CLINICAL STUDY

Characterisation of adiponectin multimers and the IGF axis in humans with a heterozygote mutation in the tyrosine kinase domain of the insulin receptor gene

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Abstract

Objective: Low levels of adiponectin, IGF-binding protein 1 (IGFBP1) and IGFBP2 and high levels of leptin correlate with several indices of insulin resistance and risk of type 2 diabetes. However, in insulin receptoropathies, plasma adiponectin is paradoxically increased despite severe insulin resistance, whereas the IGF axis is sparsely described. Here, we aimed to characterise the multimeric distribution of adiponectin and the IGF axis in humans with a heterozygous *INSR* mutation (Arg1174Gln).

Methods: Blood samples obtained from six Arg1174Gln carriers and ten lean, healthy controls before and after a euglycaemic–hyperinsulinaemic clamp were examined for plasma adiponectin multimers, leptin, total IGF1, IGF2, free IGF1, IGFBP1 and IGFBP2.

Results: Despite tenfold elevated fasting insulin and marked insulin resistance in Arg1174Gln carriers, the levels of total adiponectin, leptin, IGFBP1 and IGFBP2 were similar to those observed in controls, while total IGF1, IGF2 and free IGF1 levels were increased. The relative fraction of high-molecular weight adiponectin was increased, whereas both the absolute concentration and the fraction of low-molecular weight adiponectin were decreased in Arg1174Gln carriers. Interestingly, exogenous insulin failed to suppress total adiponectin in Arg1174Gln carriers, but reduced IGFBP1 and increased IGFBP2 as in controls.

Conclusion: The normal levels of adiponectin, IGFBP1 and IGFBP2 in the face of highly elevated insulin levels suggest an impaired ability of insulin to suppress markers of common insulin resistance in carriers of a dominant-negative *INSR* mutation. However, together with the adaptive increases in IGF1 and IGF2 and a potentially improved distribution of adiponectin multimers, these changes may contribute to rescue insulin action in insulin receptor-deficient individuals.

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Introduction

Adiponectin and leptin are prototypic adipokines secreted almost exclusively by adipocytes (1). Adiponectin levels are low in obesity and type 2 diabetes and consistently show strong correlation with several indices of insulin sensitivity (2), also in healthy populations (3). Conversely, increasing adiposity and common forms of insulin resistance are associated with increased leptin, probably due to leptin resistance in skeletal muscle (1). Correspondingly, high leptin-to-adiponectin ratios (LAR) show strong association with measures of insulin resistance in non-diabetic individuals (3).

Adiponectin circulates in blood in several multimeric subforms: low-molecular weight (LMW) trimers, medium-molecular weight (MMW) hexamers and high-molecular weight (HMW) multimers (1, 2). Of these, HMW adiponectin seems to be the most active

and predominant form (1), and accordingly, HMW adiponectin shows the same strong inverse relationship with insulin resistance and type 2 diabetes as total adiponectin (4). Although the mechanisms by which adiponectin improves insulin sensitivity in humans *in vivo* remains to be proven (5), studies on rodents and cell cultures suggest that adiponectin stimulates glucose uptake and lipid oxidation via activation of AMP-activated protein kinase in liver and skeletal muscle (1). Based on recent studies that have shown higher than normal levels of plasma adiponectin in patients with chronic heart failure, renal failure, type 1 diabetes and anorexia nervosa, it has been hypothesised that adiponectin in these conditions may protect the organism against energy deprivation (6). Elevated levels of total and HMW adiponectin have been reported in severe insulin resistance (SIR) caused by insulin receptoropathies due to either mutations in the insulin receptor gene (*INSR*) or insulin receptor antibodies

(5, 7, 8, 9). Moreover, leptin seems to be suppressed or absent, i.e. similar to that observed in lipodystrophies (5, 7). There is evidence that insulin suppresses plasma adiponectin in humans *in vivo* and down-regulates expression and/or secretion from adipocytes (5, 10, 11, 12). Assuming that this action of insulin is relatively intact in common forms of insulin resistance, fasting hyperinsulinaemia could be the cause of hypoadiponectinaemia in these conditions. In line with this, the apparently paradoxical hyperadiponectinaemia observed in SIR could be explained by complete insulin receptor dysfunction, which impairs not only insulin signalling to glucose transport but also other actions of insulin (5). To what extent a non-complete block of insulin receptor function, such as that seen in dominant-negative, heterozygous *INSR* mutations, affects adiponectin and its multimeric distribution and leptin levels remains to be established.

