Insulin use is common in type 2 diabetes mellitus and is frequently accompanied by weight gain, the composition of which is poorly understood. The present study evaluates insulin-induced body composition changes. Body weight and composition of thirty-two type 2 diabetic patients undergoing their first 12 months of insulin therapy were compared with those observed in thirty-two type 2 diabetic patients previously treated on insulin (minimum 1 year). Body composition was determined by simultaneous body water spaces (bioelectrical impedance analysis) and body density measurements. After 6 months, glycosylated Hb (HbA1c) significantly improved in the newly treated group (\(P<0.0001\)), but remained stable in those treated previously. HbA1c did not differ between 6 and 12 months in the two groups. Body weight significantly (\(P=0.04\)) changed over 12 months in those newly treated only (+2.8 kg), essentially comprising fat-free mass (\(P=0.044\)). Fat mass remained unchanged (\(P=0.85\)) as did total body water, while extracellular: total body water ratio tended to increase in those newly treated (\(P=0.059\)). Weight changes correlated with HbA1c changes (\(R^2=0.134, P=0.002\)) in the initial 6 months only. Insulin therapy leads to weight gain (28 kg), predominantly fat-free mass, over 12 months. After 6 months, newly treated patients continued gaining weight despite an unchanged HbA1c, suggesting the potential anabolic role of insulin in subsequent gains. Therefore, in the initial 6 months, weight gain can be attributed to a ‘glucose control-related effect’ and further gain appears to be due to a ‘non-glucose control-related effect’ of insulin treatment.

### Type 2 diabetes: Insulin treatment: Body composition changes: Plethysmography: Bioelectrical impedance

Insulin therapy is commonly used during the progression of type 2 diabetes mellitus, and is frequently accompanied by weight gain (Sinha et al. 1996; UK Prospective Diabetes Study Group, 1998; Mäkimattila et al. 1999; Rigalleau et al. 1999; Larger et al. 2001; Packianathan et al. 2005). The magnitude of weight gain can be quite variable between subjects and ranges from 3.7 kg (Sinha et al. 1996) to 7.5 kg (Mäkimattila et al. 1999) over the first year of treatment. It is commonly accepted that disappearance of glycosuria (Järvinen, 2001) and reduction in resting energy expenditure (Nair et al. 1984; Bogardus et al. 1986; Fontvielle et al. 1992; Gougeon, 1996; Gougeon et al. 2002) are responsible for weight gain, which is correlated with the improvement in glycaemic control (Mäkimattila et al. 1999). However, data from the UK Prospective Diabetes Study 33 (UK Prospective Diabetes Study Group, 1998) suggest that despite an identical level of metabolic control, weight gain arising from insulin treatment is greater than that following treatment with sulphonylurea. Insulin exhibits anabolic properties and, therefore, the question of the relative ‘non-glucose control-related effect’ v. ‘glucose control-related effect’ of insulin on body weight remains to be elucidated.

Insulin exerts an anabolic effect on both adipose and muscle tissue, and leads to water and Na retention (De Fronzo, 1981; Nair et al. 1987; Pacy et al. 1989; Ter Maaten et al. 2000; Packianathan et al. 2005). Its antinatriuretic effect is preserved in insulin-resistant states, such as that observed in type 2 diabetes (Ter Maaten et al. 1999). It is clear that insulin-induced weight gain (or regain) in type 1 diabetes is made of fat-free mass (FFM) (Sinha et al. 1996; DCCT Group, 2001; Gomez et al. 2001). In type 2 diabetics, the picture is less clear. Sinha et al. (1996), using dual-energy X-ray absorptiometry, showed that two-thirds of weight gain was made of fat. Other studies have derived fat mass from the measurement of total body water (TBW) and concluded similarly (Group et al. 1989; Mäkimattila et al. 1999; Rigalleau et al. 1999). However, accurate estimates of fat mass from body water assumes that hydration of FFM is known (Ritz, 1998). In certain cases, particularly during diabetes and insulin therapy, it is inappropriate to assume that K, water and bone mass all remain as constant constituents of FFM (Tsu et al. 1998; Packianathan et al. 2004). Furthermore, in diabetic patients the distribution between extracellular water (ECW) and TBW varies according to the level of metabolic control achieved (Brizzolara et al. 1996). A more accurate approach to measuring body composition changes is required in the study of the effect of insulin treatment (Packianathan et al. 2004).

