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ORIGINAL ARTICLE

Visceral, subcutaneous abdominal adiposity and liver fat content distribution in normal glucose tolerance, impaired fasting glucose and/or impaired glucose tolerance

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OBJECTIVES: To examine the specific distribution of liver fat content, visceral and subcutaneous adiposity in normal glucose tolerance (NGT/NGT), isolated impaired fasting glucose (iIFG), isolated impaired glucose tolerance (iIGT) and combined conditions (IFG+IGT), as well as with newly diagnosed type 2 diabetes (nT2D).
DESIGN: Multicenter, international observational study: cross-sectional analysis.
SUBJECTS: Two thousand five hundred and fifteen patients (50.0% women, 54.5% non-Caucasian) without previously known diabetes were recruited from 29 countries. Abdominal fat distribution was measured by computed tomography (CT). Liver fat was estimated using the CT-liver mean attenuation.
RESULTS: Compared with NGT/NGT patients, increased visceral adiposity was found in iIFG, iIGT, IFG+IGT and nT2D; estimated liver fat progressively increased across these conditions. A one-s.d. increase in visceral adiposity was associated with an increased risk of having iIFG (men: odds ratio (OR) 1.41 (95% confidence interval (CI) 1.15–1.74), women: OR 1.62 (1.29–2.04)), iIGT (men: OR 1.59 (1.15–2.01), women: OR 1.30 (0.96–1.76)), IFG+IGT (men: OR 1.64 (1.27–2.13), women: OR 1.83 (1.36–2.48)) and nT2D (men: OR 1.80 (1.35–2.42), women: OR 1.73 (1.25–2.41)). A one-s.d. increase in estimated liver fat was associated with iIGT (men: OR 1.46 (1.12–1.90), women: OR 1.81 (1.41–2.35)), IFG+IGT (men: OR 1.42 (1.14–1.77), women: OR 1.74 (1.35–2.26)) and nT2D (men: OR 1.77 (1.40–2.27), women: OR 2.38 (1.81–3.18)). Subcutaneous abdominal adipose tissue showed an inverse relationship with nT2D in women (OR 0.63 (0.45–0.88)).
CONCLUSIONS: Liver fat was associated with iIGT but not with iIFG, whereas visceral adiposity was associated with both. Liver fat and visceral adiposity were associated with nT2D, whereas subcutaneous adiposity showed an inverse relationship with nT2D in women.

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INTRODUCTION

It has been suggested that isolated impaired fasting glucose (iIFG), isolated impaired glucose tolerance (iIGT) and combined IFG+IGT are conditions of deteriorated glucose tolerance with distinct underlying pathophysiologies: patients with iIFG seem to predominantly manifest liver insulin resistance, whereas patients with iIGT have muscle insulin resistance combined with severely impaired insulin secretion.[1–4] Excess visceral fat is associated with deteriorated insulin sensitivity.[5] Indeed, visceral adiposity is characterized by an elevated rate of lipolysis, as well as with a low-grade inflammation.[6–7] Whereas visceral adiposity lipolysis accounts for $< 20\%$ of systemic circulating free fatty acid in obese patients, these free fatty acid are released directly into the portal vein thereby exposing the liver to more free fatty acid than would be predicted from systemic free fatty acid.[8] As a consequence, fat accumulates in the liver. Excess liver fat, in turn, has been associated with impairment in plasma glucose/insulin homeostasis, favoring hepatic insulin resistance[9] as well as peripheral insulin resistance.[10] However, very few studies have addressed the question of whether excess visceral fat, subcutaneous fat or liver fat could be differently associated with iIFG, iIGT or IFG+IGT, three conditions of impaired plasma glucose tolerance,[11–12] as well as with newly diagnosed type 2 diabetes (nT2D). A better understanding of the specific mechanisms underlying the prediabetic states might give a basis for the development of individualized prevention strategies for type 2 diabetes.

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In the present analysis, 2515 men and women without previously known type 2 diabetes, from 29 countries and five ethnic groups, with either normal glucose tolerance (NGT/NGT), iIFG, iIGT, IFG+IGT or nT2D were evaluated for their abdominal and liver fat distribution by computed tomography (CT) and indices of insulin sensitivity and secretion. The relative contributions of abdominal visceral adiposity, subcutaneous adiposity and liver fat content to each glucose tolerance condition were examined.

