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Metabolic imbalance of the insulin-like growth factor–I axis in Zucker diabetic fatty rats

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ABSTRACT

In healthy conditions, insulin-like growth factor–I (IGF-I) acts in a coordinated fashion with insulin to lower glycemia, mainly by increasing insulin sensitivity in peripheral tissues. The aim of this study was to explore the relationship between glucose homeostasis and the endocrine IGF-I axis in Zucker diabetic fatty (ZDF) rats. The plasma levels of glucose, insulin, growth hormone, free IGF-I, total IGF-I (associated to insulin-like growth factor binding proteins plus free), and corticosterone were measured in 13-week-old ZDF rats and in age-matched controls under fasting and postprandial conditions. The plasma IGF-I binding capacity was measured by radioligand binding. In ZDF rats, fasting total and free IGF-I levels were reduced by 22% and 92%, respectively, compared with controls. Postprandial free IGF-I was reduced by 35%, whereas total IGF-I was unaffected. The plasma IGF-I binding capacity in ZDF rats was reduced by 24% after fasting and by 13% under postprandial conditions. A clear correlation between free IGF-I and insulin was observed in postprandial controls but not in ZDF rats. A principal component analysis clearly separated ZDF and control rats into 2 main components under both fasting and postprandial conditions. The first component was determined equally by total IGF-I, bound IGF-I, the free to total IGF-I ratio, and the IGF-I binding capacity. The second component was determined mostly by glucose and insulin. Our results show a marked alteration of the plasma IGF-I levels and of the capacity of plasma to bind IGF-I, and a disturbed relationship between IGF-I and postprandial insulinemia in a rat model of type 2 diabetes mellitus.

1. Introduction

Insulin-like growth factor–I (IGF-I) is a 70–amino acid peptide related in sequence to proinsulin. It is produced locally by many cells and tissues where it can act locally as either an autocrine or a paracrine factor to regulate cell proliferation, differentiation, or anabolism. The IGF-I found in the blood is mainly due to growth hormone (GH)–dependent stimulation of IGF-I secretion from the liver. Liver-derived IGF-I in turn exerts a negative feedback regulation on GH secretion from the pituitary gland. In contrast to IGF-I produced by peripheral tissues, it is assumed that blood IGF-I has primarily endocrine and metabolic functions rather than growth factor effects [1]. The importance of endocrine IGF-I could be inferred from gene inactivation experiments in vivo. For example, in liver IGF-I–deficient (LID) mice, the plasma concentration of total IGF-I was reduced by 75%, but there was no detectable effect on postnatal body growth [2,3]. However, LID mice had increased relative liver

Authors' contributions: Frédéric De Ceuninck developed the concept, designed the experiments, analyzed the data, and wrote the paper. Jean-Olivier Rolin and Audrey Caliez performed the experiments. Louise Baschet analyzed the data and conducted all biostatistical analyses. Alain Ktorza supervised the study and gave conceptual advice.

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weights, which were attributed to a relief of IGF-I–mediated inhibition of pituitary GH secretion, with a subsequent increase in serum GH levels. These mice also had a 25% decrease in fat mass and increased plasma insulin levels, but were normoglycemic, which could be interpreted as an adequately compensated insulin resistance [4]. It was further demonstrated that, in these animals, insulin insensitivity occurred in the muscle but not in the liver or white adipose tissue [5]. It is known that sustained elevation of GH counteracts the effects of insulin, at least in part by altering the insulin receptor signaling pathway. The state of insulin resistance observed in LID mice could only be partially abolished after treatment with a GH-releasing hormone antagonist [5] as also observed in LID mice carrying an inactivating point mutation of GH [6]. These results indicated that both decreased IGF-I and increased GH levels were involved in the deterioration of insulin sensitivity.

The bioavailable form of IGF-I is unbound, that is, free IGF-I, which represents approximately 5% of the total IGF-I found in the circulation. Insulin-like growth factor–I associates with the insulin-like growth factor binding protein–3 (IGFBP-3), a protein responsible for more than 80% of its sequestration in the blood. A third component, the acid labile subunit (ALS), further stabilizes this complex [7]. As for IGF-I, both circulating ALS and IGFBP-3 are secreted by the liver and positively regulated by GH [7,8]. In offspring of LID mice crossed with ALS knockout mice, the concomitant decrease of liver IGF-I and ALS led to an 85% reduction in the plasma concentration of total IGF-I and a 10-fold increase in plasma GH levels [9]. However, free IGF-I levels were increased by 3-fold compared with LID or ALS knockout mice and were accompanied by an overall improvement in insulin sensitivity, as assessed by increased muscle and fat glucose uptake [9]. The importance of free IGF-I was further demonstrated in Wistar rats where an intravenous administration of recombinant IGF-I rapidly decreased fasting glycemia by approximately 50%, but coinjection with IGFBP-3 completely prevented the hypoglycemic effect of IGF-I [10]. Furthermore, in patients with type 2 diabetes mellitus or different forms of insulin resistance, the injection of a recombinant IGF-I/IGFBP-3 complex increased the free serum IGF-I levels, leading to a reduced fasting plasma glucose concentration, reduced glycosylated hemoglobin levels, and improved insulin sensitivity [11-13]. These studies highlighted the importance of the ratio of free to total IGF-I for the regulation of glucose homeostasis.

