Assessing and targeting insulin resistance

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CHAPTER THREE

IMPAIRED INSULIN ACTION IN THE LIVER, BUT NOT IN ADIPOSE TISSUE OR MUSCLE, IS A DISTINCT METABOLIC FEATURE OF IMPAIRED FASTING GLUCOSE IN OBESE HUMANS

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Impaired insulin action in the liver, but not in adipose tissue or muscle, is a distinct metabolic feature of impaired fasting glucose in obese humans

Abstract

Aim: Elevated basal endogenous glucose production (EGP), impaired suppression of EGP by insulin and reduced insulin-stimulated glucose disposal are cornerstones of the pathogenesis of hyperglycemia in patients with type 2 diabetes. We aimed to determine the contribution of these processes to impaired fasting glucose (IFG) levels in obese non-diabetic adults.

Methods: We included 131 obese non-diabetic adults with normal fasting glucose levels (NFG; fasting glucose <5.6 mmol/l; 62 men, 25 women; mean±SEM age 49±1 y; median (IQR) BMI 36 (34-41) kg/m^2) or IFG (fasting glucose 5.6-6.9 mmol/l; 35 men, 9 women; age 53±1 y; BMI 36 (34-42) kg/m^2) and studied basal EGP and hepatic, adipose tissue and peripheral insulin sensitivity by two-step euglycemic hyperinsulinemic clamp studies with [6,6-$^2H_2$]glucose infusion.

Results: Compared to equally obese adults with NFG, individuals with IFG did not differ in basal EGP (9.1±0.2 vs 9.8±0.3 μmol·kg^{-1}·min^{-1}, p=0.082), insulin-mediated suppression of circulating free fatty acid levels (75±1 vs 72±3%, p=0.240) and insulin-stimulated glucose disposal (26.6±1.0 vs 25.2±1.5 μmol·kg^{-1}·min^{-1}, p=0.441). Insulin-mediated suppression of EGP (68±2 vs 55±3%, p<0.001) was markedly reduced in obese subjects with IFG.

Conclusions: Hepatic insulin resistance is a distinct metabolic feature of IFG in obesity. Insulin sensitivity of free fatty acid suppression and skeletal muscle does not differ between obese people with NFG and IFG. Hepatic insulin resistance may contribute to the onset of prediabetes in obese adults.

Introduction

Individuals with impaired fasting glucose (IFG), defined as fasting glucose levels ≥5.6 and <7.0 mmol/l [1], have increased risk of future diabetes development and incident cardiovascular disease [2, 3]. Early detection and management of prediabetes or metabolic syndrome is an appealing strategy for prevention of clinically overt diabetes and its complications [1]. A better understanding of the pathogenesis of IFG may pave the way for more targeted and individualized preventive strategies.

Disturbed glucose metabolism in patients with type 2 diabetes is characterized by elevated basal endogenous glucose production (EGP), impaired suppression of EGP by insulin and reduced insulin-stimulated glucose uptake, and all contribute to the pathogenesis of hyperglycemia [4]. Currently, however, the contribution of these and other processes to the pathogenesis of IFG remains only partially elucidated. Most [5-7], but not all [8], studies suggest that inappropriately elevated EGP or hepatic insulin resistance are important contributors to IFG. Similarly, insulin-stimulated glucose disposal or peripheral insulin sensitivity in people with IFG was reduced in some studies [5, 9, 10], but was similar to controls with normal fasting glucose (NFG) in others studies [6-8, 11]. The reasons for these inconclusive results are unclear, but may relate to limited sample size of metabolic/tracer studies, inappropriately matched control groups and/or the use of different methods (e.g. insulin suppression test vs euglycemic hyperinsulinemic clamp studies).

In the present study, we aimed to determine the contribution of basal EGP, hepatic insulin resistance, insulin sensitivity of free fatty acid (FFA) suppression and peripheral insulin resistance to the pathogenesis of IFG in a large number of consecutive obese non-diabetic adults using gold-standard metabolic measurement techniques.

