweise Viszeralfett, sowie Entzündungsparamtern feststellen - beides Faktoren des metabolischen Syndroms und damit Teil der Pathophysiologie des „klassischen“ T2DM Risikophänotyps. Dies deutet darauf hin, dass Myostatin in den beim metabolischen Syndrom auftretenden gestörten Muskel-Fettgewebs-Crosstalk involviert sein könnte.

In der zweiten Analyse befassten wir uns mit Parametern, welche zur Differenzierung unserer beiden zuvor definierten Risikogruppen geeignet erschienen. Die eine Gruppe war mit Faktoren des metabolischen Syndroms assoziiert und die andere nicht. Die Ergebnisse der Untersuchungen der ausgewählten Hormone und Signalmoleküle unterstützen unsere zuvor aufgestellte Hypothese von unterschiedlichen Pathophysiologien bei diesen beiden Risiko-Subtypen. Hervorheben möchten wir die Parameter Leptin, hGH und IGF-I. Unsere Auswertungen deuten bei beiden Risikophänotypen auf eine Dysregulation der Leptin und hGH/IGF-I Signalübertragung hin. Bei dem Risikophänotyp, der nicht mit dem metabolischen Syndrom assoziiert ist, könnte eine autonome Übersekretion von IGF-I zur Suppression der hGH-Sekretion führen,

wodurch der Glukosestoffwechsel beeinträchtigt werden könnte. In Bezug auf die noch relativ unbekannte Pathophysiologie diese T2DM Risikotyps, könnten diese Ergebnisse interessante Anhaltspunkte für weitere mechanistische Untersuchungen bieten.

Meine Arbeit unterstreicht die große Heterogenität in der Entstehung von T2DM. Sie fügt weitere Erkenntnisse bezüglich potenzieller Veränderungen beim metabolischen Syndrom bei prämenopausalen Frauen hinzu, und konnte Parameter identifizieren, welche bei dem T2DM Risikotyp ohne Assoziation mit dem metabolischen Syndrom relevant zu sein scheinen. Weitere mechanistische Untersuchungen in dieser Richtung könnten in Zukunft zu phänotypspezifischen präventiven und therapeutischen Maßnahmen beitragen,

10. List of publications

Kern-Matschilles, S., Gar, C., Wanger, L., Haschka, S. J., Potzel, A. L., Hesse, N., Then, C., Seissler, J., Lechner, A., *Association of Serum Myostatin with Body Weight, Visceral Fat Volume, and High Sensitivity C-Reactive Protein But Not With Muscle Mass and Physical Fitness in Premenopausal Women.* Exp Clin Endocrinol Diabetes, 2021. 130(6): p. 393-399.

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