In healthy conditions, fasting free IGF-I is positively correlated with insulin sensitivity and inversely correlated with glucose levels [14]. However, a global picture of the relationship between the endocrine IGF-I axis and endocrine regulators of glucose homeostasis in diabetic conditions has not yet been investigated. To address this question, we used hyperglycemic insulin-resistant Zucker diabetic rats and found marked alterations in the circulating levels of components of the IGF-I axis compared with normoglycemic control rats.

2. Materials and methods

2.1. Animal preparation and experimental protocol

Thirty male Zucker diabetic fatty rats (ZDF/Gmi-fa/fa, referred to as *diabetic*) and 30 control Zucker rats (ZDF/Gmi-+/+ or +/fa, referred to as *controls*) (Charles River Laboratories L'arbresle, France) were fed a standard laboratory diet (Purina 5008, Genobios, Laval, France). At 13 weeks of age, rats were weighed and divided into 4 groups. Fifteen diabetic rats and 15 control rats were fasted overnight for 16 hours (diabetic fasted [DF] group; control fasted [CF] group) with free access to water and then killed just after the dark cycle. In parallel, 2 other groups of 15 diabetic rats and 15 control rats were fed ad libitum (diabetic postprandial [DP] group; control postprandial [CP] group) and killed at the same time. Before sacrifice, animals were anesthetized using isoflurane (Minerve, Esternay, France) and blood samples were taken via abdominal aorta puncture. These samples were then distributed into tubes containing sodium fluoride/K_3 EDTA (Greiner Bio-One, Courtaboeuf, France) and 250 U aprotinin per milliliter. Plasma was prepared by centrifugation of blood at 3500*g* for 10 minutes at 4°C and then stored in aliquots at –70°C until used. The experimental protocol was approved by the internal ethics committee.

2.2. Measurement of plasma glucose, IGF-I, insulin, GH, and corticosterone

The glucose concentration in plasma samples was measured using the oxidase method on an automated system (Cobas Mira, Roche Diagnostics, Meylan, France). Plasma insulin was determined using a rat insulin enzyme-linked immunosorbent assay kit from Mercodia (Uppsala, Sweden), plasma corticosterone using a rat corticosterone enzyme immunoassay kit from DSL (Webster, TX), and plasma GH using a mouse/rat GH enzyme-linked immunosorbent assay kit from DSL. Total IGF-I and free IGF-I were determined by immunoenzymometric assay using a rat/mouse IGF-1 immunoenzymatic assay kit from GroPep-IDS (Fountain Hills, AZ). Bound IGF-I, free to total IGF-I, and bound to total IGF-I were calculated from the total and free measured values. All assays were performed according to the manufacturer's instructions.

2.3. Determination of total IGF-I binding capacity in plasma

For the detection of total IGF-I binding capacity in plasma, 2 μL of rat plasma samples was spotted onto a 10 × 10–cm Hybond-ECL nitrocellulose membrane (GE Healthcare, Orsay, France) that had been rehydrated in ultra pure water and Tris-buffered saline (TBS). In parallel, a standard curve was generated using serial dilutions of recombinant IGFBP-3. The membrane was dried and then incubated in TBS containing 3% Nonidet-P40 for 30 minutes and then in TBS containing 0.5% gelatin for 2 hours at room temperature. After washing for 5 minutes in TBS containing 0.1% Tween-20, the membrane was dried and incubated in TBS containing 0.15% gelatin and 0.01 μCi/mL of ^{125}I-IGF-I (GE Healthcare) for 18 hours at 4°C. The membrane was washed in TBS and dried, and the radioactivity reflecting total ^{125}I-IGF-I binding in plasma samples was quantified using the IGFBP-3 standard curve with the Phosphor Imager analysis system (GE Healthcare). As IGFBP-3 does not represent all of the total IGF-I binding in the circulation (~80% of total IGF-I is bound to IGFBP-3), the results were expressed in microgram equivalent IGF-binding activity per milliliter of plasma.