Treatment of diabetes in the context of SIR is a major challenge (13, 14). The IGF axis contributes to the regulation of glucose homeostasis (15). Accordingly, therapy with recombinant IGF1 has been shown to increase insulin sensitivity in obesity and type 2 diabetes and has been recommended as a potential therapy for SIR, e.g. in patients with *INSR* mutations (13, 14, 15). In obesity and type 2 diabetes, free IGF1 and total IGF2 are increased, while IGF-binding protein 1 (IGFBP1) and IGFBP2 are down-regulated (15, 16, 17). Moreover, in cross-sectional studies, IGFBP1 and IGFBP2 levels are associated with indices of insulin sensitivity (18, 19, 20). In contrast, little is known about the IGF1/IGFBP system in relation to SIR caused by *INSR* mutations (21). Insulin suppresses hepatic expression and secretion of IGFBP1 (15), and low levels of IGFBP1 in common forms of insulin resistance are likely to be caused by fasting hyperinsulinaemia. In parallel with paradoxically high adiponectin in the face of extreme fasting hyperinsulinaemia, IGFBP1 levels seem to be elevated in *INSR* mutants due to a blunted effect of insulin on the liver (9). However, this remains to be further examined.

We have recently reported a family with nine members carrying a dominant-negative mutation in the tyrosine kinase domain of *INSR* (22). They not only clinically presented with episodes of postprandial severe hypoglycaemia but also showed marked insulin resistance when metabolically characterised. Surprisingly, insulin signalling was only modestly impaired (23). Therefore, we hypothesised that insulin signalling was in part rescued by reduced insulin clearance, but a role for adaptive changes in the IGF1 axis and hybrids of IGF1R and insulin receptors was not investigated.

To gain further insights into the role of adiponectin, leptin and IGF axis in relation to SIR, we characterised the multimeric distribution of adiponectin, leptin, LAR, circulating IGFs, IGFBP1 and IGFBP2 in carriers of a dominant-negative *INSR* mutation and matched lean, healthy controls.

Materials and methods

Study subjects

Clinical and genetic data have been reported previously (22). Six family members carrying the Arg1174Gln mutation in the tyrosine kinase domain of the *INSR* gene and ten healthy control subjects matched according to age, sex and BMI participated in the study (Table 1). Compared with the original report (22), four additional control subjects were included. The Arg1174Gln carriers were clinically characterised by episodes of hypoglycaemia in the postprandial state, and all were without diabetes and showed no clinical features of SIR. The control subjects had normal glucose tolerance and no family history of diabetes. All subjects had normal results on screening blood tests of hepatic and renal function. All subjects were instructed to refrain from strenuous physical activity for a period of 48 h before the experiment. Informed consent was obtained from all subjects before participation. The study was approved by the local ethics committee and performed in accordance with the Helsinki-II Declaration.

Study design

All study subjects were admitted to the Diabetes Research Centre at Odense University Hospital.

Table 1 Clinical, biochemical and metabolic characteristics of study subjects. Metabolic rates are expressed as mg/min per m². Data represent mean $\pm$ S.E.M.