SUBJECTS AND METHODS

Study design

The International Study of Prediction of Intra-abdominal Adiposity and its Relationships with Cardiometabolic Risk/Intra-Abdominal Adiposity (INSPIRE ME IAA) is an international prospective observational study, with a planned 3-year follow-up, in 29 countries and involving 297 physicians. The present study focuses on the baseline data. Detailed information about the protocol has been previously reported.[13] Briefly, physicians were either (i) hospital-based primary care physicians or internists, (ii) cardiologists or (iii) endocrinologists/diabetologists, and there was approximately one-third in each clinical practice group. Physicians were asked to recruit a maximum of 20 patients over a 6-month period by inviting the third patient of the day to participate in the study. If the patient refused to participate, the physician asked the next patient to volunteer. A total of 4504 men and women, of five ethnic groups (Caucasians, Blacks, Hispanic, East Asian, South-East Asian), were included between 2006 and 2008. Women whose ages were $\geqslant 45$ and $\leqslant 70$ years and men whose ages were $\geqslant 40$ and $\leqslant 70$ years were eligible to participate. Exclusion criteria were body weight exceeding 136 kg (upper limit supported by CT equipment), pregnant or breast-feeding women, current hospitalization or recent surgery (during the previous 30 days), recent cardiovascular events (30 days), ascites, hypothyroidism and hyperthyroidism, known HIV seropositivity, cancer during the past 5 years, history or planned bariatric surgery, use of anti-obesity, corticosteroids or oral retinoid drugs. The present work focused on 2515 patients without previously known type 2 diabetes. Previously known diabetes was defined as known/treated diabetes. Newly known type 2 diabetes was diagnosed by oral glucose tolerance test (OGTT) at the study examination (fasting glucose $\geqslant 7\,\mathrm{mmol\,l^{-1}}$ or 120-min OGTT glucose $\geqslant 11.1\,\mathrm{mmol\,l^{-1}}$).

Demographic characteristics and medical history were assessed by a physician-administered questionnaire, translated into every language of the different countries. Anthropometric parameters and vital signs were measured by the physician. Patients were also referred to a validated CT-imaging center to obtain abdominal and liver CT scans. Blood samples were collected and shipped using a standardized protocol for assessment, to one central laboratory, accredited by the College of American Pathologists' Laboratory Accreditation Program (MDS Pharma Services, Paris, France). A strict procedure was used for the collection and transfer of the blood samples. The study was conducted in accordance with the revised (2000) Helsinki principles, 'Good Epidemiology Practice'[14] and in accordance with local data protection regulations. The study was approved by local ethics committees, and all patients signed an appropriate informed consent.

Anthropometric measurements and body composition

Height, weight and waist circumference[15] were measured according to the standardized procedures.

CT

After a negative urinary pregnancy test for women of childbearing potential, cross-sectional areas of visceral (VAT) and subcutaneous adipose tissue (SAT) were assessed at L4–L5 by CT, using previously described procedures.[16–17] Liver fat was estimated according to the mean liver attenuation measured by CT at the T12–L1 level and by the liver to spleen attenuation ratio.[18] Personnel in all imaging centers were trained, and the procedures were validated to insure that CT image acquisition followed a standardized protocol with calibration phantoms to ensure comparability across the sites. Abdominal and liver CT images were analyzed centrally in a core laboratory (CRIUCPQ, Québec, Canada) with the Slice-O-matic software (Tomovision, Montreal, Canada).

Plasma glucose/insulin

After a 12-h overnight fast, participants had a 75-g OGTT. Blood samples were taken at 0, 30 and 120 min for the measurement of plasma glucose and at 0 and 30 min for plasma insulin concentrations. Glucose was measured by photometric methods (Roche Modular, Basel, Switzerland). Insulin was specifically assessed by immune chemiluminescent assay (Siemens Immulite 2000, Münich, Germany). Glycated hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (Variant II, Bio-Rad, Hercules, CA, USA). After the exclusion of patients with previously known type 2 diabetes, the remaining patients were categorized into four plasma glucose tolerance conditions and nT2D according to their OGTT glucose values: (i) NGT/NGT: fasting glucose $< 5.6\,\mathrm{mmol\,l^{-1}}$ and 120-min OGTT glucose $< 7.8\,\mathrm{mmol\,l^{-1}}$, (ii) iIFG: fasting glucose $\geqslant 5.6$ and $< 7\,\mathrm{mmol\,l^{-1}}$, and 120-min OGTT glucose $< 7.8\,\mathrm{mmol\,l^{-1}}$), (iii) iIGT: fasting glucose $< 5.6\,\mathrm{mmol\,l^{-1}}$ and 120-min OGTT glucose $\geqslant 7.8$ and $< 11.1\,\mathrm{mmol\,l^{-1}}$, (iv) combined fasting and 120-min conditions (IFG+IGT), and (v) fasting glucose $\geqslant 7\,\mathrm{mmol\,l^{-1}}$ and 120-min OGTT glucose $\geqslant 11.1\,\mathrm{mmol\,l^{-1}}$ (nT2D). Homeostasis model assessment-estimated insulin resistance (HOMA-IR)[19] was calculated from fasting values of glucose and insulin. The insulin secretion index was calculated by the ratio of the increment of insulin between 0 and 30 min to the glucose level at 30 min.[20] The disposition index was calculated as the ratio of insulin secretion index to HOMA-IR.

Liver enzymes

Plasma alanine transaminase, plasma aspartate transaminase and plasma gamma-glutamyl transferase were measured by photometric methods (Roche Modular).

Statistical analyses

Results are expressed as means (s.d.) for normally distributed variables and as median (25th–75th percentile) for non-normally distributed variables. Odds ratios are expressed as OR (95%, confidence interval (CI)). Data are presented as means (s.e.m.) in figures. The normal distribution of residuals was verified by stem and leaf plots and by the Shapiro–Wilk tests. Parameters with skewed distributions were log transformed (fasting insulin, HOMA-IR, disposition index, aspartate transaminase, alanine transaminase and gamma-glutamyl transferase) or transformed by square root (insulin secretion index).

