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Pronounced weight gain in insulin-treated patients with type 2 diabetes mellitus is associated with an unfavourable cardiometabolic risk profile

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ABSTRACT

Pronounced weight gain after start of insulin therapy in patients with type 2 diabetes mellitus (T2DM) may offset beneficial effects conferred by the improvement of glycaemic control. This hypothesis was tested by comparing the cardiometabolic risk profile of a group of type 2 diabetes patients with a marked increase in body weight ('gainers') after the start of insulin treatment and a similar group without any or only minimal weight gain ('non-gainers').

In a cross-sectional study, we compared two predefined groups of patients with T2DM who had been on insulin therapy for a mean of 4.0 years: 'gainers' *vs* 'non-gainers'. Cardiometabolic risk was assessed by measuring fat content and distribution (physical examination, bioelectrical impedance analysis, dual energy X-ray absorption, and magnetic resonance imaging), liver fat content (magnetic resonance spectroscopy), physical activity levels (Sensewear® armband) and plasma markers.

Each subgroup consisted of 14 patients. Gainers had significantly more total body and trunk fat (especially subcutaneous fat) compared with non-gainers. Gainers had similar liver fat content, and slightly higher levels of fat hormones. Furthermore, gainers performed significantly less physical activity. Lastly, gainers had higher total cholesterol, low-density lipoprotein cholesterol, and alanine aminotransferase levels with similar cholesterol-lowering treatment.

Patients with T2DM who show pronounced weight gain during insulin therapy have a less favourable cardiometabolic risk profile compared with patients who show no or minimal weight gain.

KEYWORDS

Insulin-associated weight gain, type 2 diabetes mellitus, cardiometabolic risk profile

INTRODUCTION

Insulin therapy is frequently needed to achieve adequate glycaemic control in patients with type 2 diabetes mellitus (T2DM), but often at the expense of weight gain. Although values differ between studies, and studies are generally of limited duration, the estimated weight gain during the first year of insulin therapy ranges from approximately 2 to 6 kg.[1] This weight gain shows large inter-individual differences, with some patients experiencing substantial insulin-associated weight gain, while others do not show any weight gain at all or even lose weight.

The determinants of insulin-associated weight gain are not entirely elucidated; most authors view the improvement in glycaemic control as the major determinant of weight gain. However, the level of improvement in glycaemic control is only weakly correlated with the increase in body weight.[2,3] A frequently mentioned clinical experience is that a subset of patients exists that shows a persistent and continuous increase in body weight over time even when stable glycaemic control has been obtained.

It is obvious that weight gain in an already overweight population is undesirable. Weight gain will deter further optimisation of insulin therapy[4] and in itself will adversely influence the cardiometabolic risk profile.[5] Little is known about the effects of insulin-associated weight gain on cardiometabolic risk in patients with T2DM. One may hypothesise that the benefits of insulin treatment

conferred by the improvement of glycaemic control may be offset by the disadvantages associated with pronounced weight gain. An increased fat mass may cause aggravation of insulin resistance, dyslipidaemia and hypertension and may increase the levels of inflammatory markers and the propensity for thrombotic events.[6] Indeed, in type 1 diabetes, patients who experienced pronounced weight gain during intensive insulin therapy showed a less favourable cardiovascular risk profile.[7] In the ACCORD trial,[8] the intensively treated group with T2DM showed increased mortality. In this group, more than 75% of the patients used insulin therapy in combination with several oral drugs. More than 25% of the patients treated in the intensive-therapy group showed a mean weight gain of >10 kg during follow-up. Although the study did not reveal any direct effect of the exaggerated weight gain on cardiovascular events, extensive weight gain might have had a negative influence on cardiometabolic risk.

In the present study, we hypothesised that pronounced weight gain during insulin therapy would be associated with an unfavourable cardiometabolic risk profile. This hypothesis was tested by comparing the cardiometabolic risk profile of insulin-treated patients with T2DM who showed weight gain at the extreme ends of the spectrum ('gainers' *vs* 'non-gainers').