2.4. Statistical analyses

Statistical analyses between diabetic and control groups, and between fasted and postprandial groups for plasma glucose, insulin, GH, corticosterone, total and free IGF-I levels, IGF-I binding capacity, and the calculated free to total IGF-I ratio were performed using a 2-way analysis of variance (ANOVA) [15] with the SAS (Cary, NC) Version 9.1 software. As insulin and GH concentrations followed a logarithmic distribution, their statistics were assessed using the log10 values. To examine correlations between 2 paired parameters, both for control and diabetic rats, Pearson correlation coefficients and their statistical significance were assessed using the R V2.6.1 software. All pairs of the 8 following parameters were analyzed: plasma glucose, log10 insulin, log10 GH, corticosterone, total IGF-I, free IGF-I, IGF-I binding capacity, and free to total IGF-I. To obtain a Gaussian distribution and to examine correlations between these parameters independently of the fasted/postprandial state, values obtained for fasted or postprandial rats of the same strain were standardized. To determine statistical differences for the same pairs of parameters between diabetic and control rats, Pearson correlation coefficients were compared using the coefficient comparison test for independent samples [16]. To limit the incidence of false-positives due to repeated multiparametric comparisons (28 analyzed parameter pairs), both Pearson P values and P values for the comparison between 2 Pearson correlation coefficients were adjusted by the method of Sidak [17].

To detect a relationship between the 9 metabolic variables measured, a principal component analysis (PCA) was applied to all groups using the R V2.6.1 software. This mathematical-statistical method decreases the complexity of large data matrices (scattering of points in a multidimensional space) by converting them into linear trends represented as principal components on an x/y plot when 2 sets of variables account for most of the variance [18].

3. Results

3.1. Plasma glucose and hormone levels in diabetic and control rats

Glucose levels were measured in diabetic and control rats, both after fasting and in postprandial conditions. In diabetic rats, glycemia was increased approximately 2-fold after fasting and 2.5-fold in postprandial conditions ($P < .001$) compared with the respective control groups (Fig. 1A). Insulin values followed a normal Gaussian distribution after logarithmic transformation. Accordingly, statistical analyses for this variable were performed on the transformed values. As observed for glycemia, insulinemia was significantly higher in diabetic rats compared with controls, with a mean increase of 5.6-fold after fasting and 8.5-fold in postprandial conditions ($P < .001$) (Fig. 1B). The plasma corticosterone levels were not significantly different between control and diabetic rats, although slightly increased levels were associated with fasting in both the control and diabetic groups ($P < .001$) (Fig. 1C). As for insulin, GH values followed a normal Gaussian distribution after logarithmic transformation. There was no statistical difference for plasma GH levels between any group of diabetic and control rats under either fasting or postprandial conditions (Fig. 1D).

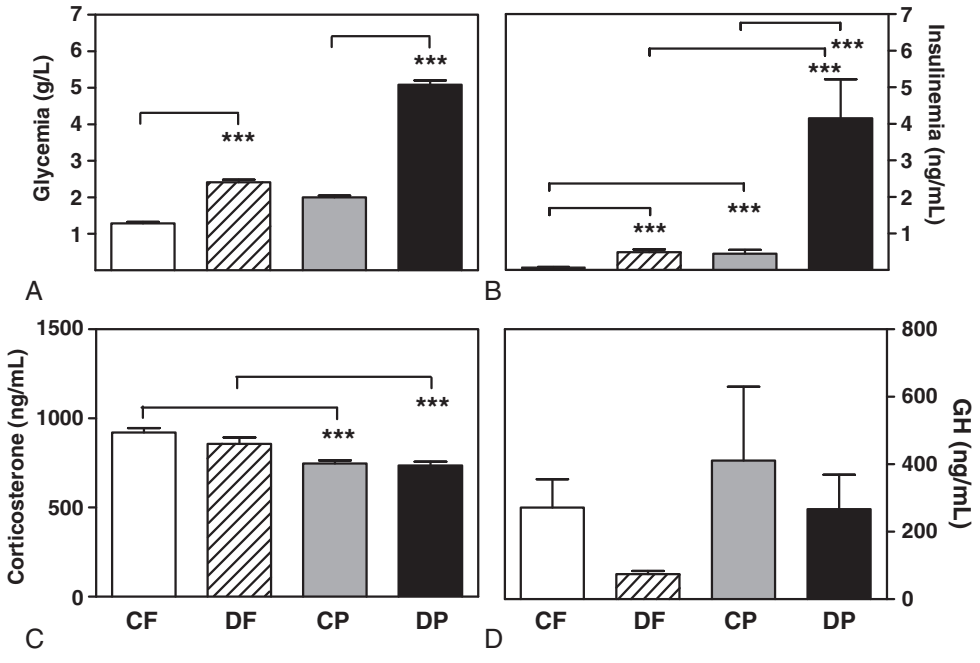

Fig. 1 – Glucose and hormone levels of 13-week-old ZDF-fa/fa rats compared with age-matched control rats. Glycemia (A), insulinemia (B), corticosterone (C), and GH (D). Open bars, CF; hatched bars, DF; gray bars, CP; black bars, DP. Values are means + SEM of measurements made in n = 14 to 15 animals per group. ***P < .001 (2-way ANOVA, assessed after log transformation for insulinemia and GH values).