Characteristics of the five groups of patients: NGT/NGT, iIFG, iIGT, IFG +IGT and nT2D were compared using analysis of variance with pairwise comparisons by *post-hoc* Tukey's test. All comparisons were adjusted for age, physician specialty and ethnicity. An interaction term was added in order to test the potential interaction of the glucose tolerance group and ethnicities in their association with the different fat depots, as well as for the potential interaction of the glucose tolerance groups with sex.

Multivariable linear regressions were performed with HOMA-IR (Model 1), insulin secretion index (Model 2) and disposition index (Model 3) as dependent variables. Independent variables were VAT, subcutaneous adipose tissue and estimated liver fat (liver fat attenuation) with a threshold of *P*-value defined at $P < 0.20$ to enter the variable into the forward regression model. Age, ethnicity and physician specialty were included as covariates in each model.

The ORs for having iIFG, iIGT, combined IFG+IGT or nT2D compared with NGT/NGT were evaluated for one-s.d. (sex specific) increases in VAT or SAT or a one-s.d. decrease in liver attenuation, in multivariable logistic regression models, adjusted for age, physician's specialty and ethnicity.

The significance level was set at $P < 0.05$. All analyses were performed using the SAS statistical package version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Anthropometric and demographic characteristics of men and women according to their glucose tolerance condition

Among the 2515 patients (50.0% women, 54.5% non-Caucasian), no differences were found between patients of different glucose tolerance conditions regarding educational level, manual versus non-manual work or smoking status (data not shown). Characteristics of men and women are reported in Tables 1 and 2, respectively. Men and women presenting with NGT/NGT were the youngest and had the smallest BMI and waist circumference.

Table 1. Characteristics of men

	NGT/NGT (n = *473*)	*iIFG* (n = *347*)	*iIGT* (n = *112*)	*IFG+IGT* (n = *179*)	*nT2D* (n = *147*)	*P-value*
Age (years)	53.9 (8.3)[b,c,d,e]	56.5 (7.9)[a]	57.4 (7.9)[a]	57.1 (8.4)[a]	57.1 (8.0)[a]	< 0.001
Body mass index (kg m^{-2})	27.0 (4.2)[b,c,d,e]	28.4 (4.4)[a,e]	27.6 (4.2)[a,e]	28.0 (4.3)[a,e]	29.4 (4.7)[a,b,c,d]	< 0.001
Waist circumference (cm)	96.1 (12.4)[b,d,e]	100.9 (11.9)[a,e]	97.7 (11.7)[e]	99.3 (12.4)[a,e]	103.3 (12.7)[a,b,c,d]	< 0.001
Plasma glucose/insulin homeostasis						
Fasting glucose (mmol l^{-1})	5.11 (0.31)	5.96 (0.32)	5.27 (0.23)	6.06 (0.38)	7.38 (2.23)	
Fasting insulin (pmol l^{-1})	49 (30–75)[b,c,d,e]	67 (42–97)[a,e]	60 (33–102)[a,e]	74 (40–118)[a]	95 (48–149)[a,b,c]	< 0.001
120-min OGTT glucose (mmol l^{-1})	5.60 (1.24)	6.00 (1.15)	8.81 (0.91)	9.09 (0.91)	13.3 (4.2)	
HbA1c (%)	5.6 (0.4)[b,d,e]	5.8 (0.4)[a,e]	5.7 (0.4)[e]	5.9 (0.4)[a,e]	6.6 (1.5)[a,b,c,d]	< 0.001
HOMA-IR	1.64 (0.97–2.55)[b,c,d,e]	2.63 (1.53–3.85)[a,e]	2.04 (1.11–3.49)[a,e]	2.88 (1.51–4.45)[a,e]	4.01 (2.31–6.47)[a,b,c,d]	< 0.001
Insulin secretion index	39 (23–59)[b,d,e]	30 (17–47)[a,e]	36 (17–65)[d,e]	25 (15–40)[a,c,e]	15 (8–26)[a,b,c,d]	< 0.001
Disposition index	24.2 (16.0–38.0)[b,c,d,e]	12.2 (7.7–20.4)[a,c,d,e]	16.4 (11.0–28.3)[a,b,d,e]	8.9 (5.3–7.8)[a,b,c,e]	4.17 (1.85–8.31)[a,b,c,d]	< 0.001
Liver enzymes						
AST (UI l^{-1})	23 (20–28)[d,e]	23 (20–29)[d,e]	24 (20–28)[e]	24 (21–31)[a,b]	32 (24–43)[a,b,c]	0.003
ALT (UI l^{-1})	25 (19–34)[d,e]	25 (19–33)[d,e]	26 (22–35)[e]	28 (22–40)[a,b]	26 (23–33)[a,b,c]	< 0.001
GGT (U l^{-1})	27 (20–42)[b,d,e]	32 (22–47)[a,e]	31 (23–46)	33 (24–53)[a]	37 (25–63)[a,b]	< 0.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostasis model assessment-estimated insulin resistance; iIFG, isolated impaired fasting glucose, iIGT, isolated impaired glucose tolerance; IFG+IGT, combined IFG+IGT; NGT/NGT, normal glucose tolerance; nT2D, newly diagnosed type 2 diabetes; OGTT, oral glucose tolerance test. Values are mean (s.d.) for normally distributed variables and median (25th–75th percentile) for non-normally distributed variables. Comparisons have been made by one-way analysis of variance and adjusted for age, physician's specialty and ethnicity. The *P*-value is reported in the right column. Fasting insulin, HOMA-IR, disposition index, AST and ALT and GGT were analyzed using the log-transformed values and insulin secretion index using square root transformation. Tukey's test was used for *post-hoc* pairwise comparisons between groups. Statistical significance ($P < 0.05$) is reported by superscript letters ([a]NGT, [b]iIFG, [c]iIGT, [d]IFG+IGT, [e]nT2D).