RESEARCH DESIGN AND METHODS

Patient groups

Patients were selected out of a cohort of patients with T2DM who started insulin therapy in our University Diabetes Clinic between 2001 and 2006. To prevent confounding with respect to influences of different types of insulin on body weight we only included patients who started and continued on biphasic insulin (NovoMix® or Mixtard® insulin), twice-daily. Patients were selected based on the weight gain after starting insulin. We defined a 'gainer' as a patient who showed an increase in body weight of ≥0.5 kg/month within the first 18 months after starting insulin therapy and ≥5% weight gain at total follow-up (i.e. at the time of cross-sectional measurement, which was different for each patient). We defined a 'non-gainer' as a patient with a maximum weight gain ≤2.5 kg at follow-up. These criteria were derived from a historical insulin-treated group (n=140), and represent the upper and lower subgroups of weight gain. Assessment at follow-up of the cardiometabolic risk profile between the two groups (gainers *vs* non-gainers) was performed. All selected patients had a minimal follow-up of 18 months. Exclusion criteria were: heart failure (NYHA class III-IV), liver or renal disease (defined by chronic renal disease stage ≥III), hypoalbuminaemia, use of alcohol of more than 2 units/day, drug abuse, use of thiazolidinedione derivatives or prednisone, and pregnancy or the intention to become pregnant during the study. Eligible patients were not on anti-obesity medications and acarbose treatment. Also weight loss surgery patients or patients who followed any other weight management program were excluded.

The inclusion and exclusion criteria were reviewed at a screening visit, where patients underwent history taking and a complete physical examination. The study protocol was approved by the local ethics committee. All patients provided written informed consent.

Cardiometabolic risk assessment

Cardiometabolic risk profile at follow-up was assessed by the following: 1) body fat distribution (weight, height, waist and hip circumference, bioelectrical impedance analysis (BIA), dual energy X-ray absorption (DEXA), and MRI),[9,10] 2) liver fat content (LFAT) by magnetic resonance spectroscopy (MRS),[11] 3) physical activity levels,[12] 4) classical risk factors, other biochemical cardiometabolic markers.[13]

Patients were tested after overnight fasting and with an empty bladder. To determine body mass index (BMI), weight (kg) was divided by height in metres, squared. Weight was measured with subjects wearing light underwear only. Scales were calibrated annually. Waist circumference was measured midway between the lower rib margin and the iliac crest at expiration, and hip circumference over the greater trochanter; waist-to-hip ratio (WHR) was calculated.

To assess fat distribution three different methods were used: BIA, DEXA and MRI. BIA was carried out using an Akern soft tissue analyser (BIA Quatum/S Body Composition Analyser model no. BIA-101, Akern Srl, Pontassieve (Florence), Italy). BIA was performed to assess total body water (TBWa) and fat-free mass (FFM). Patients rested in a supine position for approximately five minutes to equalise fluid compartments. Four surface electrodes were applied (two each to an arm and a leg). Phase sensitive sensors separated the components of the modulus into Reactance and Resistance.

Total-body DEXA scanning was performed using a Hologic QDR 4500 densitometer (Hologic Inc., Bedford, USA) to determine fat mass (total fat mass and trunk fat) and lean mass. To assess non-trunk fat, the trunk-to-leg ratio (trunk mass divided by leg mass) was calculated for each patient.

MRI measurements were performed on a Tim-Trio MR system (Siemens, Erlangen, Germany). A series of T1-weighted (flash 2D) axial MR images was acquired from a region extending from 4 cm above to 4 cm below the fourth to fifth lumbar interspace. Visceral and subcutaneous fat areas were determined based on signal intensity. Proton MR spectra (STEAM; TE/TR:20/3000ms) were obtained without water suppression from a 8-ml voxel positioned in the liver during breath holding. The water signal intensity (S_{water}) and the methylene lipid signal

intensitiy (S_{fat}) were used to calculate the percentage of liver fat by the following formula: $((S_{fat})/(S_{fat} + S_{water})) \times 100\%)$.[14] Total MR examination time was 30 minutes. Patients with pacemakers, implantable cardioverter defibrillators, metal implants, and claustrophobia were included, but did not undergo magnetic resonance imaging (MRI). In 11 gainers and 12 non-gainers MRI/MRS was performed. The remaining patients experienced claustrophobia during the MRI/MRS scan although they were not known with claustrophobia at inclusion.