Material and methods

Subjects

Subjects participated in metabolic studies in the Academic Medical Center (Amsterdam, Netherlands), results of which have been partially described [12-15]. The present study was limited to treatment-naive obese (body mass index [BMI] >30 kg/m^2) adults with stable weight (<5% weight change) for at least three months prior to the study date, who had undergone a two-step euglycemic hyperinsulinemic clamp according to standard operating procedures. Normal fasting glucose (NFG) was de-

fined as fasting glucose levels <5.6 mmol/l; impaired fasting glucose (IFG) as fasting glucose levels ≥5.6 and <7.0 mmol/l [1]. Subjects with overt diabetes (defined by one of the following: self-reported history, use of oral hypoglycemic agents and/or exogenous insulin, and/or fasting glucose ≥7.0 mmol/l) were excluded. Other exclusion criteria were substance abuse (alcohol >2 units/day, recreational drugs), use of antipsychotic or antidepressant medication, or any somatic disorder except for obesity-related conditions (e.g. secondary dyslipidemia, secondary hypertension, obstructive sleep apnea). All subjects completed a medical evaluation including history, physical examination and blood tests prior to the study date. All procedures were approved by the Academic Medical Center medical ethics committee and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

Experimental protocol

Basal endogenous glucose production, insulin-mediated suppression of EGP (hepatic insulin sensitivity) and the rate of disappearance (R_d) of glucose (peripheral/muscle insulin sensitivity) were assessed during a two-step euglycemic hyperinsulinemic clamp [16]. We also determined insulin sensitivity of FFA suppression, which in part reflects insulin sensitivity of inhibition of adipose tissue lipolysis as plasma FFA concentration and tracer-determined FFA release are strongly correlated in obese subjects [17].

Subjects were studied after an overnight fast. A primed continuous infusion of the stable isotopically labeled glucose tracer [6,6-$^2H_2$]glucose (prime 11 μmol/kg; continuous 0.11 μmol·kg^{-1}·min^{-1}; >99% enriched; Cambridge Isotopes, Andover, MA, USA) was started at 0800 h, and continued until the end of the experiment. After two hours of tracer equilibration (1000 h), infusion of human recombinant insulin (Novo Nordisk Farma, Alphen aan de Rijn, Netherlands) was started at a rate of 20 mU·m^{-2}·min^{-1} (step 1). During hyperinsulinemia, plasma glucose was measured every 10 min and 20% glucose, enriched with 1% [6,6-$^2H_2$]glucose to approximate plasma enrichment, was infused at a variable rate to maintain plasma glucose at 5.0 mmol/l. After two hours of low-dose insulin infusion (1210 h), the rate was increased to 60 mU·m^{-2}·min^{-1} for an additional two hours (step 2). Three (after tracer equilibration) or five (at the end of each step of insulin infusion) arterialized venous blood samples with five-min intervals were drawn for determination of tracer enrichment and hormones.

Analytical techniques

Plasma glucose concentration was determined with the glucose oxidase method using

a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barleben/Magdeburg, Germany) or a Beckman autoanalyzer (Beckman, Fullerton, CA, USA). Abnormal fasting plasma glucose levels were confirmed by immediate repeat testing in a new blood sample [1]. Insulin and cortisol were determined by immunoassay on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA), with intra-assay variation of 4-5% and 3-6%, respectively, and inter-assay variation of 5% and 5-7%, respectively. Glucagon was determined by radioimmunoassay (Linco Research, St Charles, MO, USA), with intra-assay variation of 4-8% and inter-assay variation of 6-11%. Plasma FFA were determined by an enzymatic colorimetric method (NEFA C test kit [Wako Chemicals, Neuss, Germany]), with intra-assay variation of 1% and inter-assay variation of 4-15%. Enrichment of [6,6-$^{2}H_{2}$]glucose (tracer-to-tracee ratio) in plasma and exogenous glucose infusate was determined by gas chromatography-mass spectrometry as described [18]. Body composition was determined by bioelectrical impedance analysis (Maltron BF-906, Rayleigh, UK). Indirect calorimetry was performed using a ventilated hood system (Vmax Encore 29n, CareFusion, San Diego, CA, USA).