Table 2. Characteristics of women

	NGT/NGT (n = *629*)	*iIFG* (n = *234*)	*iIGT* (n = *148*)	*IFG+IGT* (n = *127*)	*nT2D* (n = *119*)	P-*value*
Age (years)	55.5 (6.9)[d,e]	56.6 (6.2)	57.2 (6.8)	58.2 (6.4)[a]	57.9, (6.8)[a]	< 0.001
Body mass index (kg m^{-2})	26.4 (5.1)[b,d,e]	28.9 (5.4)[a]	27.3 (5.6)	28.6 (5.6)[a]	28.3 (5.0)[a]	< 0.001
Waist circumference (cm)	86.4 (12.7)[b,c,d,e]	99.3 (14.1)[a]	89.5 (12.5)[a]	92.7 (13.5)[a]	93.4 (13.5)[a]	< 0.001
Plasma glucose/insulin homeostasis						
Fasting glucose (mmol l^{-1})	5.03 (0.33)	5.92 (0.33)	5.14 (0.28)	5.94 (0.34)	6.56 (1.32)	
Fasting insulin (pmol l^{-1})	42 (25–68)[b,c,d,e]	71 (43–97)[a]	54.0 (32–90)[a,d,e]	71 (49–95)[a,c]	77 (49–118)[a,c]	< 0.001
120-min OGTT glucose (mmol l^{-1})	5.74 (1.17)	6.12 (0.94)	8.86 (0.90)	9.07 (0.92)	12.95 (2.83)	
HbA1c (%)	5.6 (0.3)[b,c,d,e]	5.9 (0.4)[a,e]	5.8 (0.4)[a,d,e]	6.0 (0.4)[a,c,e]	6.4 (1.1)[a,b,c,d]	< 0.001
HOMA-IR	1.32 (0.80–2.27)[b,c,d,e]	2.61 (1.61–3.87)[a,c,e]	1.80 (1.05–2.94)[a,b,d,e]	2.69 (1.70–3.73)[a,c]	3.28 (1.98–4.87)[a,b,c]	< 0.001
Insulin secretion index	37 (24–56)[d,e]	34 (21–54)[e]	31 (21–54)[e]	25 (18–38)[a]	20 (10–30)[a,b,c]	< 0.001
Disposition index	28.9 (17.6–46.6)[b,c,d,e]	14.3 (8.8–21.4)[a,c,d,e]	18.8 (14.2–28.4)[a,b,d,e]	10.3 (6.9–16.1)[a,b,c,e]	6.0 (3.6–13.1)[a,b,c,d]	< 0.001
Liver enzymes						
AST (UI l^{-1})	21 (18–26)[c,d,e]	21 (18–26)[e]	22 (19–28)[a]	22 (19–28)[a]	25 (16–35)[a,b]	< 0.001
ALT (UI l^{-1})	18 (14–24)[e]	21 (18–26)[e]	20 (15–29)[a]	22 (19–28)[a]	23 (18–29)[a,b]	0.011
GGT (U l^{-1})	17 (13–26)[c,d,e]	20 (14–31)[e]	22 (15–32)[a]	23 (16–37)[a]	25 (18–41)[a,b]	< 0.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-estimated insulin resistance; iIFG, isolated impaired fasting glucose, iIGT, isolated impaired glucose tolerance; IFG+IGT, combined IFG+IGT; NGT/NGT, normal glucose tolerance; nT2D, newly diagnosed type 2 diabetes; OGTT, oral glucose tolerance test. Values are mean (s.d.) for normally distributed variables and median (25th–75th percentile) for non-normally distributed variables. Comparisons have been made by one-way analysis of variance and adjusted for age, physician's specialty and ethnicity. The *P*-value is reported in the right column. Fasting insulin, HOMA-IR, disposition index, AST and ALT and GGT were analyzed using log-transformed values and insulin secretion index using square root transformation. Tukey's test was used for *post-hoc* pairwise comparisons between groups. Statistical significance ($P < 0.05$) is reported by superscript letters ([a]NGT, [b]iIFG, [c]iIGT, [d]IFG+IGT, [e]nT2D).