Physical activity was measured using a SenseWear Pro Armband™ (Body Media, Pittsburgh, PA, USA).[15,16] The device was placed on the right upper arm over the triceps muscle for five consecutive days. Measurements were only used for calculations if >90% of data were available. SenseWear Innerview professional software 6.1 was used to analyse the data.

Classical risk factors as blood pressure, smoking habits, lipids, renal function and albumin excretion ratio (AER) were determined. Blood pressure was measured at the right arm with the patient in a supine position after a minimum of five minutes rest. All patients had already taken their antihypertensive drugs. Blood pressure was determined twice by a manual sphygmomanometer. The average blood pressure (mean systolic and diastolic blood pressure) was calculated. Furthermore, fasting blood samples were drawn to assess: HbA_{1c}, lipids, alanine aminotransferase (ALAT), and creatinine (all determined by standard laboratory methods). Renal function expressed as glomerular filtration rate (GFR) was calculated by the modified diet in renal disease (MDRD) formula.[17] The adipocytokines, adiponectin and leptin were determined by using DuoSet ELISA development system kits (R&D systems, Minneapolis, USA), free fatty acids (FFA) using Cobas Mira Plus® (Roche Diagnostics Ltd., Basal, Switzerland), and the inflammatory markers high-sensitive C-reactive protein (hsCRP) by Dako ELISA (Glastrup, Denmark), IL-6 and 18 by Luminex® Corporation assay (Austin, Texas, USA)).

Statistical analyses

Differences between groups were analysed by unpaired Student's *t*-test and Mann-Whitney U test as appropriate. For comparing dichotomous variables the χ^2 test was used. All calculations were made using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Data are expressed as means ± SD, unless otherwise indicated. A p value <0.05 was considered significant in all statistical comparisons.

RESULTS

A total of 14 patients were included in each group. *Table 1* shows the characteristics of the patients. All patients were Caucasian. The two groups (gainers *vs* non-gainers) were compared after a mean of 4.0±1.6 years insulin therapy (i.e. mean follow-up); as per protocol, all patients were still on biphasic insulin. Gainers were on insulin longer than non-gainers (4.6±1.7 *vs* 3.7±2.1 year, p=0.03).

Table 1. *Patient characteristics after a mean of four years of insulin therapy*

	Gainers	Non-gainers
Gender (male/female)	8/6	10/4
Age (year)	63±7	65±7
Diabetes duration (years)	9 (2-25)	12 (2-22)
Insulin therapy (years)	4.6±1.7	3.7±2.1 *
Medication (n)		
• Insulin alone	7	5
• Insulin + Metformin	6	7
• Insulin + SU	0	1
• Insulin + Metformin + SU	1	1
Insulin dose (U/day)	68±37	60±37
Insulin dose (U/kg)	0.7±0.4	0.7±0.4

Data are means±SD or median (range); *P<0.05; SU = sulfonylurea derivative.

Change in weight and HbA_{1c} after start of insulin therapy

As per definition, gainers showed a substantially larger weight gain (+11 kg [range +5.2 to +19.6 kg]) compared with the non-gainers (-1.2 kg [range -7.6 to +2.5 kg], *figure 1*). Of note, body weight at the start of insulin therapy was slightly lower in gainers (83± 5 *vs* 87±13 kg, p=NS). After four years of insulin therapy, BMI, waist circumference and WHR were not significantly different between the two groups (BMI 32.3±5.6 *vs* 29.0±4.5 kg/m^2, waist circumference 110±15 *vs* 106±13 cm, WHR 1.05±0.12 *vs* 1.03±0.10, for gainers and non-gainers, respectively, p=NS). Mean HbA_{1c} decreased from 9.9±2.6 to 7.2±0.7 in gainers *vs* 8.9±1.3 to 7.4±0.9 % in non-gainers (p=NS for the difference in gainers *vs* the difference in non-gainers).