Calculations

Glucose fluxes (EGP and R_d) were calculated using modified versions of the Steele equations for the steady state (basal) or non-steady state (during insulin infusion) as previously described [19, 20], and expressed as μmol·(kg body weight)$^{-1}$·min^{-1}. Insulin-mediated suppression of EGP and FFA were assessed during step 1 of the clamp (low-dose insulin infusion), while insulin-stimulated glucose disposal was assessed during step 2 (high-dose insulin infusion).

Statistical analyses

Data are presented as mean ± standard error of the mean (SEM) or median (interquartile range [IQR]) unless stated otherwise. Depending on type and distribution of variable, we used two-sided independent samples t, Mann-Whitney U or χ^2 tests to compare results between NFG and IFG groups. There was a small difference in age between groups (**Table 1**) and we repeated all between-group comparisons of continuous variables using analysis of covariance (ANCOVA) to adjust for age. Correlations between two normally distributed variables were evaluated by Pearson's correlation coefficient (r). We performed multiple logistic regression analysis to assess independent associations between metabolic parameters and IFG. Non-normally distributed data entered into the adjustment model were log-trans-

Table 1. Baseline characteristics of included subjects.

	Normal fasting glucose	Impaired fasting glucose	p
n	87	44	-
Sex (M/F)	62 / 25	35 / 9	0.307
Age (y)	49 ± 1	53 ± 1	0.022
BMI (kg/m^2)	36.4 (33.7 – 40.9)	36.2 (33.5 – 41.7)	0.554
Body fat (%)	46.9 ± 1.1	45.4 ± 2.0	0.477
Glucose (mmol/l)	5.1 (4.8 – 5.4)	6.0 (5.8 – 6.4)	< 0.001[a]
Triglycerides (mmol/l)	1.3 (0.9 – 1.8)	1.1 (0.9 – 1.9)	0.525
Cholesterol (mmol/l)	4.8 ± 0.1	4.4 ± 0.2	0.193
LDL (mmol/l)	3.2 ± 0.1	3.1 ± 0.1	0.351
HDL (mmol/l)	1.1 ± 0.0	1.0 ± 0.0	0.821
Alanine aminotransferase (U/l)	35 ± 2	32 ± 4	0.441
Aspartate aminotransferase (U/l)	26 (20 – 36)	26 (4 – 30)	0.336
Gamma-glutamyl transpeptidase (U/l)	32 (28 – 41)	24 (18 – 37)	0.133
C-reactive protein (mg/l)	8.1 ± 1.4	4.0 ± 1.0	0.143
Resting energy expenditure (kcal/day)	1957 ± 47	2029 ± 46	0.310

Data are presented as mean ± SEM or median (IQR) and compared by two-sided independent samples t or Mann-Whitney U test, respectively. Impaired fasting glucose was defined as fasting plasma glucose concentration ≥ 5.6 mmol/l and < 7.0 mmol/l. [a] By study design.

formed. Findings were considered statistically significant if $p<0.05$. Analyses were performed using IBM SPSS Statistics 22 (Armonk, NY, USA).

Results

Participants

We included 131 obese subjects. Eighty-seven subjects had fasting glucose levels <5.6 mmol/l and were assigned to the NFG group; 44 subjects had fasting glucose

Table 2. Data during two-step euglycemic hyperinsulinemic clamp studies in included subjects.