Abdominal CT scan data are illustrated in Figure 1. Men and women with iIFG, iIGT, IFG+IGT and nT2D had higher VAT than men and women with NGT/NGT. As reflected by the progressive decrease in liver attenuation values, liver fat progressively increased across glucose tolerance conditions in both genders. Liver enzymes followed the same trends (Tables 1 and 2). There were no significant interaction between the glucose tolerance groups and ethnicities regarding their association with VAT, SAT or liver fat. However, there were significant interactions between the glucose tolerance group and sex for their association with VAT ($P < 0.001$), SAT ($P < 0.001$) and estimated liver fat ($P < 0.001$). Further analyses were therefore conducted separately for men and women.

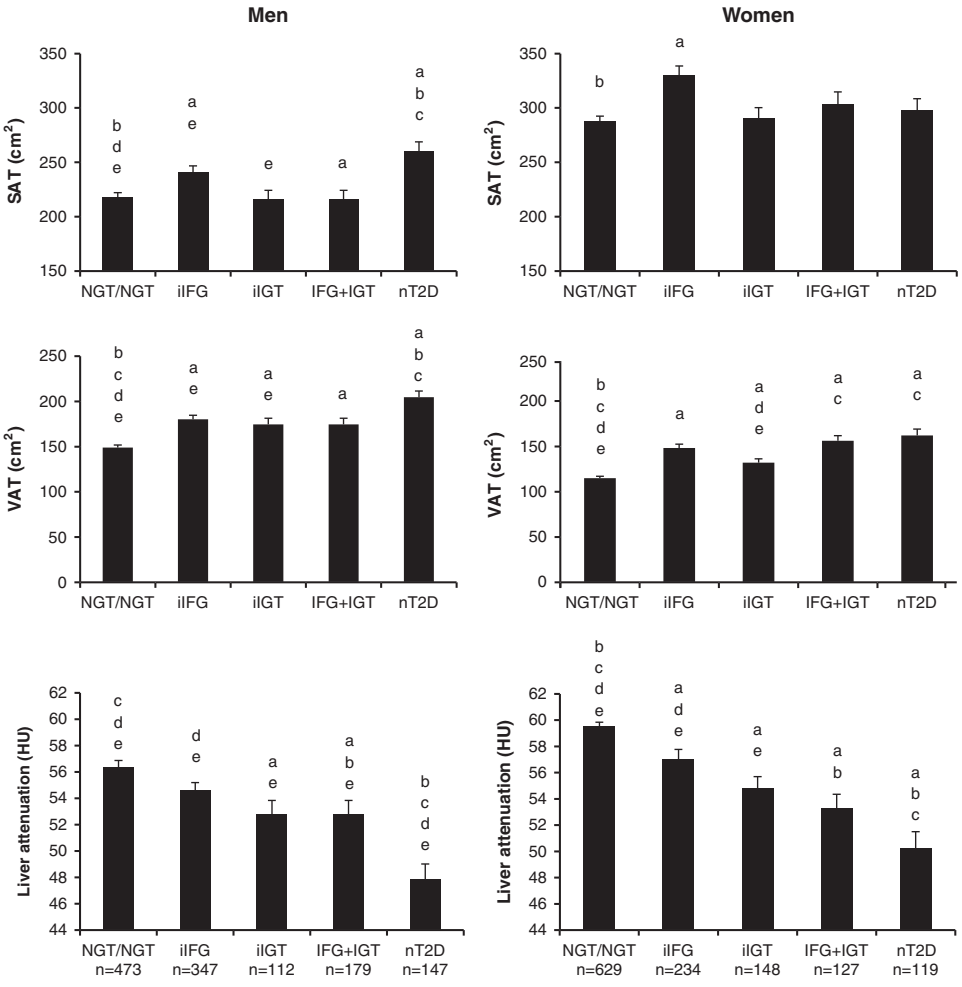

Figure 1. Abdominal adipose tissue and liver fat distribution according to glucose tolerance condition in INSPIRE ME IAA. Comparisons were made by one-way analysis of variance and were adjusted for age, physician's specialty and ethnicity. Statistical significance ($P < 0.05$) is reported by superscript letters ([a]NGT/NGT, [b]iIFG, [c]iIGT, [d]IFG+IGT, [e]nT2D).

Plasma glucose/insulin homeostasis according to glucose tolerance condition

Plasma fasting glucose and 120-min OGTT glucose are presented in Tables 1 and 2 without any statistical analysis as men and women were classified in the different glucose tolerance conditions according to these criteria. In both genders, HbA1c levels were lower in patients with NGT/NGT compared with patients having impaired fasting and/or glucose tolerance who in turn had lower HbA1c levels than patients with nT2D. Accordingly, the values of HOMA-IR were found the lowest in men and women with NGT/NGT, intermediate in iIFG, iGT and IFG+IGT and the highest in nT2DM. The disposition index decreased progressively from NGT/NGT to iIGT, iIFG, IFG+IGT and nT2D in both men and women.

The respective contributions of VAT, SAT and liver fat to the variance of the different markers of plasma glucose/insulin homeostasis are reported in Table 3. VAT, SAT and estimated liver fat were all independently associated with HOMA-IR in men as in women, whereas only VAT and estimated liver fat were independently and inversely associated with the disposition index. SAT was the only independent variable correlated with insulin secretion index.