Cardiometabolic risk profile at follow-up

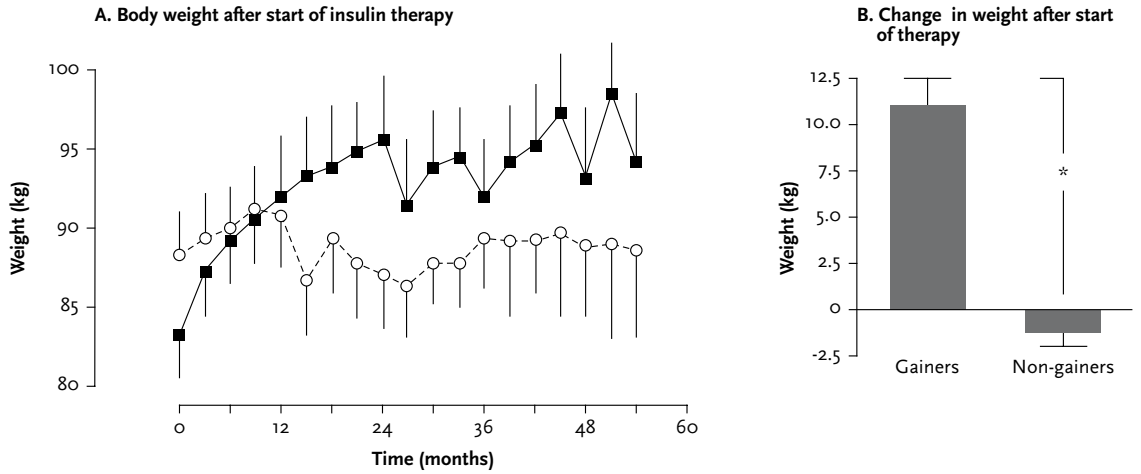
Fat distribution

Gainers had significantly more total body fat (32.4±9.4 *vs* 24.6±7.7 kg, p=0.03) and more trunk fat compared with non-gainers (18.3±5.5 *vs* 14.1±4.2 kg, p=0.04). Gainers had slightly higher TBWa compared with non-gainers (37.7±7.5 *vs* 35.9±7.1 litres, p=NS). FFM was comparable in gainers and non-gainers as measured by DEXA (58.4±9.1 *vs* 59.6±8.7 kg, p=NS) and by BIA. In both groups the trunk-to-leg ratio was similar.

As measured by MRI, gainers had significantly higher subcutaneous fat than non-gainers (2.5±0.8 *vs* 1.8±0.8 litres, p=0.04), while visceral fat was similar (1.7±0.7 *vs* 1.5±0.7 litres, p=NS).

Sixteen patients (70%) had LFAT levels above the upper reference value of 5.5%.[18] Gainers and non-gainers had similar LFAT (9.6±2.7 *vs* 9.3±1.6 %, respectively, p=NS).

Figure 1. *A. (left panel) Observed change in weight after start of insulin therapy (t=0) comparing gainers (closed squares) and non-gainers (open circles). B. (right panel) Absolute change in weight after start of insulin therapy. * P<0.05.*



Physical activity levels

The average total energy expenditure at follow-up was significantly lower in gainers than in non-gainers (2275±385 *vs* 2632±734 kcal/day, p=0.005). Physical activity duration expressed as metabolic equivalent (MET) ≥3, which is consuming ≥3 kcal/kg of body weight per hour, tended to be lower in gainers compared with non-gainers (56±51 *vs* 83±80 min/day, p=0.06). Also the amount of vigorous activity (MET >6) was lower in gainers than in non-gainers (2.4±3.4 *vs* 6.5±5.5 minutes, p=0.03). The amount of sedentary activity (MET 0-3) and moderate activity (MET 3-6) was similar between the two groups, as was the number of steps per day (5416±3543 *vs* 5282±3681), and the total duration of rest (514±139 *vs* 484±148 minutes) and sleep (409±137 *vs* 394±135 minutes), all gainers *vs* non-gainers, respectively.

Cardiometabolic markers

Prior to the start of insulin therapy the classical cardiometabolic risk markers (i.e. BMI, blood pressure, lipid profiles, smoking, GFR and AER) were similar in the two groups (*table 2*).

The cardiometabolic risk markers of the two groups measured in the present study are shown in *table 3*. Blood pressure was similar in gainers compared with non-gainers, as was the average number (2.1 *vs* 2.2) and dose of antihypertensive medication. Total cholesterol and low-density lipoprotein cholesterol (LDL-cholesterol) were significantly higher in gainers than in non-gainers, as was the level of high-density lipoprotein cholesterol (HDL-cholesterol), despite similar use of statins at equipotent doses.