The aim of the present study was therefore to evaluate the effect of 12 months of insulin treatment on the body composition of newly treated type 2 diabetic patients and to analyse the

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**Abbreviations:** BIA, bioelectrical impedance analysis; ECW, extracellular water; FFM, fat-free mass; HbA1c, glycosylated Hb; NEWt, newly insulin-treated patients; PREV, previously insulin-treated patients; TBW, total body water.

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influence of metabolic control of weight gain. The control group consisted of type 2 diabetic patients who had been treated with insulin for at least 1 year prior to the study. This renders two comparable groups, thus eliminating potential confounding factors that could influence body composition, such as diet and lifestyle changes, which are recommended to patients, as well as their age, BMI and the effect of time. This extends work previously published (Sallet et al. 2004), where 6 months changes in body composition were described.

Subjects and methods

Patients
Sixty-four type 2 diabetic patients, mean age 65.7 (SD 9.3) years, were recruited from within the diabetes department of the University Hospital of Angers between June and September 2001. For thirty-two patients (seventeen women and fifteen men), the evaluation was carried out before and during the first 12 months of receiving insulin therapy (NEW). Their body weight 6 months before starting treatment was not significantly different from weight at time T0. Thirty-two other patients (nine women and twenty-three men) undergoing insulin therapy for at least 1 year prior to commencing the study served as the control group (PREV). All patients were treated on two to three injections of insulin per day in order to obtain glycaemic and glycosylated Hb (HbA1c) levels close to those recommended (Recommandations de l’ANAES, 2000). Subject exclusion criteria included a reduced autonomy, which could interfere with plethysmographic measurement or any condition leading to water and salt retention (general heart failure, liver failure or severe kidney failure as defined by a plasma creatinine clearance <30 ml/min). All patients were advised by a physician and a dietitian regarding their diet and physical activity in order to have it adapted in accordance with their body weight. All patients were fully informed of the purpose of the study and gave their voluntary consent to take part in the investigation. The University Hospital ethical committee accepted the protocol.

Study design
Each patient was measured within a single consultation, on three separate occasions, under identical conditions and separated by a 6-month interval. Each evaluation comprised a full clinical examination (weight, height and blood pressure), HbA1c measurement, bioelectrical impedance analysis (BIA) and a plethysmographic measurement.

Height, weight and BMI
Height was measured in bare feet and in undergarments, with heels together and touching the base of a vertical scale, which was set to the nearest 0.1 cm (SECA, Hamburg, Germany). Weight was measured after breakfast, in undergarments, on a plethysmographic weighing scale, which was calibrated to the nearest 0.01 kg. The scale was calibrated prior to each test.

Biological parameters
HbA1c was determined using the HPLC technique (Variant Hæmoglobin A1c Recorder Pack; Bio-Rad, Hercules, CA, USA).

Impedance analysis
Resistance and reactance were measured at three different frequencies (5, 50 and 100 kHz) using a BIA system (Analyzor 4; Spengler, Cachan, France). The procedure was carried out after the patient had been resting in a supine position for at least 30 min. Current-inducing spot externally applied electrodes were positioned on the dorsal side of the right hand between the metacarpal bones of the second and third fingers and on the dorsal side of the right foot between the metatarsal bones of the second and third toes. The two receiver electrodes were placed between the distal radial and cubital styloids of the right wrist and between the malleoli of the right ankle. BIA measured at a low frequency (5 kHz) allowed the quantity of ECW to be determined (the equations of which are given on p. 933), while measurements taken at higher frequencies (100 kHz) reflected TBW (the equations of which are given on p. 933).