Respective contributions of VAT, SAT and estimated liver fat to iIFG, iIGT or combined IFG+IGT

Multivariable logistic regression analyses, including VAT, SAT and liver attenuation, and adjusted for age, physician's specialty and ethnicity were used to quantify their respective contributions to the different glucose tolerance conditions (Figure 2). For a one-s.d. increase in VAT, the ORs of having iIFG were 1.41 (1.15–1.74) for men and 1.62 (1.29–2.04) for women compared with NGT/NGT men and women. For a one-s.d. decrease in liver attenuation, the ORs of having iIFG were not significant in either gender: 1.00 (0.83–1.21) for men and 1.14 (0.93–1.40) for women. One-s.d. increase in SAT was not associated with significant ORs of having iIFG, neither in men (OR 1.11 (0.91–1.36)) nor in women (OR 1.09 (0.89–1.33)).

In men, one-s.d. variation in either VAT or liver attenuation was associated with significant ORs of having iIGT compared with NGT/NGT (OR 1.59 (1.15–2.21) and OR 1.46 (1.12–1.90) for one-s.d. increase in VAT and one-s.d. decrease in liver attenuation, respectively). In women, only the decrease in liver attenuation was associated with a significant OR of having iIGT compared with women with NGT/NGT (OR 1.30 (0.96–1.76) and OR 1.81 (1.41–2.35) for VAT and liver attenuation, respectively). In both genders, a one-s.d. increase in SAT did not lead to significant ORs of having iIGT compared with NGT/NGT (OR 0.77 (0.53–1.08) and OR 0.81 (0.61–1.06) in men and women, respectively).

Increases in VAT and decreases in liver attenuation were both associated with significant ORs of having combined IFG+IGT compared with NGT/NGT in men (OR 1.64 (1.27–2.13) and OR 1.42 (1.14–1.77) for one-s.d. increase in VAT and one-s.d. decrease in liver attenuation, respectively), as well as in women (OR 1.83 (1.36–2.48) and OR 1.74 (1.35–2.26) for VAT and liver attenuation, respectively).

Table 3. Respective contribution of VAT, SAT and estimated liver fat to insulin resistance, the insulin secretion index and the disposition index

Dependent variable	*Independent variables*	*Men*		*Women*	P-*value*
Model 1		β (95% CI)	*P*-value	β (95% CI)	*P*-value
HOMA-IR	$R^2 = 31\%$			$R^2 = 30\%$	
	VAT	0.0014 (0.0011 0.0017)	< 0.001	0.0023 (0.0019 0.0027)	< 0.001
	SAT	0.0010 (0.0008 0.0013)	< 0.001	0.0003 (0.0002 0.0005)	< 0.001
	Liver fat	−0.0045 (−0.0063–0.0027)	< 0.001	−0.0051 (−0.0069 −0.0032)	< 0.001
Model 2		β (95% CI)	*P*-value	β (95% CI)	*P*-value
Insulin secretion index	$R^2 = 5\%$			$R^2 = 5\%$	
	VAT			0.0029 (−0.0002 0.0059)	0.006
	SAT	0.0053 (0.0036 0.0069)	< 0.001	0.0023 (0.0007 0.0038)	0.004
Model 3		β (95% CI)	*P*-value	β (95% CI)	*P*-value
Disposition index	$R^2 = 8\%$			$R^2 = 12\%$	
	VAT	−0.0013 (−0.0018–0.0008)	< 0.001	−0.0021 (−0.0026–0.0016)	< 0.001
	SAT	−0.0002 (−0.0006 0.0001)	0.198		
	Liver fat	0.0045 (0.0018 0.0072)	0.001	0.0048 (0.0022 0.0075)	< 0.001

Abbreviations: CI, confidence interval; HOMA-IR, homeostasis model assessment-estimated insulin resistance; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. A multivariable forward linear regression was performed with HOMA-IR (log transformed) (Model 1), insulin secretion index (transformed by square root) (Model 2) and disposition index (log transformed) (Model 3) as dependent variables. Independent variables were VAT, SAT and estimated liver fat (liver fat attenuation) with a threshold of *P*-value defined at $P < 0.20$ to enter the variable into the forward regression model. Age, ethnicity and physician specialty were included as covariates in each model.