3.2. *Total and free plasma IGF-I levels in diabetic and control rats*

The plasma levels of total IGF-I (IGF-I bound to circulating IGFBPs plus free IGF-I) and free bioavailable IGF-I were measured in diabetic and control rats under both fasting and postprandial conditions (Fig. 2). The low variability observed in each group suggested a tight regulation of total and free IGF-I. Both total and free IGF-I levels were significantly increased in postprandial compared with fasting conditions in control and diabetic rats. Total IGF-I was significantly lower in fasted diabetic rats than in controls (1090 ± 27 and 1398 ± 21 ng/mL, respectively; $P < .001$) (Fig. 2A), but it was unchanged under postprandial conditions. A severe decrease of fasting free IGF-I levels was observed in diabetic rats compared with controls (7.6 ± 2.0 and 94.5 ± 6.6 ng/mL, respectively; $P < .001$) (Fig. 2B). Accordingly, the percentage of free to total fasting IGF-I fell from 6.7% in controls to as low as 0.7% in diabetic rats ($P < .001$). Under postprandial conditions, the free IGF-I levels were decreased by 35% in diabetic rats compared with controls, which was a significantly lower reduction than that seen after fasting (significant interaction, $P < .001$). The percentage of free to total postprandial IGF-I was 8.2% in control rats and 5.3% in diabetic rats ($P < .001$).

3.3. *IGF-I binding capacity in the plasma of diabetic and control rats*

Insulin-like growth factor binding proteins are the main determinants of IGF-I stability and bioavailability in the circulation. To compare the total IGF-I binding capacity in the plasma of diabetic and control rats, 2 μL of plasma was spotted onto a nitrocellulose membrane and hybridized with ^{125}I-IGF-I followed by autoradiography (Fig. 3A). As observed for IGF-I levels, the total IGF-I binding capacity was very homogeneous within each group. It was significantly increased under postprandial conditions in both control and diabetic rats ($P < .001$, Fig. 3B). Furthermore, compared with the control group, the IGF-I binding capacity in the plasma of diabetic rats was significantly reduced after fasting (–24%, $P < .001$) and postprandial conditions (–13%, $P < .001$).

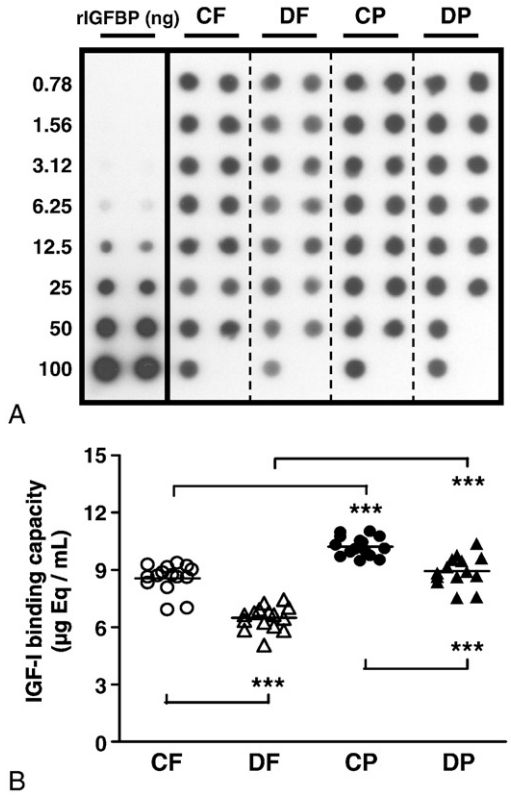

Fig. 3 – A, Dot blot autoradiographic analysis of ^{125}I-IGF-I binding activity in the plasma of 13-week-old ZDF-fa/fa rats compared with age-matched control rats. B, Phosphor Imager analysis of IGF-I binding activity in the plasma of CF (open circles), DF (open triangles), CP (black circles), and DP (black triangles) rats. Values were quantified using a standard curve drawn from the intensities of IGFBP-3 spots in panel A. Data are means + SEM of measurements made in n = 14 to 15 animals per group. ***P < .001 (2-way ANOVA).

3.4. *Correlation analysis and PCA*

A correlation analysis was performed to identify parameters differentially regulated in control and diabetic rats (Tables 1 and 2). To identify differences due solely to the pathological

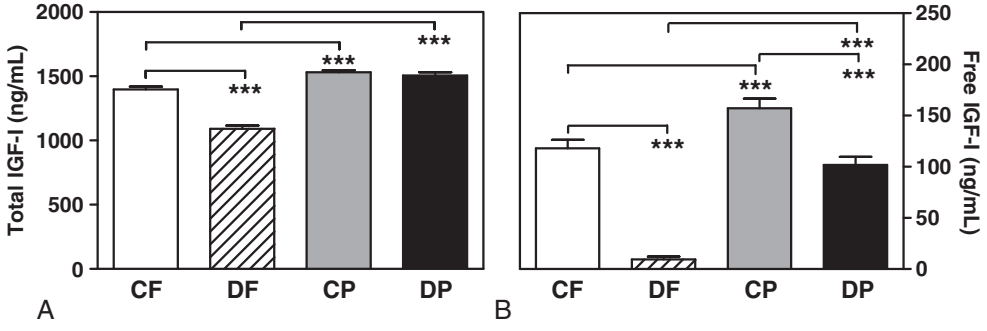

Fig. 2 – Total (A) and free (B) plasma IGF-I levels of 13-week-old ZDF-fa/fa rats compared with age-matched control rats. Open bars, CF; hatched bars, DF; gray bars, CP; black bars, DP. Values are means + SEM of measurements made in n = 14 to 15 animals per group. ***P < .001 (2-way ANOVA).