	Normal fasting glucose	Impaired fasting glucose	*p*
Basal (fasting)			
Insulin (pmol/l)	99 ± 5	114 ± 8	0.084
Glucagon (ng/l)	77 ± 3	70 ± 3	0.102
Cortisol (nmol/l)	240 ± 14	261 ± 17	0.351
FFA (mmol/l)	0.65 ± 0.03	0.61 ± 0.03	0.533
First step of insulin infusion			
Insulin (pmol/l)	276 ± 9	261 ± 12	0.313
Glucagon (ng/l)	65 ± 3	66 ± 3	0.773
Cortisol (nmol/l)	246 ± 12	251 ± 16	0.818
FFA (mmol/l)	0.16 ± 0.01	0.18 ± 0.02	0.386
Second step of insulin infusion			
Insulin (pmol/l)	715 ± 18	736 ± 26	0.509
Glucagon (ng/l)	53 ± 2	52 ± 3	0.892
Cortisol (nmol/l)	244 ± 13	228 ± 14	0.448
FFA (mmol/l)	0.04 ± 0.00	0.05 ± 0.01	0.357

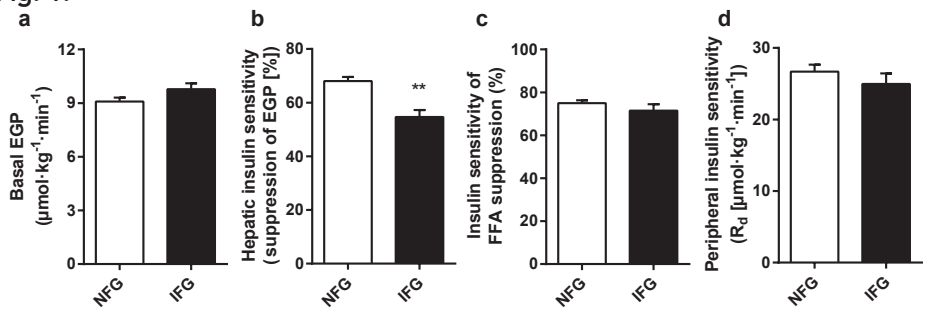
Data are presented as mean ± SEM and compared by two-sided independent samples t test.

levels ≥5.6 and <7.0 mmol/l and were assigned to the IFG group. Subjects with IFG were slightly, but significantly older than subjects with NFG (**Table 1**). Other baseline clinical and biochemical characteristics were comparable between groups (**Table 1**). Adjusting for age did not alter the results (p>0.05 for all baseline variables, except fasting glucose).

Basal glucose metabolism and insulin sensitivity

Plasma enrichments of the glucose tracer during each stage of the clamp are presented in **Supplementary Table 1**. Fasting plasma insulin levels (**Table 2**) and the

Fig. 1.



(a) *Basal endogenous glucose production (EGP),* **(b)** *hepatic insulin sensitivity (expressed as the insulin-mediated suppression of basal EGP),* **(c)** *insulin sensitivity of free fatty acid (FFA) suppression, and* **(d)** *peripheral insulin sensitivity (expressed as the rate of disappearance [R_d] of glucose) in obese adults with normal (NFG) or impaired fasting glucose (IFG). ** p<0.001.*

basal rate of EGP **(Fig. 1a**) trended to be higher in obese subjects with IFG, but these differences were not statistically significant (p=0.084 and p=0.082, respectively). When basal EGP was expressed relative to lean body mass, there was no difference between obese subjects with NFG or IFG (14.6±0.4 vs 15.1±0.8 μmol·(kg fat-free mass [FFM])$^{-1}$·min^{-1}, p=0.543).

Insulin infusion during the clamp resulted in two steps of comparable hyperinsulinemia among NFG and IFG subjects (**Table 2**), indicating similar rates of insulin clearance between groups. During the first step of low-dose insulin infusion, suppression of EGP ranged from 24.9% to 100%, with complete suppression (100%) in only one obese subject (**Supplementary Fig. 1**), demonstrating that the insulin dose was adequate to detect differences in hepatic insulin sensitivity in the present cohort of obese subjects.

