Development of hyperinsulinemia and insulin resistance during the early stage of weight gain

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OVERWEIGHT AND OBESITY represent a worldwide increasing problem that greatly raises the risk of the development of cardiovascular disease, severe metabolic disorders such as type 2 diabetes mellitus or various malignances (22), and related mortality (5). Obesity is associated with insulin resistance and subsequent hyperinsulinemia, which has been known for more than 40 years (21, 35), and it is considered to be a core component in the pathophysiology of obesity-related comorbidities (19).

Increased peripheral plasma insulin concentrations can be attributed to stimulation of pancreatic insulin secretion. On the other hand, insulin is extracted to a considerable and varying degree during its passage through the liver, which is an important physiological mechanism for the postprandial increase of peripheral plasma insulin concentrations (29, 39). Several studies have reported that both mechanisms of action contribute to the pathophysiologically relevant hyperinsulinemia of overweight and obese subjects. In the basal state, hypersecretion (3, 13, 14, 31) as well as decreased hepatic insulin clearance (38) have been demonstrated. Similarly, both mechanisms are relevant during stimulation by a meal (31, 37), an oral glucose load (3, 13, 28), or an intravenous glucose load (17, 18, 27, 31, 41, 42), respectively.

Insulin sensitivity and plasma insulin concentrations are significantly different when groups of normal-weight and obese subjects with substantial differences of body weight were compared. A few cross-sectional studies have examined insulin sensitivity and release in “normal-weight” subjects (6, 14, 16, 41), which, however, comprise not only normal (body mass index (BMI) < 25 kg/m²) but also overweight subjects (BMI 25–29.9 kg/m²). These studies demonstrate that among normal-weight subjects a wide interindividual variation of β-cell function and insulin sensitivity has to be considered, which makes it difficult to demonstrate significant alterations between groups with only small differences in body weight. Thus, cross-sectional studies are not an ideal basis for the analysis of the endocrine and metabolic consequences that might occur during small changes in body weight.

A solution to this problem would be a longitudinal study of weight gain, which permits an intra-individual comparison of the various parameters before and after a defined increase of body weight. An approach of this kind has been reported by Sims et. al. (40). They demonstrated an increase of basal as well as stimulated insulin concentrations following an oral glucose load paralleled by insulin resistance after a period of intentional weight gain. The increase in body weight, however, was ~20 kg, which is equivalent to a change in BMI by ~7 kg/m².

As yet, it is unknown whether the development of insulin resistance and hyperinsulinemia requires a critical mass of excess body fat in the overweight (BMI 25–29.9 kg/m²) or even obese range (BMI ≥ 30 kg/m²) or whether individual insulin sensitivity already decreases when weight gain occurs below this level.

Therefore, in the present study we have examined the effect of intentional weight gain (increase of BMI by 2 kg/m²) within
the range of normal body weight (BMI < 25 kg/m²) on basal, postprandial, and intravenous (iv) glucose-stimulated plasma concentrations of insulin, C-peptide, and glucose in healthy subjects to better understand the role of weight gain in the absence of other important but also confounding factors, i.e., sex, age, physical activity, lifestyle factors, and genetic background, that are difficult to stratify in a cross-sectional analysis.

MATERIALS AND METHODS

Ten healthy male subjects (body weight 71.1 ± 3.03 kg, height 1.80 ± 0.02 m, age 26.2 ± 1.26 yr) were studied in random order on three different occasions after a 12-h overnight fast. The subjects were nonsmokers, were taking no medication, and had no family history of hypertension, hyper- or dyslipidemia, or type 2 diabetes mellitus in parents, grandparents, or any first-degree relatives. After informed consent was obtained, all examinations were performed according to the guidelines of the Ethics Committee of the Technical University of Munich and in accordance with the principles of the Declaration of Helsinki. The study was approved by the Ethics Review Board of the Department of Internal Medicine II in agreement with the Ethics Committee’s guidelines.

All subjects were unrestrained eaters with stable body weights for ≥12 mo before enrollment in the study. They had no history of dieting. The subjects were instructed by a dietitian to increase daily food intake by 300–500 kcal in addition to what they were consuming. The energy surplus was achieved by food items of higher energy density considering individual taste preferences, palatability, etc., or by increasing meal size, portion size of snacks, or the number of meals, respectively. A further alternative to increase energy intake was the exchange of water to energy-containing beverages such as fruit juice or lemonades. In addition, the subjects were instructed to maintain their lifestyles and physical activities as usual. Prior to the experiments they consumed a weight-maintaining diet containing 40–50% carbohydrate, 15–20% protein, and 30–40% fat. The increased body weight (BMI + 2 kg/m²) had to be maintained stable for a period of 4 wk before the second group of experiments.

Three experiments were performed in random order before and after weight gain, with a 4-day interval between test days. On the first occasion, each subject was given a standard test meal consisting of bread, butter, and marmalade (260 kcal, 62% carbohydrate, 32% fat, protein). The test meal had to be consumed within 10 min. On the second occasion, 75 g of glucose dissolved in 300 ml of water was consumed. During both tests, blood samples were drawn at −15, 0, 15, 30, 60, 90, 120, 150, and 180 min from an antecubital vein. On the third occasion, glucose was infused intravenously with the aim to imitate a moderate and high physiological increase of plasma glucose as previously described (8). In brief, polyethylene cannulae were inserted into an antecubital vein (for the infusion of glucose) and retrogradely into a wrist vein heated at 60°C in a heated pad for intermittent blood sampling of arterialized blood (8).

After a loading dose, the glucose infusion (20%) was adjusted to clamp plasma glucose concentrations at 1.38 mmol/l (25 mg/dl) above baseline for 30 min; thereafter, the infusion rate was increased to raise plasma glucose by 2.75 mmol/l (50 mg/dl) for an additional 30 min. Bedside glucose measurements were done in 2-min intervals to adjust the infusion rate. Samples for hormonal and glucose analysis were drawn in 10-min intervals.