Table 1 – Pearson correlation coefficients in control rats

	Glycemia						
Log10 insulin	0.41	**Log10 insulin**					
Log10 GH	–0.087	–0.18	**Log10 GH**				
Total IGF-I	0.18	0.17	0.098	**Total IGF-I**			
Free IGF-I	0.35	**0.55 ($P = .052$)**	0.016	0.74***	**Free IGF-I**		
IGF-I BC	0.058	–0.11	0.11	0.72***	0.36	**IGF-I BC**	
Free to total IGF-I	0.37	**0.57***	0.013	0.66**	0.99***	0.31	**Free to total IGF-I**
Corticosterone	0.18	0.51	–0.098	–0.16	0.12	–0.075	0.17

Pearson correlation coefficients for parameter pairs are shown together with their statistical significance. Values in bold underline Pearson correlation coefficients significantly different from those calculated for the corresponding pairs in ZDF-fa/fa rats in Table 2. BC indicates binding capacity.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

Table 2 – Pearson correlation coefficients in ZDF-fa/fa rats

	Glycemia						
Log10 insulin	–0.019	**Log10 insulin**					
Log10 GH	0.16	–0.13	**Log10 GH**				
Total IGF-I	0.0072	–0.25	0.12	**Total IGF-I**			
Free IGF-I	0.22	**–0.28**	–0.0075	0.71***	**Free IGF-I**		
IGF-I BC	–0.093	–0.31	–0.14	0.68**	0.47	**IGF-I BC**	
Free to total **IGF-I**	0.24	**–0.24**	–0.036	0.68**	0.98***	0.44	Free to total **IGF-I**
Corticosterone	0.26	0.14	–0.41	–0.025	0.096	–0.0088	0.12

Pearson correlation coefficients for parameter pairs are shown together with their statistical significance. Values in bold underline Pearson correlation coefficients significantly different from those calculated for the corresponding pairs in control rats in Table 1. BC indicates binding capacity.

** $P < .01$.

*** $P < .001$.

condition, the effects dependent on the fasted/postprandial state were removed by grouping the fasted and postprandial animals before analysis (for separate correlation analyses as a function of the fasted/postprandial state, see supplemental table). Positive correlations were found in control and diabetic rats between free IGF-I, IGF-I binding capacity, the free to total IGF-I ratio, and total IGF-I. As expected, free IGF-I was strongly correlated to the free to total IGF-I ratio in both groups. In control rats, a weak positive correlation was observed for insulin with free IGF-I ($r = 0.55$, $P = .052$) and for insulin with the free to total IGF-I ratio ($r = 0.57$, $P < .05$) (Table 1). However, these pairs of parameters were not found to be correlated in diabetic rats ($r = –0.28$ and –0.24 respectively, not significant) (Table 2). This difference between control and diabetic rats was statistically significant (P comparison = .024 for the insulin-free IGF-I pair and P comparison = .029 for the insulin-free to total IGF-I pair).

To further analyze the observed differences, control and diabetic rats were separated into fasted (Fig. 4, panels CF and DF) and postprandial (Fig. 4, panels CP and DP) groups. The insulinemia of control rats was correlated with free IGF-I levels under postprandial ($r = 0.819$, $P < .01$) (CP), but not fasting (CF), conditions. However, free IGF-I levels did not correlate with postprandial insulinemia in diabetic rats ($r = –0.371$, not significant) (DP). This difference between control and diabetic rats was significant ($P < .05$). Similar results were obtained for correlation analyses between insulinemia and the free to total IGF-I ratio (not shown). In control rats, the Pearson correlation coefficients for these 2 parameters were $r = 0.096$ after fasting and $r = 0.833$ ($P < .01$) in postprandial conditions. In diabetic rats, the correlation coefficients were $r = –0.005$ after fasting and $r = –0.336$ in postprandial conditions. The absence of correlation between postprandial insulinemia and the free to total IGF-I ratio in diabetic rats in comparison with controls was statistically significant ($P < .05$).