Insulin-mediated suppression of EGP was reduced by 20% in subjects with IFG (54.6±3 vs 68.1±2%, *p*<0.001; **Fig. 1b**), indicating that hepatic insulin sensitivity is reduced in IFG. Suppression of circulating FFA during insulin infusion, which in part reflects insulin-mediated suppression of adipose tissue lipolysis, did not differ between subjects with NFG and with IFG (**Fig. 1c**), indicating similar insulin sensitivity of FFA suppression.

Peripheral insulin sensitivity, assessed as the insulin-stimulated R_d of glucose during the second step of insulin infusion, was low in all obese subjects compared to a healthy non-obese reference population [16], but did not differ between groups when expressed as absolute value (**Fig. 1d**) or relative to FFM or basal glucose

uptake (**Supplementary Table 2**).

Levels of glucoregulatory hormones did not differ in the basal state or during both steps of insulin infusion between groups (**Table 2**). Adjusting for age did not alter the results ($p<0.001$ for suppression of EGP; $p>0.05$ for all other outcomes).

Factors associated with impaired fasting glucose

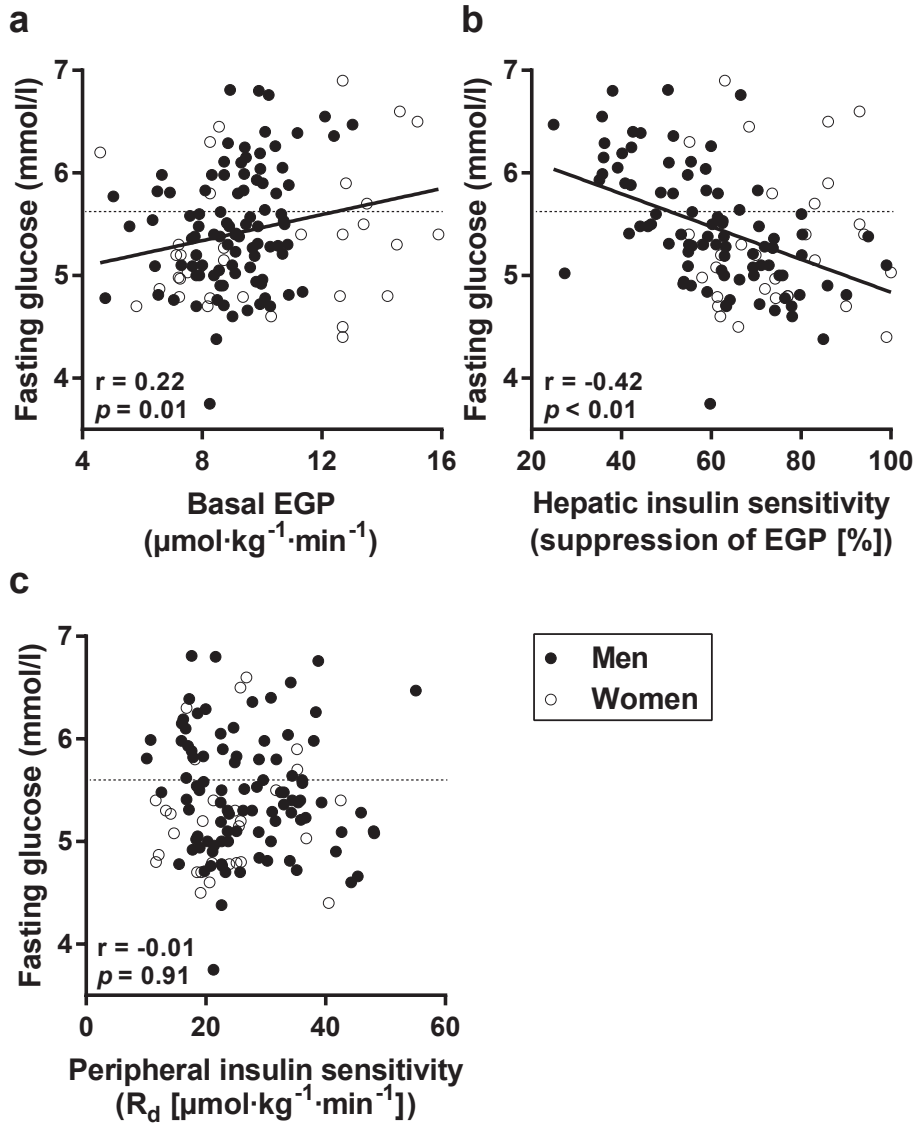
In all obese subjects, the fasting glucose concentration correlated positively with the basal rate of EGP (**Fig. 2a**). In addition, there was a strong inverse linear correlation between fasting glucose concentration and insulin-mediated suppression of EGP (**Fig. 2b**), indicating that higher fasting glucose levels are associated with hepatic insulin resistance. Multiple logistic regression analysis further showed that both the basal rate of EGP (odds ratio [95% confidence interval], 1.48 [1.12–1.95], $p=0.006$) and the insulin-mediated suppression of EGP (0.94 [0.91-0.97], $p=0.001$) were independently associated with the presence of IFG in obese subjects, after correction for sex, age and BMI (log-transformed). There was no association between fasting glucose levels and peripheral insulin sensitivity in univariate (**Fig. 2c**) or multivariate analysis (not shown).