Plethysmography
Air displacement plethysmographic measurements were carried out using the Bod-Pod body composition system (Life Measurement, Inc, Concord, CA, USA) according to the manufacturer’s instructions and recommendations (Fields et al. 2002). The test was carried out while the patients were in their undergarments and were wearing a swimming cap. The patient was initially weighed on an electronic scale. The procedure involved the calibration of the system when empty and then when a 50-litre metal cylinder was placed inside. The patient was then seated within a 450-litre chamber inside the machine, and asked to remain still with hands positioned on their thighs and breathing normally. A minimum of two consecutive body volume measurements (Vb), each lasting 50 s, were carried out. If the two volumes differed from each other by more than 150 ml, a third measurement was undertaken. Body density (Dh) was calculated by the machine’s integrated computer using the equation: Dh = weight/Vb. Predicted respiratory volumes were considered in the calculations.

Anthropometric markers
In the following equations, weight is expressed in kg, height in cm and body density in g/cm³.

TBW was calculated according to the equation:

TBW (litres) = 2.896 + 0.366 × Height 2/100 + 0.137 × Weight + 2.485 × gender,

for subjects over 65 years, using 1 male and 0 females (Vache et al. 1998), or according to the equation:

TBW (litres) = 0.454796 × Height 2/100 + 0.139523 × Weight + 3.432026.

for subjects less than 65 years, regardless of gender (Segal et al. 1991). Z100 represents the impedance at 100 kHz. We have shown in Sallet et al. (2004) that these equations are valid in diabetic subjects, irrespective of metabolic control.

We have separately validated these equations. Forty-three other diabetic patients (age 61-4 (SD 10-2) years; BMI 31.7 (SD 6.04) kg/m²) had, on the same day, a measurement of TBW using 2H dilution and BIA (Z100). The mean difference between
The two estimates of TBW was 20.34 (SD 2.3) kg, with no indication of bias as assessed by Bland and Altman analysis (Bland & Altman, 1986). ECW was calculated using the same equation regardless of gender or age. In the group of forty-three type 2 diabetic patients, we have shown that equations traditionally used to estimate ECW, such as that of Segal et al. (1991), are inaccurate. Segal's equation induces a bias of 1.87 (SD 1.76) litres when compared with Bromide dilution, which is referred to as a reference technique (Ritz, 1998). We have validated a new set of equations based on the Bromide dilution technique:

$$ECW (\text{litres}) = 0.367 + 0.093 \times \text{Weight} + 0.157 \times \text{Height}^2 - 2.05 \times \text{Z}$$

where SEE is the standard error of the estimate.

The percentage of fat mass was determined using a three-compartmental model using the following equation (Siri, 1961):

$$\% \text{Fat} = \frac{2.1176 \times \text{Density} - 20.78 \times \text{TBW}^{0.78} - 1.3151}{0.72}$$

This method has been shown to provide accurate measurements of body fat (0.2 %) and FFM when compared to dual-energy X-ray absorptiometry (Clasey et al., 1999). It does take variation of hydration into account.

FFM, determined by a three-compartmental model, was calculated from weight and % fat.

FFM was also determined with a two-compartmental model using BIA according to the equation: FFM 2-comp (kg) = TBW / 0.732.

Statistical analysis
Results are expressed as means and standard deviations. A comparison between baseline values (T0) and at 6 months (T6) and 12 months (T12) among the NEWt and the PREVt was carried out using ANOVA for repeated measurements with the interaction between time and treatment conducted. $P < 0.05$ was considered significant in all statistical comparisons.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>NEWt</th>
<th>PREVt</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T6</td>
<td>T12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.6</td>
<td>65.8</td>
<td>0.94</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.4</td>
<td>85.8</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2</td>
<td>30.6</td>
<td>0.70</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.48</td>
<td>7.78</td>
<td>0.12</td>
</tr>
</tbody>
</table>

HbA1c, glycosylated Hb; NEWt, newly insulin-treated patients; PREVt, patients treated on insulin for at least 1 year previously.