Because simple correlation analyses do not extract the maximal amount of information from large data matrices, a PCA was used to analyze the possible relationship between the large set of variables measured and the 4 groups studied. Principal component analysis allows the conversion of a complex scattering of sample points in multidimensional space into linear trends (principal components) that can be evaluated visually to assess similarities or differences between individuals or variables [18]. The PCA identified 2 components accounting for 76% of the total variability (Fig. 5). The first principal component (PC1, direction along which the samples showed the largest variation) explained 53% of the variability and was determined equally by total IGF-I, bound IGF-I, free IGF-I, the free to total IGF-I ratio, and IGF-I binding capacity (Fig. 5A). All 5 parameters were found to be strongly associated as demonstrated by their tight grouping. The second principal component (PC2) explained 23% of the variability and was represented mostly by insulin and glucose. Corticosterone could not be explained by the PC1 or PC2, demonstrating a lack of association with some other parameters. Growth hormone was near the center of the circle; therefore, its relationship with other parameters was not

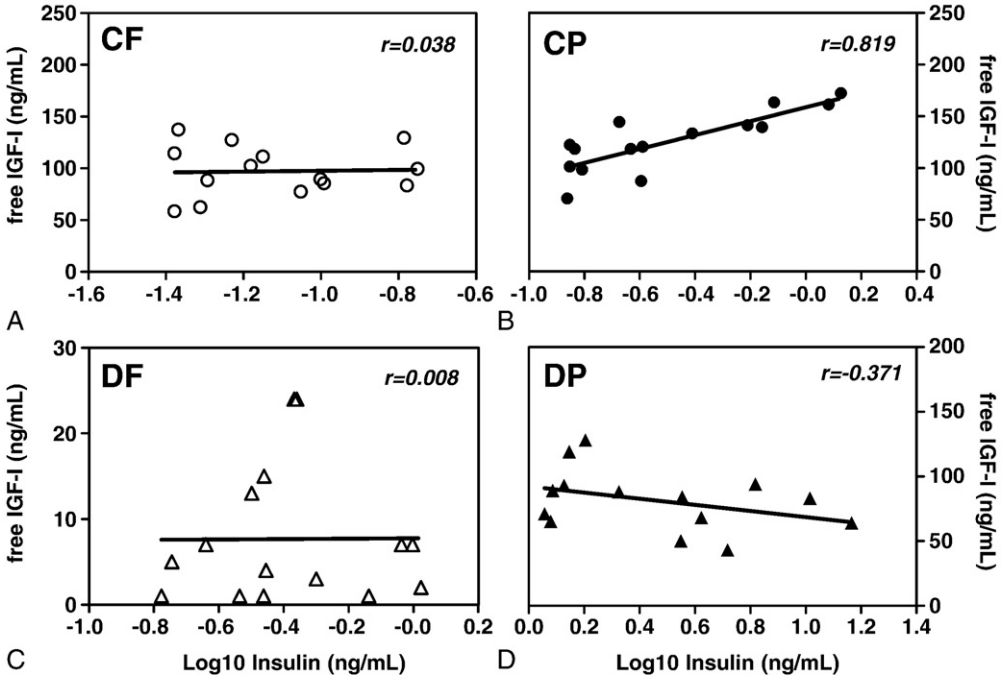

Fig. 4 – Correlation analyses between free IGF-I and insulin. A, Control fasted rats (open circles). B, Control postprandial rats (black circles). C, Diabetic fasted rats (open triangles). D, Diabetic postprandial rats (black triangles).

possible using this 2-dimensional representation. As shown on the right panel (Fig. 5B), all groups were strikingly well separated by the PCA, with a high level of homogeneity within each group. By projecting samples on the x-/y-axes, PC1 (x) appeared to separate mostly animals according to the fasted/postprandial state (black vs empty symbols), whereas PC2 (y) appeared to separate mostly control and diabetic rats (circles vs triangles). In postprandial conditions, control rats were noticeably more closely associated to the 5 IGF-I parameters than diabetic rats.

4. Discussion

Under physiological conditions, the endocrine function of IGF-I is regulated at a primary level by 6 high-affinity IGFBPs