Discussion

Data from the present study demonstrate that IFG in the context of human obesity is characterized by distinct metabolic disturbances. Using gold standard metabolic tracer techniques, we evaluated the contribution of basal and insulin-mediated metabolic fluxes to the pathogenesis of IFG in obese humans. Compared to similarly obese adults with NFG, subjects with IFG had significantly reduced insulin-mediated suppression of EGP, whereas there was no difference in basal EGP, insulin-mediated suppression of circulating FFA and insulin-stimulated glucose disposal. This indicates that reduced insulin action in the liver is a distinct metabolic feature of IFG in obese subjects. Although the cross-sectional design of the present study does not allow us to conclude a causal relationship, our findings provide strong experimental evidence suggesting that the liver plays an important role in the progression from compensated insulin resistance to prediabetes.

In fasted healthy humans, the rate of glucose uptake from the blood is matched by the basal rate of EGP in order to maintain the fasting plasma glucose concentration between 3.9 and 5.0 mmol/l [21, 22]. Under fasting conditions, the rate of EGP

Fig. 2.



Relationships between fasting plasma glucose levels and **(a)** *basal endogenous glucose production (EGP),* **(b)** *hepatic insulin sensitivity or* **(c)** *peripheral insulin sensitivity in obese adults.*

is primarily regulated by counterregulatory hormones and the autonomic nervous system [23]. Insulin, secreted in response to rising or elevated plasma glucose levels, is an effective suppressor of EGP [24], ensuring that EGP is appropriately low

under hyperglycemic conditions. In our study, fasting levels of glucagon, cortisol and insulin were not different in obese individuals with IFG and with NFG. In accordance, the basal rate of EGP was not different between groups. This suggests that regulation of EGP by glucoregulatory hormones under fasting conditions is similar in obese humans with IFG and NFG. Alternatively, the basal rate of EGP may have been elevated in obese subjects with IFG, but did not reach statistical significance due to insufficient power. This is supported by our finding that the basal rate of EGP and fasting glucose levels were weakly, but nonetheless significantly correlated in all subjects. However, the small difference in basal EGP was likely not sufficient to explain the entire difference in fasting glucose levels between subjects with NFG and IFG, indicating that other mechanisms may still be explored. Since the basal rate of EGP was not optimally adapted to the prevailing fasting glucose concentration in IFG, the ability of glucose to directly suppress its own production may be impaired, suggesting reduced glucose effectiveness in IFG [25], or the plasma set point for glucose homeostasis may be disturbed.