There were no differences between the two groups with respect to smoking habits. Creatinine was slightly lower

Table 2. *Baseline cardiometabolic risk markers comparing gainers and non-gainers*

	Gainers	Non-gainers	p-value
Classical risk factors			
SBP (mmHg)	148±26	153±26	NS
DBP (mmHg)	85±12	88±8	NS
Total cholesterol (mmol/l)	5.1±1.0	4.5±0.9	NS
LDL-cholesterol (mmol/l)	3.0±1.0	2.3±0.9	NS
HDL-cholesterol (mmol/l)	1.2±0.2	0.9±0.2 *	0.01
Triglycerides (mmol/l)	2.0±1.1	3.3±2.4	NS
Smoking (n)	11	12	NS
Creatinine (µmol/l)	92.6±29.7	99.5±28.9	NS
GFR (MDRD); ml/min/1.73m²	79.0±34.6	64.5±0.19	NS
Albumin excretion ratio (µg/min)	29.8±24.6	39.1±53.4	NS
Liver enzymes			
ALAT (U/l)	36.8±18.4	36.2±20.4	NS

Data are means ± SD; SBP = systolic blood pressure; DBP = diastolic blood pressure; LDL = low-density lipoprotein; HDL = high-density lipoprotein; GRF = glomerular filtration rate; MDRD = modified diet in renal disease; ALAT = alanine aminotransferase. All data were analysed by Student *t*-test, except for the albumin excretion ratio (AER) data, which were analysed with Mann-Whitney *U*-test. * P<0.05.

Table 3. *Cardiometabolic risk markers comparing gainers and non-gainers at follow-up*

	Gainers	Non-gainers	P-value
Classical risk factors			
SBP (mmHg)	150±23	146±31	NS
DBP (mmHg)	81±10	80±11	NS
Total cholesterol (mmol/l)	4.8±0.9	3.8±0.8 *	0.001
LDL-cholesterol (mmol/l)	2.9±0.8	2.1±0.4 *	0.006
HDL-cholesterol (mmol/l)	1.2±0.2	1.0±0.2 *	0.03
Triglycerides (mmol/l)	2.1±1.4	1.7±0.6	NS
Smoking (n)	2	2	NS
Creatinine (µmol/l)	85±29	97±38	NS
GFR (MDRD; ml/min/1.73 m²)	110±28	85±33 *	0.04
AER (µmol/min)	113±228	74±143	NS
FFA (µmol/l)	0.6±0.2	0.6±0.3	NS
Fat hormones			
Leptin (ng/ml)	43.3±26.7	29.7±21.3	NS
Adiponectin (µg/ml)	3.0±1.4	2.1±1.0	NS
Inflammatory markers			
hsCRP (pmol/ml)	4.8±3.3	3.7±4.2	NS
IL-6 (pg/ml)	11.5±18.3	5.9±2.9	NS
IL-18 (pg/ml)	139±39	133±46	NS
Liver enzymes			
ALAT (U/l)	33.1±11.4	23.8±9.1 *	0.04

Data are means ± SD. SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL = low-density lipoprotein, HDL = high-density lipoprotein, GRF = glomerular filtration rate; MDRD = modified diet in renal disease; AER = albumin excretion ratio; ALAT = alanine aminotransferase. All data were analysed by Student *t*-test, except for the data on AER, leptin, hsCRP, IL-6 and 18 which were analysed by nonparametric Mann-Whitney *U* test. * P<0.05.

in gainers than in non-gainers and urinary albumin excretion appeared quantitatively higher in gainers, but the differences were not significant. Calculated GFR was significantly higher in the gainers group. FFA and adiponectin levels were similar between the two groups. Leptin, and the inflammatory cytokines were slightly higher in gainers than in non-gainers. ALAT levels were significantly higher in gainers.

DISCUSSION

The main finding of this cross-sectional pilot study is that patients who develop pronounced weight gain after long-term insulin therapy have more total, trunk and subcutaneous fat, perform less physical activity and show slightly higher cholesterol and ALAT levels and GFR compared with those who do not gain weight. All together these findings suggest that this group of 'gainers' may have an unfavourable cardiometabolic risk profile compared with 'non-gainers'.