	Control group	Arg1174Gln group	P value
Male/female	6/4	5/1	NS
Age (years)	46.8 $\pm$ 2.0	41.7 $\pm$ 5.6	0.48
BMI (kg/m ²)	24.4 $\pm$ 0.7	25.4 $\pm$ 1.0	0.52
Fat-free mass	49.0 $\pm$ 2.9	48.5 $\pm$ 2.0	0.91
HbA1c (%)	5.1 $\pm$ 0.1	5.7 $\pm$ 0.2	0.039
Total cholesterol (mmol/l)	5.2 $\pm$ 0.2	5.7 $\pm$ 0.4	0.36
LDL-cholesterol (mmol/l)	3.0 $\pm$ 0.2	3.6 $\pm$ 0.3	0.14
HDL-cholesterol (mmol/l)	1.6 $\pm$ 0.1	1.5 $\pm$ 0.2	0.66
Plasma triglycerides (mmol/l)	1.3 $\pm$ 0.3	1.3 $\pm$ 0.2	NS
Serum C-peptide (pmol/l)	424 $\pm$ 42	553 $\pm$ 64	0.16
Plasma glucose basal (mmol/l)	5.3 $\pm$ 0.1	5.6 $\pm$ 0.2	0.21
Plasma glucose clamp (mmol/l)	5.2 $\pm$ 0.1	5.3 $\pm$ 0.1	0.39
Serum insulin basal (pmol/l)	18 $\pm$ 2	177 $\pm$ 31	0.0011
Serum insulin clamp (pmol/l)	332 $\pm$ 10 [†]	1313 $\pm$ 127*	0.0011
Serum C-peptide basal (pmol/l)	424 $\pm$ 42	553 $\pm$ 64	0.16
Serum C-peptide clamp (pmol/l)	394 $\pm$ 57	527 $\pm$ 101	0.42
Insulin clearance (ml/min per m ²)	759 $\pm$ 110	192 $\pm$ 45	<0.001
Plasma FFA basal (nmol/l)	529 $\pm$ 56	382 $\pm$ 77	0.23
Plasma FFA clamp (nmol/l)	17 $\pm$ 4 [†]	118 $\pm$ 48*	0.0061
GDR basal	75 $\pm$ 3	76 $\pm$ 3	0.66
GDR clamp	352 $\pm$ 21 [†]	200 $\pm$ 34*	0.0067
Glucose oxidation basal	48 $\pm$ 4	48 $\pm$ 6	0.59
Glucose oxidation clamp	128 $\pm$ 7 [†]	100 $\pm$ 14*	0.11
Lipid oxidation basal	39 $\pm$ 2	37 $\pm$ 3	0.74
Lipid oxidation clamp	10 $\pm$ 2 [†]	21 $\pm$ 6*	0.19
NOGM basal	27 $\pm$ 4	27 $\pm$ 5	0.74
NOGM clamp	225 $\pm$ 18 [†]	100 $\pm$ 24*	0.0048

* $P < 0.05$ and [†] $P < 0.01$ vs basal. NOGM, non-oxidative glucose metabolism.

Denmark. After a 10 h overnight fast, the subjects underwent a euglycaemic–hyperinsulinaemic clamp (2 h equilibrium period followed by 3 h insulin infusion, 40 mU/m² per min) combined with indirect calorimetry and muscle biopsies. Please refer to our previous report (22) for further details about calculation of glucose disposal rates (GDR), glucose oxidation, lipid oxidation, non-oxidative glucose metabolism (NOGM) and insulin clearance. Blood samples were drawn in the basal and insulin-stimulated state. Plasma glucose, serum insulin, C-peptide and FFA were measured using commercial assays as described previously (22). Fat-free mass was determined by the bioimpedance method.

Total adiponectin and adiponectin multimers

Serum total adiponectin was measured using a validated in-house time-resolved immunofluorometric assay (TR-IFMA) as described previously (24), having an intra-assay coefficient of variance (CV) <5% and interassay CV <10%. The three major subforms of circulating adiponectin, HMW, MMW and LMW subforms were isolated by fast protein liquid chromatography (FPLC), followed by immunoassay of the obtained fractions corresponding to the three major subforms. The FPLC method has been described previously in detail elsewhere (25). The intra-assay CV values for the relative concentrations of the HMW, MMW and LMW subforms were <4, 6 and 3% respectively. The interassay CV values were 6, 12 and 7% respectively (25).

Leptin

Leptin was determined by a validated in-house TR-IFMA (26) based on commercial reagents (R&D Systems, Abingdon, UK: two MABs (cat. no. MAb 398 for coating and BAM 398 for detection) and recombinant human leptin as standard (cat. no. 398-LP)) and carried out essentially as the adiponectin TR-IFMA. The recovery of exogenous leptin added to serum averaged 96.8 ± 0.2%, the intra-assay CV averaged <5% and the interassay CV averaged <10% (>50 assay setups).

Total IGF1, IGF2, free IGF1 and IGFBP1 and -2

All measurements were performed in duplicate or triplicate (free IGF1) within the same assay run. With the exception of free IGF1 (see below), all intra-assay CVs were <5%. Serum total IGF1 and total IGF2 were determined after acid ethanol extraction using non-competitive TR-IFMAs as described previously (27). Serum-free IGF1 was determined using ultrafiltration by centrifugation, as described previously (28). The intra-assay CV including ultrafiltration and immunoassay averaged 19%. Serum IGFBP1 was determined with an in-house RIA as described by

Westwood *et al.* (29, 30), with modifications. Serum IGFBP2 was measured using an in-house TR-IFMA as described previously (30).