Individuals with IFG did not have higher fasting insulin levels compared to obese subjects with NFG, despite their elevated fasting glucose concentration. Although the present study may have been unable to pick up a small increase in fasting insulin levels, this suggests that pancreatic glucose sensing and/or insulin secretion may be impaired. In line, previous studies have demonstrated reduced insulin secretion in IFG [21, 26].

Importantly, in obese subjects with IFG, hepatic insulin resistance became apparent during low-dose insulin infusion. The data support the view that hepatic (rather than peripheral) insulin resistance is a major metabolic disturbance in IFG [26], extending previous experimental findings to a large number of well-matched obese adults. Importantly, insulin infusion during the first step of the hyperinsulinemic clamp resulted in circulating insulin levels similar to typical postprandial levels of insulin [5], suggesting that hepatic insulin resistance may be evident under postprandial conditions and contribute to postprandial hyperglycemia.

Since elevated circulating FFA may impair insulin action on suppression of EGP and glucose disposal [27], we evaluated plasma FFA concentrations during the hyperinsulinemic glucose clamp studies. The data show that plasma FFA concentrations during step 1 of the clamp, and insulin-mediated effects (suppression) on the plasma FFA concentration, did not differ between obese subjects with NFG or IFG, suggesting that elevated FFA did not contribute to the impaired insulin action on EGP suppression in subjects with IFG.

We previously reported that normal hepatic insulin sensitivity, defined from the

reference range (central 0.95 fraction) of two-step hyperinsulinemic clamp data in healthy non-obese men, is insulin-mediated suppression of EGP in the range of 46.5–100% during the first step of insulin infusion [16]. Notably, when we plot the probability of having IFG against the insulin-mediated suppression of EGP (**Supplementary Fig. 2**), the previously found cutoff for hepatic insulin resistance (i.e. EGP suppression <46.5%) matches the steep part of the probability plot: when EGP suppression is lower than 46.5%, obese adults have a much higher probability of having IFG. These findings provide a biological rationale for the reported reference range and suggest that below-normal hepatic insulin sensitivity is indeed associated with clinically relevant progression of metabolic disease in the context of human obesity.

Current clinical guidelines recommend testing for (pre)diabetes and metabolic syndrome in asymptomatic people who are overweight and have one or more risk factors for diabetes [1]. Severe obesity is especially associated with prediabetes [28]. The high prevalence of IFG in our obese cohort (44 out of 131 subjects, or 34%), in line with previously reported numbers [28, 29], further warrants special caution in clinical care for severely obese patients. Notably, intensive lifestyle modification programs and/or pharmacological interventions can decrease the rate of type 2 diabetes onset in high-risk (prediabetes) patients [1, 30, 31]. Current preventive strategies, however, do not distinguish between patients with different categories of increased risk for diabetes: IFG, impaired glucose tolerance (IGT) or combined IFG/IGT [1]. Given the different pathophysiologic mechanisms that contribute to these metabolic states [26], patients in different categories will likely benefit from different interventions. For instance, a subgroup analysis in the Diabetes Prevention Program trial suggested greater effect of metformin, which primarily targets hepatic glucose production/hepatic insulin resistance [32], in subjects with higher fasting glucose levels [30].

Conclusions

Obese adults with IFG have distinctly impaired insulin action in the liver, but not on suppression of FFA or in skeletal muscle, compared to similarly obese individuals with NFG. Both hepatic insulin resistance and impaired insulin secretion may contribute to the progression from compensated insulin resistance to prediabetes and hyperglycemia in obese adults. Obese adults with IFG may benefit from interventions that specifically target hepatic insulin resistance.

Author contributions

KWH researched data, performed the analysis, contributed to discussions about the results and wrote the manuscript. PWG researched data, contributed to discussions about the results, and critically reviewed and approved the final manuscript. MTA was responsible for laboratory analyses, and critically reviewed and approved the final manuscript. MRS, MN, JAR and MJS contributed to discussions about the results, and critically reviewed and approved the final manuscript. MJS is the guarantor of this work.