* For details of procedures, see p. 902.
Body composition at baseline (T0), after 6 months (T6) and after 12 months (T12) *

(Values are means and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T6</th>
<th>T12</th>
<th>T0</th>
<th>T6</th>
<th>T12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBW (litres)</td>
<td>39·0</td>
<td>38·4</td>
<td>38·7</td>
<td>40·9</td>
<td>40·4</td>
<td>41·6</td>
</tr>
<tr>
<td>ECW (litres)</td>
<td>16·8</td>
<td>16·6</td>
<td>16·8</td>
<td>18·4</td>
<td>18·0</td>
<td>18·5</td>
</tr>
<tr>
<td>ECW:TBW</td>
<td>42·5</td>
<td>42·9</td>
<td>43·2</td>
<td>44·7</td>
<td>44·4</td>
<td>44·4</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>1·014</td>
<td>1·017</td>
<td>1·019</td>
<td>1·021</td>
<td>1·020</td>
<td>1·021</td>
</tr>
<tr>
<td>Fat mass 3-comp (kg)</td>
<td>31·4</td>
<td>30·6</td>
<td>30·9</td>
<td>30·6</td>
<td>30·4</td>
<td>30·6</td>
</tr>
<tr>
<td>FFM 3-comp (kg)</td>
<td>50·51</td>
<td>51·11</td>
<td>51·35</td>
<td>52·01</td>
<td>50·96</td>
<td>51·25</td>
</tr>
<tr>
<td>Fat mass 2-comp (kg)</td>
<td>27·2</td>
<td>29·4</td>
<td>30·3</td>
<td>30·4</td>
<td>30·4</td>
<td>30·8</td>
</tr>
<tr>
<td>FFM 2-comp (kg)</td>
<td>53·3</td>
<td>52·7</td>
<td>52·9</td>
<td>51·5</td>
<td>51·2</td>
<td>51·5</td>
</tr>
</tbody>
</table>

* For details of procedures, see p. 932.

Discussion

In the present study we have shown that newly insulin-treated type 2 diabetic patients gained 2.8 kg in body weight over 12 months, while type 2 diabetic patients who were already under insulin treatment did not. The weight gain was composed of mainly FFM. We also show that HbA1c change is seen to be a determinant of weight gain in the first 6 months, but this is no longer the case after 6 months. BIA alone is insufficient to evaluate the composition of the weight gain emerging in these patients.

Weight gain is often associated with insulin treatment (Groop et al. 1989; Lindstrom et al. 1994; Chow et al. 1995; Sinha et al. 1996; Yki-Jarvinen et al. 1997; UK Prospective Diabetes Study Group, 1998; Mäkimmätäla et al. 1999; Packianathan et al. 2005); however, previous studies show that this weight gain is very variable and its composition remains unclear. Additionally, there is still debate as to whether it is the ‘non-glucose control-related effect’ of insulin per se or the improvement in metabolic control that is responsible for weight gain.