So far, hardly any studies have investigated the effects of long-term insulin therapy on body weight as a primary endpoint. Follow-up in most studies is limited to 6 to 12 months, with a reported increase in body weight of approximately 2 to 6 kg.[1] Whether ongoing weight increase occurs in insulin-treated patients while stable glycaemic control has been obtained is less clear. Recently, Aas *et al.*[19] reported a mean weight gain approaching 4 kg over three years in insulin-treated participants of the DIGAMI study. Approximately 30% of the weight gain took place beyond one year of therapy. Also Kooy *et al.*[20] found ongoing weight increase in a group of insulin-treated patients who were followed for over four years. The limited findings from literature match with general clinical experience and suggest that – at least in a subset of patients – weight continues to increase during insulin treatment without further improvement in glycaemic control. The 'gainers' selected in the present study may represent this group.

The two groups seemed to differ with respect to body weight before the start of insulin, with the gainers starting at a lower weight, although this difference was statistically not significant. This may suggest that gainers had lost more weight before the onset of insulin and thus simply regained more weight after starting insulin, as has been suggested before.[21] As the initial HbA_{1c} level was also slightly higher in the gainers group (not statistically significant), a relative contribution from 'initial regain' cannot be fully excluded. Indeed, gainers suffered a mean of 4.3 kg±6.4 kg weight loss within 12 months prior to the start of insulin therapy. However, the observed sustained increase in body weight during long-term insulin treatment cannot be attributable to exaggerated weight loss before therapy.

The adverse effect of a sustained increase in body weight during insulin treatment as found in the present study is supported by previous findings. Yki-Järvinen *et al.*[4] reported higher blood pressure and lipid levels in patients with an exaggerated weight increase. In a recent report, initiation of insulin treatment after myocardial infarction

was associated with a significant increase in weight and incidence of re-infarction, although the latter was not clearly explained by the increased weight.[19] In addition, elevated levels of adipokines were found in a group of patients treated with insulin who gained weight compared with a group treated by lifestyle intervention that lost weight, despite similar glycaemic levels. All together these data, though limited, suggest that insulin-associated weight gain may indeed negatively affect cardiometabolic risk profile.

We found that the group of subjects with pronounced weight increase had higher total, trunk and subcutaneous fat. Long-term effects of insulin-associated weight gain on body composition have not been reported, but in short-term studies, insulin treatment showed an increase in fat mass but also FFM, in line with the anabolic effect of insulin.[22] The present study did not reveal a difference in lean body mass between the two groups. Although this does not exclude a beneficial effect of insulin treatment in itself on lean body mass, it does show that the exaggerated weight increase is explained by an increase in fat only.

Most studies suggest that visceral rather than subcutaneous fat is associated with insulin-resistance and may confer increased cardiometabolic risk.[23-25] From this point of view, the currently reported increase in subcutaneous fat mass may not necessarily incur to a strongly elevated cardiometabolic risk, but may still contribute to an adverse cardiometabolic risk profile.[26] It could be hypothesised that gainers exhibit higher levels of adipocytokines (e.g. leptin, IL-6) at the level of subcutaneous adipose tissue compared with non-gainers. Furthermore, it could be argued that dietary content (e.g. ceramide intake) might influence body weight and metabolic effects on adipose tissue.[27] Unfortunately, we did not perform subcutaneous fat biopsies in the two groups and took a standardised questionnaire survey for assessing patients dietary habits.