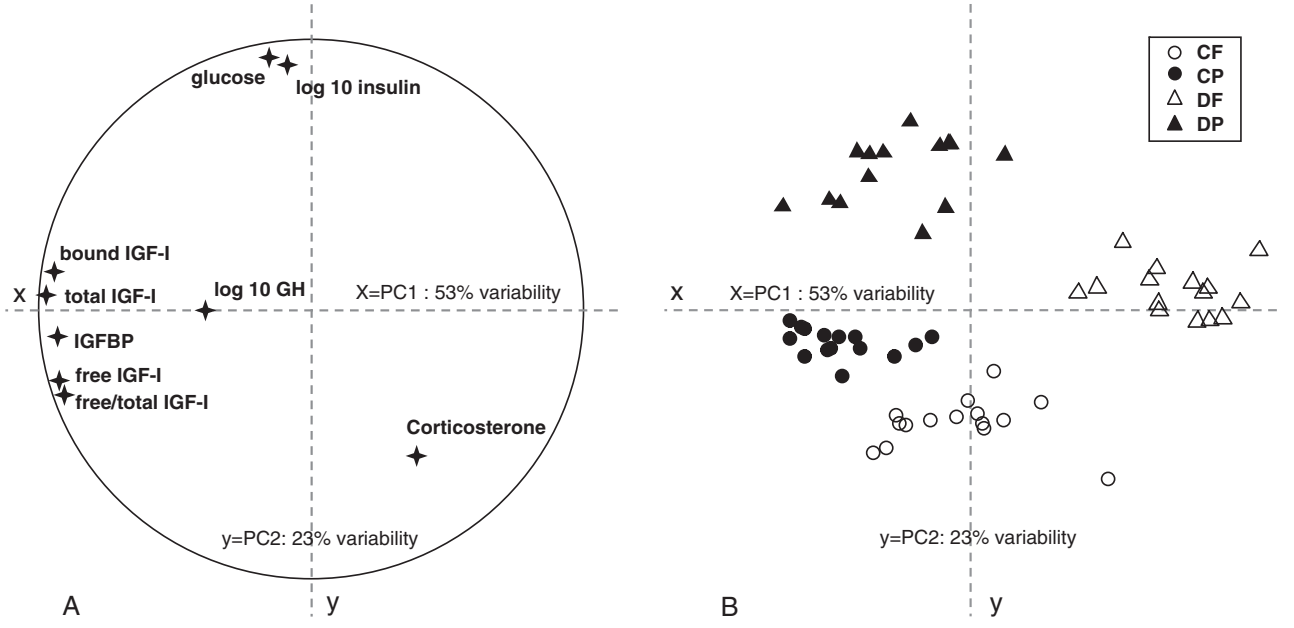

Fig. 5 – Principal component analysis of IGF-I variables, GH, insulin, glucose, and corticosterone in fed or fasted 13-week-old ZDF-fa/fa rats and age-matched control rats. A, Two-dimensional PCA representation of the 9 variables. B, Plot analysis of the 4 animal groups: CF (open circles), DF (open triangles), CP (black circles), and DP (black triangles).

that control its bioavailability and by 4 cell surface receptors: IGF-I, IGF-II, insulin, and IGF-I/insulin hybrid receptors [19,20]. At a second level, IGF-I and IGFBPs are regulated by hormones [21-24], nutrition [24-26], and IGFBP proteases [27]. Whereas insulin circulates in the plasma at picomolar concentrations, plasma total IGF-I reaches nanomolar concentrations. The hypoglycemic effect of IGF-I is only 5% of that of insulin, although theoretically it could be 50 times greater because of its abundance [28]. However, this does not occur because IGF-I is neutralized by IGFBPs. Accordingly, even small disturbances of the well-balanced association between IGF-I and its binding proteins in the circulation may interfere with the control of glucose homeostasis. Despite the central role of fasting and feeding in the regulation of glycemia, insulinemia, and IGF-I levels, plus the known involvement of IGF-I in the regulation of glucose homeostasis, the relative importance of the IGF-I axis in normoglycemic, hyperglycemic, fasted, and fed conditions has not been studied. To address this question, we chose the ZDF-fa/fa rat model that closely reflects human type 2 diabetes mellitus, thanks to its dual defect in both β-cell function and peripheral insulin resistance. Hyperinsulinemia and hyperglycemia, which can be considered as a physiological response to feeding in healthy conditions, were further increased in ZDF rats in the fed state, as well as after fasting, indicating a state of insulin resistance and inadequate ability of the pancreatic β-cells to compensate for the elevated glycemia. We found that profound changes occurred in the circulating levels of components of the IGF-I axis in this rat model of type 2 diabetes mellitus.

One of the most noticeable changes in these diabetic rats was the decrease of total IGF-I under fasting conditions, together with the reduction of IGF-binding capacity in plasma. As the half-life of IGF-I in biological fluids depends on its binding to IGFBPs, it is likely that the lower concentration of total IGF-I could be, at least in part, a consequence of this decrease of binding capacity. Compared with control rats, the plasma IGF-I binding capacity was reduced in diabetic rats in postprandial conditions, although to a smaller extent than after fasting. This reduction, however, was not accompanied by a decrease of total IGF-I levels. With respect to these findings, it is interesting to note that sera from patients with type 2 diabetes mellitus had significantly higher IGFBP-3 proteolytic activity than sera from age-matched healthy subjects [29]. Similarly, children with untreated type 1 diabetes mellitus had a 2-fold decrease of plasma IGFBP-3 levels when compared with age-matched healthy children [30]. This decrease could be attributed to increased IGFBP-3 proteolytic activity, and it was reversed after insulin treatment. In addition, IGF-I was demonstrated to be more susceptible to proteolytic activity in the serum of streptozotocin-treated diabetic rats than in control rats and the administration of insulin rapidly and markedly decreased this protease activity [31]. In accordance with these observations, the sustained levels of total IGF-I in diabetic rats in postprandial conditions in the present study, despite a reduction of IGF-binding capacity in plasma, may be explained by a reduction of IGFBP-3 and/or IGF-I degradation favored by high postprandial insulinemia. A reduction of IGF-I degradation in fed compared with fasted diabetic rats was also suggested by the smaller decrease of free IGF-I levels at the fed state.