Statistical analysis

Data calculation and statistical analysis were performed using the SPSS for Windows Version 10.0 program. Data are presented as mean ± S.E.M. Differences between the groups or within the groups were evaluated using the Mann–Whitney *U* test for unpaired data and the Wilcoxon signed rank test for paired data respectively (two sided). Correlation analysis was performed using Spearman's correlation analysis. Significance was accepted at the level of *P* < 0.05.

Results

Clinical and metabolic characteristics

As reported previously (22), Arg1174Gln carriers had pronounced fasting hyperinsulinaemia and slightly elevated HbA1c levels (within the normal range) compared with lean, healthy controls (Table 1). Nevertheless, fasting plasma glucose and serum C-peptide were similar between groups. Exogenous insulin infused at a rate of 40 mU/m² per min raised serum insulin to fourfold higher levels in the Arg1174Gln group than in controls due to reduced clearance of insulin (Table 1). There was no effect of insulin on serum C-peptide in either group. In both groups, insulin infusion increased GDR, glucose oxidation and NOGM and suppressed lipid oxidation and plasma FFA (Table 1). However, the effect of exogenous insulin on GDR and NOGM was significantly reduced in the Arg1174Gln group.

Adiponectin and leptin

Despite fasting hyperinsulinaemia and marked insulin resistance, total plasma adiponectin levels in Arg1174Gln carriers were not different from those observed in lean, healthy controls (Table 2). However, the absolute concentrations of LMW adiponectin were reduced in Arg1174Gln carriers (*P* < 0.05), whereas HMW and MMW adiponectin did not differ between groups (Fig. 1A). When evaluating the relative distribution of the adiponectin subforms, the HMW fraction was increased in Arg1174Gln carriers (*P* < 0.05), whereas the LMW fraction was reduced compared with lean, healthy subjects (*P* < 0.001) both before and after exogenous insulin (Fig. 1B). The MMW fraction was also decreased in Arg1174Gln carriers, but only after infusion of insulin (*P* = 0.04). Administration of insulin in physiological concentrations over 3 h caused a small but significant reduction in total adiponectin (7%; *P* = 0.02) and MMW adiponectin (9%; *P* = 0.02) in lean, healthy controls, whereas no

Table 2 Adiponectin, leptin and LAR. Data represent mean $\pm$ s.e.m.

	Control group	Arg1174-Gln group	P value
Plasma adiponectin basal (mg/l)	12.1 $\pm$ 1.5	11.5 $\pm$ 1.8	0.91
Plasma adiponectin clamp (mg/l)	11.3 $\pm$ 1.6*	11.3 $\pm$ 1.7	0.91
Serum leptin basal (μ g/l)	6.9 $\pm$ 1.9	5.6 $\pm$ 1.0	0.91
Serum leptin clamp (μ g/l)	6.6 $\pm$ 1.7	4.7 $\pm$ 1.2*	0.52
LAR basal	0.61 $\pm$ 0.198	0.55 $\pm$ 0.13	0.72
LAR clamp	0.62 $\pm$ 0.17	0.48 $\pm$ 0.14	0.66

* $P < 0.05$ vs basal. LAR, leptin-to-adiponectin ratio.

effect of exogenous insulin was observed in Arg1174Gln carriers despite fourfold higher insulin levels at the end of the clamp. No differences in serum leptin levels or LAR ratios were found between Arg1174Gln carriers and lean, healthy individuals (Table 2). However, in Arg1174Gln carriers, exogenous insulin caused a small reduction in serum leptin levels ($P = 0.03$).

IGF axis

Arg1174Gln carriers showed increased circulating levels of total IGF1 ($P = 0.007$), total IGF2 ($P = 0.01$) and free IGF1 ($P = 0.007$) compared with lean, healthy individuals (Fig. 2). However, despite insulin resistance, and fasting hyperinsulinaemia, serum levels of IGFBP1 and IGFBP2 in Arg1174Gln carriers did not differ from those observed in lean, healthy individuals (Fig. 3). Moreover, the ability of exogenous insulin to acutely suppress IGFBP1 ($P = 0.03$) and increase IGFBP2 ($P = 0.05$) was intact in Arg1174Gln carriers.