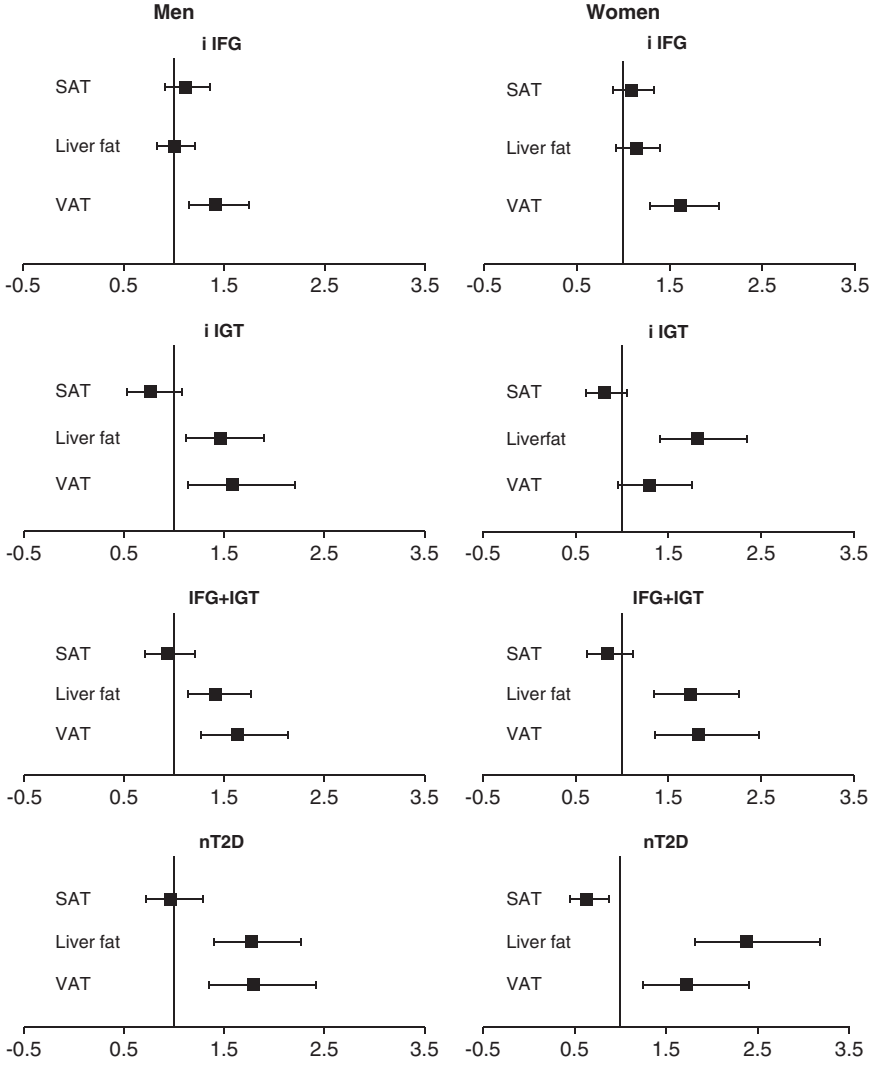

Figure 2. Odds ratios for impaired glucose tolerance compared with normal glucose tolerance, for increased VAT, liver fat and SAT in INSPIRE ME IAA. The ORs for having either an iIFG, iIGT, IFG+IGT or nT2D were calculated for a one-s.d. increases in VAT and SAT and for a one-s.d. decrease in liver attenuation (sex-specific s.d.). Analyses were performed with a multivariable logistic regression model, including VAT, liver attenuation, SAT, age, physician's specialty and ethnicity.

Finally, a one-s.d. increase in SAT was not associated with significant ORs of having IFG+IGT in either gender (OR 0.93 (0.71–1.21) and OR 0.84 (0.63–1.12) in men and women, respectively).

Increases in VAT and decreases in liver attenuation were both associated with significant ORs of having nT2D compared with NGT/NGT in men (OR 1.80 (1.35–2.42) and OR 1.77 (1.40–2.27) for one-s.d. increase in VAT and one-s.d. decrease in liver attenuation, respectively), as well as in women (OR 1.73 (1.25–2.41) and OR 2.38 (1.81–3.18) for VAT and liver attenuation, respectively). A one-s.d. increase in SAT was not associated with significant ORs of having nT2D in men (OR 0.97 (0.72–1.29)), whereas it was associated with a decrease in odds of having nT2D in women (OR 0.63 (0.45–0.88)).

DISCUSSION

The present study reports the specific abdominal fat distribution in men and women having an impaired glucose tolerance condition or nT2D compared with patients with normal glucose tolerance. Excess VAT was found in all the impaired glucose tolerance groups and nT2D compared with the normal glucose tolerance group. Liver fat progressively increased across the conditions of NGT/NGT to iIFG, iIGT, IFG/IGT to nT2D. Only excess visceral adiposity was found to be associated with iIFG. An increase in SAT showed an inverse relationship with nT2D, in women only.

Respective contributions of VAT, SAT and estimated liver fat to impaired fasting glucose, impaired glucose tolerance and both conditions

The present results showed that liver fat progressively increased from NGT/NGT to iIFG, iIGT and IFG+IGT patients. A similar observation was also made by Van der Zijl *et al.*[11] in 330 Caucasian patients without type 2 diabetes. In their study, patients with NGT, iIFG, iIGT and IFG+IGT showed a progressive increase in liver fat that was, in turn, inversely associated with insulin sensitivity, as measured by euglycemic hyperinsulinic clamp. In the present work, a one-s.d. increase in liver fat (as estimated by a one-s.d. decrease in the liver Hunsfield unit) was associated with an increased risk of having iIGT or IFG+IGT, but not iIFG, and these relationships were adjusted for visceral and subcutaneous adiposity. In accordance with previous reports,[1,4,12,21] the present study found that iIFG was associated with a higher hepatic insulin resistance (estimated by HOMA-IR) than iIGT in women. However, the estimated liver fat was higher in patients with iIGT than in patients with iIFG in both genders, a finding concordant with the study published by Kantartzis *et al.*[12] In this study, intramuscular fat was also measured by magnetic resonance spectroscopy and was found to be more closely associated with excess liver fat than with excess VAT. Moreover, peripheral insulin sensitivity, measured by a euglycemic hyperinsulinemic clamp, showed a continuous decrease from NGT/NGT to iIFG, iIGT and IFG+IGT. Therefore, in patients with iIGT, the excess liver fat could be the marker of a multi-organ ectopic fat deposition, which could explain the more pronounced peripheral insulin resistance in these individuals.