References

1. American Diabetes Association. Standards of medical care in diabetes—2015. Diabetes Care 2015;38 (Suppl 1):S1-S93.
2. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. Lancet 2012;379:2279-90.
3. Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. J Am Coll Cardiol 2010;55:1310-7.
4. Rizza RA. Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy. Diabetes 2010;59:2697-707.
5. Bock G, Chittilapilly E, Basu R, et al. Contribution of hepatic and extrahepatic insulin resistance to the pathogenesis of impaired fasting glucose: role of increased rates of gluconeogenesis. Diabetes 2007;56:1703-11.
6. Bock G, Dalla Man C, Campioni M, et al. Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 2006;55:3536-49.
7. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 2006;55:1430-5.
8. Faerch K, Vaag A, Holst JJ, Glumer C, Pedersen O, Borch-Johnsen K. Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action. Diabetologia 2008;51:853-61.
9. Weyer C, Bogardus C, Pratley RE. Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 1999;48:2197-203.
10. Kim SH, Reaven GM. Isolated impaired fasting glucose and peripheral insulin sensitivity: not a simple relationship. Diabetes Care 2008;31:347-52.
11. Meyer C, Pimenta W, Woerle HJ, et al. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. Diabetes Care 2006;29:1909-14.
12. de Weijer BA, Aarts E, Janssen IM, et al. Hepatic and peripheral insulin sensitivity do not improve 2 weeks after bariatric surgery. Obesity 2013;21:1143-7.
13. Vrieze A, Out C, Fuentes S, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol 2014;60:824-31.

14. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913-6.e7.
15. van der Valk F, Hassing C, Visser M, et al. The effect of a diiodothyronine mimetic on insulin sensitivity in male cardiometabolic patients: a double-blind randomized controlled trial. PLoS ONE 2014;9:e86890.
16. ter Horst KW, Gilijamse PW, Koopman KE, et al. Insulin resistance in obesity can be reliably identified from fasting plasma insulin. Int J Obes 2015;39:1703–9.
17. Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. Obesity 2009;17:1872-7.
18. Ackermans MT, Pereira Arias AM, Bisschop PH, Endert E, Sauerwein HP, Romijn JA. The quantification of gluconeogenesis in healthy men by (2) H2O and [2-(13)C]glycerol yields different results: rates of gluconeogenesis in healthy men measured with (2)H2O are higher than those measured with [2-(13)C]glycerol. J Clin Endocrinol Metab 2001;86:2220-6.
19. Finegood DT, Bergman RN, Vranic M. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. Diabetes 1987;36:914-24.
20. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420-30.
21. Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care 2006;29:1130-9.
22. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectrum 2004;17:183-90.
23. Thorens B. Brain glucose sensing and neural regulation of insulin and glucagon secretion. Diabetes Obes Metab 2011;13 Suppl 1:82-8.
24. Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999;48:1198-214.
25. Tonelli J, Kishore P, Lee DE, Hawkins M. The regulation of glucose effectiveness: how glucose modulates its own production. Curr Opin Clin Nutr Metab Care 2005;8:450-6.
26. Nathan DM, Davidson MB, DeFronzo RA, et al. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care

2007;30:753-9.
27. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction.Eur J Clin Invest 2002;32 Suppl 3:14-23.
28. Guerrero-Romero F, Rodriguez-Moran M, Perez-Fuentes R, et al. Prediabetes and its relationship with obesity in Mexican adults: The Mexican Diabetes Prevention (MexDiab) Study. Metab Syndr Relat Disord 2008;6:15-23.
29. Chen CM, Yeh MC. The prevalence and determinants of impaired fasting glucose in the population of Taiwan. BMC Public Health 2013;13:1123.
30. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.
31. Lilly M, Godwin M. Treating prediabetes with metformin: Systematic review and meta-analysis. Canadian Family Physician 2009;55:363-9.
32. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clinical Science 2012;122:253-70.