Correlation analysis

In Arg1174Gln carriers, total adiponectin and absolute concentrations of MMW and LMW subforms were closely correlated with insulin action on GDR (all $r = 0.94$; $P = 0.005$), glucose oxidation (all $r = 0.83$; $P = 0.04$), lipid oxidation (all $r = -0.94$; $P = 0.005$) and FFA (all $r = -0.94$; $P = 0.005$). HMW adiponectin also correlated with insulin action on GDR ($r = 0.89$; $P = 0.02$), lipid oxidation ($r = -0.89$; $P = 0.02$) and FFA ($r = -0.89$; $P = 0.02$). Furthermore, IGFBP1 was strongly associated with basal FFA ($r = 0.99$; $P < 0.001$) and basal leptin correlated with HDL-cholesterol ($r = -0.89$; $P = 0.02$) in Arg1174Gln carriers.

In lean, healthy controls, no correlations between adiponectin or its subforms and measures of glucose and lipid metabolism were observed. However, IGFBP1 correlated inversely with fasting insulin ($r = -0.70$; $P = 0.02$) and positively with insulin-stimulated GDR ($r = 0.70$; $P = 0.03$) and NOGM ($r = 0.70$; $P = 0.03$), whereas IGFBP2 was negatively related to HbA1c ($r = -0.79$; $P = 0.004$) and FFA clamp ($r = -0.79$; $P = 0.006$) in controls. Moreover, total IGF1 was positively associated with HbA1c ($r = 0.72$; $P = 0.02$)

in controls. Basal leptin correlated positively with glucose oxidation ($r = 0.67$; $P = 0.05$) and FFA ($r = 0.68$; $P = 0.046$) during clamp.

Total IGF1 and free IGF1 were strongly associated in both Arg1174Gln carriers ($r = 1.00$; $P < 0.001$) and controls ($r = 0.81$; $P = 0.009$). Moreover, IGFBP2 correlated inversely with basal serum leptin in Arg1174Gln carriers ($r = -0.89$; $P = 0.02$) as well as in healthy control subjects ($r = -0.83$; $P = 0.005$).

Discussion

Recent studies have shown apparently paradoxical high adiponectin in humans with SIR caused either by *INSR* mutations or insulin receptor antibodies (7, 8). Here, we have characterised the multimeric distribution of adiponectin and the IGF axis in carriers of a dominant-negative mutation in the tyrosine kinase domain of *INSR* (Arg1174Gln). The major findings are that total adiponectin and absolute levels of HMW adiponectin

Figure 1 Absolute levels (A) and fractions (B) of HMW, MMW and LMW adiponectin in ten healthy subjects (Con) and six carriers of a dominant-negative mutation (Arg1174Gln) in the tyrosine kinase domain of the insulin receptor gene (Mut) before (white bars) and at the end (black bars) of a 3 h, 40 mU/m² per min euglycaemic-hyperinsulinaemic clamp. Data are mean $\pm$ s.e.m. * $P < 0.05$ and § $P < 0.001$ vs Con; # $P < 0.05$ vs basal.

Figure 2 Baseline serum levels of total IGF1 (A), total IGF2 (B) and free IGF1 (C) in ten healthy controls (white bars) and six carriers of a dominant-negative mutation (Arg1174Gln) in the tyrosine kinase domain of the insulin receptor gene (black bars). Data are mean ± S.E.M. ## $P < 0.001$ and # $P < 0.05$ vs controls.

are not decreased in these insulin receptor-deficient patients despite fasting hyperinsulinaemia and marked insulin resistance. The plasma levels of other markers for common insulin resistance, such as leptin, IGFBP1 and

IGFBP2, were also completely normal in Arg1174Gln carriers. Our results are consistent with the hypothesis that high fasting levels of insulin cannot suppress these markers of common insulin resistance in patients with dysfunctional insulin receptors (5). Circulating levels of total IGF1, IGF2 and free IGF1 were elevated, probably reflecting a compensatory increase. Interestingly, the distribution of adiponectin complexes showed an increased ratio of HMW adiponectin and decreased levels of LMW adiponectin, and total plasma adiponectin and its subforms strongly correlated with insulin action on glucose metabolism and lipid oxidation in Arg1174Gln carriers. These findings indicate that the preservation of adiponectin levels together with an improved distribution and adaptive increases in IGF1 and -2 may contribute to rescue insulin action in insulin receptor-deficient patients.