In all the studies listed earlier, there are methodological issues that can explain some of the different findings obtained between studies. Packianathan et al. (2004) have provided a comprehensive analysis of these methodological issues. Based on a four-compartmental approach, considered the reference technique, Packianathan et al. (2004) show that most of the simpler methods may not be accurate and precise enough to be used to estimate body composition changes in the specific field of diabetic patients. As another example, and in type 1 diabetics, Carlson & Campbell (1993), using underwater weighing, reported that weight gain was due solely to an increase in fat mass. On the contrary, Sinha et al. (1996), using dual-energy X-ray absorptiometry, reported that weight gain was made of 60% FFM.
Furthermore, BIA estimates body water spaces from the resistance opposed by the body to an alternative current. It follows that hydration has to remain constant for body compartments (FFM hence fat mass) to be calculated from body water spaces (Ritz, 1998). It may also be, as suggested by Packianathan et al. (2004), that changes in the hydration status lead to erroneous evaluations of TBW itself. Insulin induces water and Na retention (De Fronzo, 1981; Ter Maaten et al. 2000) hence may modify hydration. Hydration can also be modified by improving glycaemic control (Brizzolara et al. 1996; Bagg et al. 2001). This casts some doubts on results obtained with a two-compartmental model approach such as BIA or isotopic dilution. Indeed, we did show a slight change in hydration (no change in TBW:FFM ratio, but a change in ECW:TBW ratio). Packianathan et al. (2005) did show changes in TBW:FFM ratio. This means that it is unsafe to derive FFM from TBW in such situations.

Therefore, the composition of weight gained during insulin treatment is not well understood. Insulin exerts an anabolic action on both lipid and protein metabolism, and favours water and salt retention (De Fronzo, 1981; Nair et al. 1987; Pacy et al. 1989; Ter Maaten et al. 2000). However, Packianathan et al. (2004) did not show a change in total body proteins. One should expect a combination of FFM and fat mass changes. When not using a four-compartmental analysis, one of the best models of body composition analysis is therefore one that takes into account changes in hydration. The three-compartmental method, as used by Packianathan et al. (2004), which relies on the simultaneous measurement of body volume and water spaces, avoids the difficulties of changes in hydration. Classy et al. (1999) have demonstrated that the determination of body composition using another three-compartmental model has been very accurate (0·2 %) in healthy subjects when compared with gold standard techniques. Our present data suggest that water spaces did not change, but that body density increased in the newly treated group over the 12-month period. If whole body density increases, it means that a dense compartment of the body has increased its relative mass. Here it is not water, but it could be protein and/or glycogen as insulin has anabolic properties (De Fronzo, 1981; Nair et al. 1987; Pacy et al. 1989; Ter Maaten et al. 2000). Using only TBW leads to a methodological error since weight increases, while TBW does not. It is therefore regarded as fat (in a two-compartment approach) disregarding the change in dense components discussed earlier. We rule out the possibility that the estimate of TBW by BIA could have systematically biased the results. Indeed, Packianathan et al. (2004) have shown that BIA with manufacturer’s equations is wrong. We have verified earlier that BIA was accurate when compared with ¹H dilution. We suggest that there are almost no changes in hydration avoiding the bias suggested by Packianathan et al. (2004). Furthermore, the density increased, which is independent of technical errors on TBW. Therefore, we consider that the three-compartmental method is appropriate and the present study suggests that most of the weight gained is FFM. Packianathan et al. (2005) recently published a similar study using a four-compartmental model. The results differ from ours since they show a predominant increase in fat mass. However, patients differed at the beginning of their study (being in worst metabolic situation) and put on more weight over 6 months than in the present study. It may be that body composition changes differ between studies (despite using appropriate methodologies) because of the degree of metabolic derangement, the intensity of weight regain, the degree of physical activity, all factors that should be controlled for.

This points at a very important issue. It should be considered that due to the extreme variability of the metabolic conditions affecting type 2 diabetic patients at the time of insulin treatment (varying insulin sensitivity, varying residual insulin secretion capacity, varying body weight and composition, varying metabolic control on macronutrient metabolisms, etc.) weight changes will be variable. Furthermore, body composition changes will also be variable, and could be made of fat in some subjects or FFM in others, or various combinations. This composition of weight gain is an important parameter influencing the future health of the patients. To study those changes future work is necessary on a large number of ‘variable’ subjects with reference techniques such as those described by Packianathan et al. (2004).