Free IGF-I, rather than total IGF-I, is of crucial importance for the regulation of glucose homeostasis because it is the bioactive form of IGF-I [32]. In overnight-fasted Wistar rats, the decrease of glycemia induced by a single intravenous injection of IGF-I could be blocked by the coadministration of IGFBP-3 [10], demonstrating that free, rather than total, IGF-I was implicated in the hypoglycemic effect. Thus, in the present study, the dramatic reduction of free IGF-I levels during fasting in diabetic rats probably contributed significantly to the observed fasting hyperglycemia. After a meal, free IGF-I levels are known to increase because of the insulin-induced down-regulation of IGFBP-1 in the liver [21-23,33]. In agreement with these findings, we found that the free IGF-I concentration in fed control rats was positively correlated with insulinemia. This correlation was completely abolished in fed diabetic rats, which could possibly be explained by insulin resistance in the liver, a hallmark of the ZDF rat model. Free IGF-I is also known to have an important role in the regulation of β-cell function and shares intracellular signaling pathways with insulin [34]. It was reported that a loss of action of both IGF-I and insulin in β-cells was associated with diabetes and that therapeutic improvement of insulin and IGF-I signaling in β-cells might protect against type 2 diabetes mellitus [35]. In the present study, the loss of correlation between postprandial plasma free IGF-I and insulin may be of importance in the altered β-cell function in ZDF rats, but the implication of autocrine/paracrine IGF-I on this pathological process deserves further investigation.

Because of a possible cross talk between corticosteroids, GH, IGF-I, and IGFBPs in the regulation of glucose homeostasis [22,36-38], corticosterone, the primary glucocorticoid in rats, and GH were also measured in this study. We did not find any correlation for corticosterone or GH levels with any other parameter measured in either diabetic or control rats. The PCA led to a similar conclusion. This lack of correlation may be due to the need to include other parameters such as circulating hormones, hypothalamic factors, and neurotransmitters, which are also important for GH regulation, to obtain a more global and physiological approach.

The 3 main findings of the present study underline the importance of the endocrine IGF-I axis in an animal model of type 2 diabetes mellitus: a decreased ability of plasma to bind IGF-I, a decrease of free IGF-I levels especially under fasting conditions, and a loss of correlation between postprandial IGF-I and insulin. These findings raise further questions that will need to be addressed in the future. Notably, it will be important to know whether the administration of IGF-I in prediabetic ZDF rats can prevent or delay the appearance of alterations in glucose homeostasis and/or whether it can alleviate such alterations in a fully established diabetic state. It also remains to be demonstrated whether these alterations of the IGF-I axis in ZDF rats are only relevant to this model or whether they are a common feature of insulin-resistant models. To our knowledge, no study has addressed this question in-depth. However, the observation that serum total IGF-I was also reduced in leptin-receptor–deficient db/db mice [39], as well as in Goto-Kakizaki rats [40], a polygenic model of type 2 diabetes mellitus, suggests that dysregulation

of the endocrine IGF-I axis in relation with disturbed glucose homeostasis is not restricted to ZDF rats.

Importantly, the translational potential of the present findings also deserves further discussion. In nondiabetic individuals, high plasma IGF-I levels were found to be associated with a reduced [41] or increased [42] risk of developing impaired glucose tolerance or type 2 diabetes mellitus. Conversely, low IGF-I levels were found to be associated with a higher incidence of prediabetic state or type 2 diabetes mellitus [42,43]. There have only been a few reports about serum IGF-I levels in patients with type 2 diabetes mellitus compared with healthy subjects, with conflicting results [44-49]. The differences in IGF-I or IGFBPs levels reported in these studies were probably influenced by several factors such as the number of patients, their weight (lean vs obese), their nutritional state, and the impact of medical treatments. However, in general, these studies suggested that the total plasma IGF-I was an important factor determining glucose homeostasis, with a potentially protective role of elevated IGF-I levels against the development of glucose intolerance and type 2 diabetes mellitus. Although the experimental model reported in the present study does not exactly reflect drug-naive type 2 diabetes mellitus in humans, it does suggest that the correction of defects in the IGF-I axis may bring some benefits to these patients. Indeed, several studies demonstrated that acute administration or short-term treatment with recombinant IGF-I or an IGF-I/IGFBP-3 complex (allowing free IGF-I levels to increase) improved glucose and lipid metabolism in patients with type 2 diabetes mellitus [11,50-53]. Interestingly, IGF-I had greater metabolic activity in skeletal muscle cells from subjects with than those without type 2 diabetes mellitus, suggesting that IGF-I administration could be a viable strategy to circumvent insulin insensitivity [54]. However, continuous administration of recombinant IGF-I caused serious safety concerns that precluded further therapeutic advancements [55-58].

In summary, we report herein a marked dysregulation of the endocrine IGF-I axis in ZDF-fa/fa rats. These findings could provide an attractive model to examine the ability of existing antidiabetic treatments to correct abnormalities of the IGF-I axis in relationship with their ability to improve glucose homeostasis, or to promote safer treatments targeting the IGF-I axis.

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