Based on fasting insulin levels above 150 pmol/l, all but one of the Arg1174Gln carriers could be categorised as having SIR (9). Moreover, all but one of these six carriers of an *INSR* mutation showed plasma adiponectin above 7 mg/l, and they all showed plasma IGFBP1 above 20 µg/l. Based on the observations done mostly in patients with homozygous or compound heterozygous mutations in *INSR* mutations (7), these criteria have been proposed to separate cases of dysfunctional insulin receptors from other causes of SIR (9). However, in agreement with the normal rather than high levels of adiponectin in the Arg1174Gln carriers, the lowest adiponectin levels were previously observed in two patients with heterozygote *INSR* (7). Nevertheless, it appears that also in Arg1174Gln carriers, chronic fasting hyperinsulinaemia fails to suppress adiponectin similar to that observed in cases with biallelic *INSR* mutations. Indeed, our data confirm that measures of adiponectin and IGFBP1 may help to point out dysfunctional insulin receptors in cases with SIR. However, compared with common forms of insulin resistance such as obesity and type 2 diabetes, other measures may be better discriminators. Thus, Arg1174Gln carriers are characterised by highly increased fasting insulin-to-C-peptide ratios (well above 0.1) due to a fourfold decrease in the clearance of insulin from the circulation (22).

Compared with individuals with common insulin resistance, the levels of adiponectin were surprisingly normal in the Arg1174Gln carriers in the face of highly elevated fasting insulin levels. Evidence from genetically modified mouse models (5) evidence from studies showing the presence of high adiponectin levels in type 1 diabetes (12) and evidence from studies showing insulin infusion for 3–5 h suppresses plasma adiponectin in healthy subjects (10, 11); all indicate that insulin negatively regulates adiponectin expression and/or secretion from adipocytes. In line with this the apparently paradoxical hyperadiponectinaemia observed in SIR could be explained by complete insulin receptor dysfunction, which impairs not only insulin signalling to glucose transport but also other actions of insulin (5).

Figure 3 Serum levels of IGFBP1 (A) and IGFBP2 (B) in ten healthy controls and six carriers of a dominant-negative mutation (Arg1174Gln) in the tyrosine kinase domain of the insulin receptor gene before (white bars) and at the end (black bars) of a 3 h 40 mU/m² per min euglycaemic-hyperinsulinaemic clamp. Data are mean $\pm$ S.E.M. * P < 0.05 and ** P < 0.001 vs basal.

Accordingly, fasting hyperinsulinaemia is believed to be the cause of reduced plasma adiponectin in common forms of insulin resistance. In support of this hypothesis, humans with loss-of-function mutation in Akt2 show SIR together with low levels of adiponectin (7). Assuming that carriers of a dominant-negative *INSR* mutation have about 25% fully functional insulin receptors (31), it was expected that the tenfold higher insulin levels would be enough to suppress plasma adiponectin below normal levels. This was, however, not the case, and our findings agree with reports of normal adiponectin levels (6–10 mg/l) in the presence of extreme fasting hyperinsulinaemia (> 1500 pmol/l) in two other cases of heterozygote *INSR* mutations (7). In support of an attenuated response to insulin in Arg1174Gln carriers, a 3 h insulin infusion raised insulin levels to 1300 pmol/l but failed to suppress plasma adiponectin. Thus, it appears that 25% fully functional insulin receptors in adipocytes are not sufficient to induce a suppression of plasma adiponectin despite tenfold elevated insulin levels. This suggests that other mechanisms may be operating to keep plasma adiponectin high in insulin receptor-deficient individuals.

In the majority of studies, total and HMW adiponectin are reduced in obesity and type 2 diabetes and show stronger association with insulin resistance, risk of type 2 diabetes and coronary artery diseases than MMW and LMW adiponectin (32, 33, 34, 35, 36, 37, 38). In this context, the finding of normal total and HMW adiponectin and the relative increase in the fraction of HMW adiponectin and the decrease in absolute levels and fraction of LMW adiponectin may play a beneficial role for the Arg1174Gln carriers. However, in a recent study, LMW adiponectin was the only decreased subform in obesity and type 2 diabetes (39). This indicates that the role of LMW adiponectin in the pathogenesis of insulin resistance and type 2 diabetes remains to be fully defined, and hence, further studies are required to safely conclude that high HMW and low LMW adiponectin represent an improved multimeric distribution of adiponectin. This is, to some extent, also reflected by the observation that all three subforms of adiponectin showed tight correlation with measures of insulin action on glucose and lipid metabolism in the small group of Arg1174Gln carriers, but not in the controls. This suggests the possibility that adiponectin may play a relatively greater role for preservation of insulin sensitivity in insulin receptor-deficient individuals and that the paradoxically high levels of adiponectin in insulin receptoropathies may represent a compensatory increase rather than simply being the consequence of dysfunctional insulin receptors.