It is known that hepatic fat accumulation is associated with (hepatic) insulin resistance in non-alcoholic fatty liver disease and is also a predictor of cardiometabolic disease.[27] Juurinen *et al.*[28] showed that after seven months of insulin therapy (basal insulin) patients had improvement of hepatic insulin sensitivity and reduction of hepatic fat content. LFAT content after longer periods of insulin treatment has not been studied. The present results found no statistically significant differences between the two groups with respect to LFAT content as measured by MRS. Both groups had substantial percentages of LFAT (~10%), which is in line with results reported in literature.[29] The number of subjects in whom LFAT measurements were successful in the study was relatively low and thus the lack of a difference may represent a power problem, especially as the slightly higher ALAT levels suggest that the group of gainers may have had slightly higher LFAT. Alternatively, higher ALAT levels may confer an elevated cardiovascular risk, independent of LFAT.[30,31]

MDRD-GFR was higher in the gainers, which was associated with a tendency towards lower serum creatinine and an increased albumin excretion rate. Together these results suggest the existence of glomerular hyperfiltration, which in itself has been listed as a cardiovascular risk marker.[32]

(Low-grade) inflammation and adipocytokines (i.e. leptin) are associated with obesity and cardiovascular disease.[33-35] In line with this association, leptin, IL-6, IL-18 and hsCRP tended to be higher in gainers. Adiponectin levels are negatively associated with obesity and with cardiovascular endpoints. Adiponectin levels in both groups were similar, suggesting that insulin-associated weight gain does not necessarily translate in a (further) decrease in adiponectin levels.

Physical activity is a strong predictor of future cardiovascular disease and a determinant of body weight.[36] Gainers had lower levels of total energy expenditure compared with non-gainers, and performed less vigorous exercise compared with non-gainers. Due to the cross-sectional design of our study, it cannot be determined whether the decreased level of physical exercise is the cause of the exaggerated insulin-associated weight gain or the consequence. However, no matter the cause or consequence, a low physical exercise level remains a cardiovascular risk factor. It can be speculated, for instance, that (pronounced) weight gain in insulin-treated patients and change in physical activity is associated with a decrease in mood or tendency towards depression. In this study we did not assess (changes in) mood or depression score. Further prospective work is warranted in order to investigate the relationship between insulin-associated weight gain and level of physical activity.

The study has a number of limitations. The cross-sectional comparison cannot determine whether the unfavourable cardiometabolic risk profile observed in the gainers is the direct consequence of insulin-associated weight gain. The study cannot determine whether part of the observed weight gain is due to the 'natural' course of body weight associated with ageing; this would require a control group of either matched non-diabetic subjects, or subjects with T2DM on oral medication. The study also has a number of strengths. There is a rather homogenous population, similarly treated patients from a single centre, all on biphasic insulin. We used a set of sophisticated techniques to quantify body fat distribution and physical activity.

The results of our study may have clinical implications. As it seems that pronounced weight gain during (long-term) insulin therapy is associated with a less favourable cardiometabolic risk profile, it may be important to determine which patients are most at risk for weight gain. This would require assessment of predictive factors and

lifestyle characteristics before onset of insulin treatment, which, however, are largely unknown. Most authors view the change in glycaemic control (i.e. change in HbA_{1c}) as the major determinant of insulin-associated weight gain.[2,3] Although part of the short-term insulin-associated weight gain may be explained by change in HbA_{1c} in this study, this cannot explain sustained weight gain after long-term insulin therapy when stable or even increased HbA_{1c} is observed. Further prospective studies are needed to improve identification of patients who are at risk for extensive body weight increase and develop interventions to prevent the weight gain.

In conclusion, the present study suggests that pronounced weight gain during (long-term) insulin therapy in patients with T2DM is associated with an unfavourable cardiometabolic risk profile. Further work is required to determine the individual risk factors for exaggerated weight increase, to assess long-term consequences and to develop potential interventions.