The second methodological issue relates to the choice of control group. Ideally, for type 2 diabetic patient comparisons, the control group should comprise obese persons exhibiting similar metabolic control and undergoing a similar diet. This is the reason why we chose previously insulin-treated type 2 diabetic patients. Given that the two groups were similar in age, body composition, weight and disease, the results provide important information regarding the effect of starting insulin treatment and improving metabolic control because the effect of time was cancelled out between the groups. In some studies, type 1 diabetic patients have served as the control group (Sinha et al. 1996; Rigalleau et al. 1999). Their initial body composition is, however, different from that of type 2 diabetic patients (type 1 being typically leaner subjects, dehydrated, with muscular wasting). Finally, obese non-diabetic patients are similar only with regard to body composition. Therefore, the influence of the control group on body weight and body composition changes are likely to create some variability between studies.

Our present results suggest that there are two effects of insulin on weight gain in insulin-treated type 2 diabetic subjects: one is a ‘glucose control-related effect’; the other is a ‘non-glucose control-related effect’. Indeed, HbA1c and body weight, in the present study, varied in the group of newly insulin-treated diabetic patients exclusively, over the initial 6 months. Meanwhile, body weight and HbA1c remained unchanged in the PREVt patients. It is an established fact that weight gain accompanies an improvement in glycaemic control in type 2 diabetes (Groop et al. 1989; Lindstrom et al. 1994; UK Prospective Diabetes Study Group, 1995, 1998; Sinha et al. 1996; Yki-Jarvinen et al. 1997; Makimattila et al. 1999; Rigalleau et al. 1999). This has been observed with insulin treatment (Groop et al. 1989; Lindstrom et al. 1994; UK Prospective Diabetes Study Group, 1995, 1998; Sinha et al. 1996; Yki-Jarvinen et al. 1997; Makimattila et al. 1999; Rigalleau et al. 1999) but also when glycaemic control is optimised with oral anti-diabetic agents solely or when given in combination with insulin (Bagg et al. 2001). In those studies, which were carried out over 3–12 months, the observed weight gain was greater than that seen in the present study, varying between 3·7 and 7·5 kg. This occurred despite similar improvements in glycaemic control, with HbA1c decreasing by an average of 2·3 % (Groop et al. 1989; Chow et al. 1995; Sinha et al. 1996). Although weight gain in the present study was moderate, it was sufficient to elicit changes in body composition. Correlations showed that changes in body compartments were associated with overall weight change.

After 6 months, NEWt patients continued gaining weight although their HbA1c did not change. This suggests that after an initial improvement, glycaemic control does not determine further weight gain. Weight gain is commonly associated with
the improvement of glycaemic control by the disappearance of glycosuria, and/or the decrease in resting energy expenditure (Nair et al. 1984; Bogardus et al. 1986; Fontvielle et al. 1992; Gougeon, 1996; Järvinen, 2001; Gougeon et al. 2002). However, over a few weeks of treatment it is difficult to conceive that the disappearance of glycosuria still plays a role. Furthermore, the decrease in resting energy expenditure can be quite variable, at a mean –3% in patients treated (Bogardus et al. 1986). None of those parameters was measured in the present study.

In the UK Prospective Diabetes Study, despite a similar level of glycaemic control, weight gain arising from insulin treatment is greater than that following treatment with sulphonylureas (UK Prospective Diabetes Study Group, 1998). There may be therefore a ‘non-glucose control-related effect’ of insulin, which could be related to the anabolic effects of insulin (De Fronzo, 1981; Ter Maaten et al. 2000). This deserves further research.

Conclusion

The present study shows that compared with adequate controls newly insulin-treated type 2 diabetic patients increased their body weight by 2.8 kg weight over 12 months. Weight gain is composed primarily of FFM. It appears that while an improvement in glycaemic control determines weight gain in the initial 6 months, it is no longer the case after this point, suggesting the potential anabolic role of insulin in further weight gain. Future work is necessary to understand the variability of the composition of weight changes.

References