To our knowledge, this is the first case-control study of the IGF axis in carriers of a dominant-negative *INSR* mutation. We report increased levels of total IGF1, total IGF2 and free IGF1. While total IGF2 and free IGF1 have been reported to be increased in obesity and type 2 diabetes (15, 16, 17), in most studies no change or even a decrease in total IGF1 has been reported (15, 16, 17, 40, 41). Moreover, lower levels of total IGF1 may increase the risk of type 2 diabetes (42) and are associated with poorer glycaemic control in type 2 diabetes (41). In obesity and type 2 diabetes, the increase in free IGF1 and total IGF2 is considered as compensatory to insulin resistance, with the increase in free IGF1 being mainly a consequence of reduced IGFBP1 (15). Whether the increase in total IGF1 in Arg1174Gln carriers is caused by increased synthesis or reduced clearance cannot be determined from this study. Previously, low levels of total IGF1 have been reported in a case with biallelic *INSR* mutations (43). In this case, increased clearance of IGF1 was found and proposed to explain the failure of IGF1 therapy. The higher levels of both total and free IGF1 in the Arg1174Gln carriers suggest that IGF1 therapy of carriers of dominant-negative *INSR* mutations would have only limited beneficial effects. The higher free IGF1, despite normal IGFBP1, seems to be directly explained by the increase in total IGF1. Thus, a strong correlation between total and free IGF1 was preserved in Arg1174Gln carriers. The elevated total and free

IGF1 levels may help to preserve not only insulin sensitivity (40) but also β -cell function (15) in the Arg1174Gln carriers. In this context, it is of interest that so far none of the ten family members in three generations carrying the Arg1174Gln mutation in *INSR* have developed fasting hyperglycaemia or type 2 diabetes (22). Of course, we cannot exclude that these family members share other gene variants that are in linkage disequilibrium with the *INSR* mutation and that protects against β -cell failure despite marked insulin resistance.

The intact acute regulation of IGFBP1 and IGFBP2 by insulin suggests that 25% normal insulin receptors in the tissues secreting IGFBP1 and IGFBP2 are sufficient, although the elevated levels of clamp insulin may also elicit insulinomimetic effects on IGF1 receptors or hybrids in, e.g. the liver (15). However, similar to adiponectin, the highly elevated fasting insulin levels in Arg174Gln carriers were not sufficient to cause reduced levels of IGFBP1 and IGFBP2, as observed in common forms of insulin resistance. This suggests that the effects of chronically elevated insulin levels on circulating markers of insulin resistance cannot simply be extrapolated from the effects of prolonged insulin infusion.

Of interest, we found a strong inverse relationship between fasting levels of IGFBP2 and leptin, both in the Arg1174Gln carriers and in the control subjects. Thus, the normal serum leptin does not seem to be the cause of normal serum IGFBP2 in these insulin receptor-deficient individuals. At first sight, this contrasts with the recent finding that leptin administration to *ob/ob* mice (which lack leptin) induces hepatic *IGFBP2* mRNA and increases circulating IGFBP2 and that this response reduces glucose and insulin resistance (44). Moreover, pharmacological IGFBP2 levels were shown to reverse both type 1 and type 2 diabetes in mice (44), and based on the findings in mice, leptin therapy for type 1 diabetes in humans is currently debated (45). Indeed, serum IGFBP2 increased in two of three patients with lipodystrophy in response to leptin therapy for 6 months (46). However, consistent with our results, another recent study demonstrated the same inverse relationship between leptin and plasma IGFBP2 in humans (46). In addition, low IGFBP2 and high leptin are consistently associated with decreased insulin sensitivity even in healthy populations (15). This suggests that the ability of leptin to increase IGFBP2 is restricted to leptin-deficient conditions, although this also warrants further studies on humans.

In conclusion, in carriers of a dominant-negative *INSR* mutation, markers of common insulin resistance such as adiponectin, leptin, IGFBP1 and IGFBP2 are normal in the context of highly elevated fasting insulin levels and marked insulin resistance. Together with adaptive changes in the IGF axis and a potentially improved multimeric distribution of adiponectin, this may help to preserve insulin action in insulin receptor-deficient patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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