Distribution of the different fat depots in patients with nT2D

VAT is associated with type 2 diabetes, in men as well as in women and whatever the ethnicity.[13,22] Liver fat has also been reported to independently predict the incidence of type 2 diabetes,[13,23] unless the fatty liver resolves.[24] Recent studies have shown that liver fat might be more closely associated to insulin resistance than visceral adiposity.[25] However, both visceral and liver fat are associated with hepatic insulin resistance in patients having type 2 diabetes.[9] In the present study, we demonstrate that both fat depots are independently associated with nT2D in a multiethnic population and in both sexes. Liver fat progressively worsened from normal glucose tolerance to prediabetes and to type 2 diabetes. As the markers of glucose/insulin homeostasis, liver fat progressively worsen from normal glucose tolerance to prediabetes and effective type 2 diabetes. Excess visceral fat is more homogenously distributed across prediabetic and diabetic conditions. It is of interest to note that the three locations of fat deposition are independently associated with insulin resistance as measured by HOMA-IR; however, only the increase in subcutaneous adipose tissue is linked to an increased insulin secretion. It is a possibility that the more the patient β-cell function allows secreting insulin, the more the subcutaneous, insulin-sensitive adipose tissue will grow secondary to lipogenesis activation. A low disposition index has been shown to predict the risk of developing type 2 diabetes,[26] because it reports the insulin secretion capacity relative to the degree of insulin resistance. Liver fat and VAT accumulations, but not subcutaneous fat accumulation, were related to the decrease in disposition index. That is, subjects accumulating subcutaneous fat progressively increase their insulin resistance but are able to maintain an adapted insulin secretion to overcome insulin resistance. Subcutaneous fat shows an inverse relationship with nT2D in women in the present study. Previous studies have shown that subcutaneous fat does not have a deleterious effect on plasma glucose/insulin homeostasis.[27] Its superficial content, as opposed to the deep abdominal subcutaneous fat, has been shown to have an inverse relationship with fasting plasma glucose and HbA1c.[28] Larger subcutaneous thigh fat was independently associated with more favorable fasting glucose level in elderly men of the Health, Aging and Body Composition Study.[29] Finally, patients presenting with peripheral lipodystrophies suffer from severe insulin resistance.[30]

Strengths and weaknesses of the study

The present study is a large international, observational study that assesses a large panel of anthropometric, demographic and cardiometabolic variables with precise and centralized measurements of abdominal adiposity and liver fat content measured by CT. The present study did not detail the specificities of each ethnic group due to the limited number of patients per group studied. Fat distribution by ethnicity in the INSPIRE ME IAA study has been reported elsewhere.[31] Although the distribution of fat was different according to ethnicity, a one-s.d. increases in VAT and liver fat were similarly associated with hypertension, type 2 diabetes, hypertriglyceridemia, low high-density lipoprotein cholesterol level and high C-reactive protein level in all the ethnic groups. Accordingly, we did not find in the present study any significant interaction between the glucose tolerance group and ethnicities regarding their association with the different fat depots.

Liver fat content was estimated from CT liver attenuation, which is not the gold standard imaging technique (magnetic resonance spectroscopy)[32] to assess liver fat. However, liver attenuation has been validated to be inversely and strongly correlated with liver fat content assessed by histopathological analysis.[33,34] It has also been shown that liver fat content is highly correlated with visceral adiposity.[35,36] Accordingly, in the present study, VAT and liver fat estimated by liver attenuation showed a highly significant correlation ($r = -0.32$ in men and $r = -0.40$ in women, $P < 0.001$).

CONCLUSIONS

In this large international cohort of patients managed by 297 physicians from 29 countries, we found that patients with iIFG, iIGT, combined IFG+IGT and nT2D had excess abdominal fat with specific distributions between visceral, abdominal subcutaneous adiposity and estimated liver fat. The OR to present with iIFG was associated with an increase in VAT only, whereas the OR to present with iIGT, IFG+IGT or nT2D were associated with increases in both VAT and estimated liver fat. This observation suggests that liver fat reflects multi-organ ectopic fat deposition, which is associated with iIGT, instead of being only a marker of increased

hepatic insulin resistance, which is more characteristic of iIFG. When completed, the prospective part of the present study will allow us to better understand which of VAT or estimated liver fat is predictive of increased risks of developing type 2 diabetes in patients with NGT/NGT, iIFG, iIGT or IFG+IGT.

CONFLICT OF INTEREST

Jean-Pierre Després has received a research grant from Eli Lilly, has received speaker's fees from Abbott, AstraZeneca, Merck, GlaxoSmithKline and Pfizer Canada Inc. and is on the consultant/advisory board of Novartis, Theratechnologies, Torrent Pharmaceuticals Ltd, Abbott and Sanofi. All the other authors declare no conflict of interest.

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