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REFERENCES

1. Holman RR, Thorne KI, Farmer AJ, et al. Addition of biphasic, prandial, or basal insulin to oral therapy in type 2 diabetes. N Engl J Med. 2007;357(17):1716-30.
2. Salle A, Ryan M, Guilloteau G, Bouhanick B, Berrut G, Ritz P. 'Glucose control-related' and 'non-glucose control-related' effects of insulin on weight gain in newly insulin-treated type 2 diabetic patients. Br J Nutr. 2005;94(6):931-7.
3. Jacob AN, Salinas K, Adams-Huet B, Raskin P. Weight gain in type 2 diabetes mellitus. Diabetes Obes Metab. 2007;9(3):386-93.
4. Yki-Järvinen H, Ryysy L, Kauppila M, et al. Effect of obesity on the response to insulin therapy in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1997;82(12):4037-43.
5. Anderson JW, Kendall CW, Jenkins DJ. Importance of weight management in type 2 diabetes: review with meta-analysis of clinical studies. J Am Coll Nutr. 2003;22(5):331-9.
6. Aas AM, Seljeflot I, Torjesen PA, Diep LM, Thorsby PM, Birkeland KI. Blood glucose lowering by means of lifestyle intervention has different effects on adipokines as compared with insulin treatment in subjects with type 2 diabetes. Diabetologia. 2006;49(5):872-80.
7. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Diabetes Control and Complications Trial. JAMA. 1998;280(2):140-6.
8. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med. 2008;358(24):2545-59.
9. Wilson PW, Bozeman SR, Burton TM, Hoaglin DC, Ben-Joseph R, Pashos CL. Prediction of first events of coronary heart disease and stroke with consideration of adiposity. Circulation. 2008;118(2):124-30.
10. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J (Clin Res Ed). 1984;289(6454):1257-61.
11. Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki-Jarvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology. 2008;135(1):122-30.
12. Sesso HD, Paffenbarger RS, Jr, Lee IM. Physical activity and coronary heart disease in men: The Harvard Alumni Health Study. Circulation. 2000;102(9):975-80.
13. Qasim A, Mehta NN, Tadesse MG, et al. Adipokines, insulin resistance, and coronary artery calcification. J Am Coll Cardiol. 2008;52(3):231-6.
14. Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol. 1999;276:E977-89.
15. St-Onge M, Mignault D, Allison DB, Rabasa-Lhoret R. Evaluation of a portable device to measure daily energy expenditure in free-living adults. Am J Clin Nutr. 2007;85(3):742-9.
16. Mignault D, St-Onge M, Karelis AD, Allison DB, Rabasa-Lhoret R. Evaluation of the Portable HealthWear Armband: a device to measure total daily energy expenditure in free-living type 2 diabetic individuals. Diabetes Care. 2005;28(1):225-7.
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130(6):461-70.
18. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462-8.
19. Aas AM, Ohrvik J, Malmberg K, Ryden L, Birkeland KI. Insulin-induced weight gain and cardiovascular events in patients with type 2 diabetes. A report from the DIGAMI 2 study. Diabetes Obes Metab. 2009;11(4):323-9.
20. Kooy A, de Jager J, Lehert P, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. Arch Intern Med. 2009;169(6):616-25.
21. Larger E, Rufat P, Dubois-Laforgue D, Ledoux S. Insulin therapy does not itself induce weight gain in patients with type 2 diabetes. Diabetes Care. 2001;24(10):1849-50.
22. Wolfe RR. Effects of insulin on muscle tissue. Curr Opin Clin Nutr Metab Care. 2000;3(1):67-71.
23. Kuk JL, Church TS, Blair SN, Ross R. Does measurement site for visceral and abdominal subcutaneous adipose tissue alter associations with the metabolic syndrome? Diabetes Care. 2006;29(3):679-84.
24. Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. Diabetes. 2004;53(8):2087-94.
25. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39-48.
26. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30(5):1212-8.
27. Kolak M, Westerbacka J, Velagapudi VR, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. Diabetes. 2007;56(8):1960-8.
28. Juurinen L, Tiikkainen M, Hakkinen AM, Hakkarainen A, Yki-Jarvinen H. Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes. Am J Physiol Endocrinol Metab. 2007;292(3):E829-35.

29. Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology. 2007;133(2):496-506.

30. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis. 2007;191(2):235-40.

31. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ. 2004;328(7446):983.

32. Tomaszewski M, Charchar FJ, Maric C, et al. Glomerular hyperfiltration: a new marker of metabolic risk 1. Kidney Int. 2007;71(8):816-21.

33. Koenig W, Sund M, Frohlich M, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation. 1999;99(2):237-42.

34. Engeli S, Feldpausch M, Gorzelniak K, et al. Association between adiponectin and mediators of inflammation in obese women. Diabetes. 2003;52:942-7.

35. Reilly MP, Iqbal N, Schutta M, et al. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. J Clin Endocrinol Metab. 2004;89:3872-8.

36. Mora S, Lee IM, Buring JE, Ridker PM. Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. JAMA. 2006;295:1412-9.