Analyses. Samples for insulin and C-peptide were collected into plastic tubes containing 1.2 mg EDTA and 500 kIU Trasylol and into NaF-containing tubes for the determination of glucose. They were kept chilled in an ice bath until centrifugation at 2,000 rpm for 15 min at 4°C. The separated plasma was stored at −20°C until the time of assay. All samples of one subject were run in duplicate in the same assay.

Insulin was determined by radioimmunoassay (RIA) with <20% cross-reactivity to proinsulin (DPC, Los Angeles, CA); C-peptide was measured by radioimmunoassay (CIS Biointernational, Gil-Sur-yvette Cedex, France). Leptin was measured by RIA purchased from Linco Research (St. Charles, MO). Ghrelin was determined by RIA purchased from Phoenix as described previously (11). Glucose was measured by the hexokinase method (Roche Diagnostics, Mannheim, Germany). Body fat was determined by bioelectrical impedance analysis (BIA) (1).

Calculations. Insulin sensitivity in the basal state was determined by homeostasis model assessment insulin resistance (HOMA-IR) index (26). HOMA-IR was calculated as [(fasting blood glucose (mg/dl) × fasting insulin (µU/ml)] ÷ 405. Insulin secretion was determined by the kinetic model of Eaton et al. (10), which had been validated for this purpose (25, 32), using the C-peptide kinetic constants generated by Polonsky et al. (34), employing the following formula of Eaton et al. (10):

\[
\text{Insulin secretion, pmol/min} = \frac{\text{C-peptide, pmol/ml}^*}{\text{Glucose, mmol/l}}
\]

Table 1. Body weight and baseline metabolic and endocrine parameters before and after weight gain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Weight Gain</th>
<th>After Weight Gain</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>71.1 ± 3.03</td>
<td>77.3 ± 3.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.8 ± 0.7</td>
<td>23.8 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>14.2 ± 1.6</td>
<td>17.2 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin, mmol/l*</td>
<td>0.15 ± 0.03</td>
<td>0.27 ± 0.06</td>
<td>0.026</td>
</tr>
<tr>
<td>Ghrelin, pmol/l*</td>
<td>155 ± 6.1</td>
<td>160.84 ± 39.1</td>
<td>0.026</td>
</tr>
<tr>
<td>Insulin, pmol/l*</td>
<td>11.5 ± 3.9</td>
<td>25.1 ± 4.54</td>
<td>0.001</td>
</tr>
<tr>
<td>C-peptide, pmol/l*</td>
<td>0.59 ± 0.01</td>
<td>0.73 ± 0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose, mmol/l*</td>
<td>4.8 ± 0.07</td>
<td>5.1 ± 0.09</td>
<td>0.005</td>
</tr>
<tr>
<td>Insulin secretion, pmol/min*</td>
<td>37.9 ± 5.0</td>
<td>45.0 ± 5.0</td>
<td>0.016</td>
</tr>
<tr>
<td>Insulin clearance, l/min*</td>
<td>5.0 ± 0.82</td>
<td>2.3 ± 0.42</td>
<td>0.001</td>
</tr>
<tr>
<td>C-peptide/insulin ratio *</td>
<td>76.6 ± 12.9</td>
<td>36.6 ± 5.8</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>0.35 ± 0.12</td>
<td>0.8 ± 0.14</td>
<td>0.002</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>4.08 ± 0.2</td>
<td>4.43 ± 0.2</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.5 ± 0.21</td>
<td>2.76 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>0.33 ± 0.04</td>
<td>0.46 ± 0.07</td>
<td>0.14</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>1.98 ± 0.3</td>
<td>2.22 ± 0.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>3.3 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 (95% confidence intervals are in parentheses). BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance; Hb A1c, glycosylated hemoglobin. *Mean value on the basis of all baseline values obtained during the 3 experiments.
where \( k_1 = 0.057, k_2 = 0.54, \) and \( k_3 = 0.060 \) (34).

Integrated insulin concentrations were 56% higher after weight gain, whereas insulin sensitivity (HOMA-IR) decreased. Basal insulin secretion rose significantly, by 20%, paralleled by a 50% reduction of insulin clearance as well as direct calculation. In addition to the alterations of carbohydrate metabolism, small but significant changes were observed for total and LDL cholesterol and triglyceride concentrations, whereas VLDL, HDL, and LDL/HDL ratio remained unchanged.

**Test meal.** In response to the test meal, insulin concentrations rose from a baseline of 12.2 ± 2.4 pmol/l to a maximum of 97.7 ± 21.0 pmol/l at 60 min, returning thereafter to baseline. After weight gain the maximal postprandial increase to 177.4 ± 43.1 pmol/l at 60 min was significantly greater (\( P = 0.02; \) Fig. 1). The integrated postprandial insulin concentration was 30% higher (Table 2).

C-peptide concentrations increased similarly within 60 min to a maximum of 1.76 ± 0.27 nmol/l pre- and to 1.94 ± 0.26 nmol/l after weight gain (\( P = 0.41, \) not significant). The integrated AUC of C-peptide and also glucose concentrations did not differ significantly after weight gain (Table 2). Postprandial insulin secretion increased similarly in both groups, whereas insulin clearance, on the other hand, decreased significantly, by ~40%.

**Oral glucose load.** Before weight gain insulin concentrations rose in response to oral glucose to a maximum of 193.9 ± 28.5 pmol/l at 60 min, returning thereafter to baseline levels. After weight gain the maximal increase was 320.4 ± 55.6 pmol/l, which was already reached at 30 min (\( P = 0.008 \)). Integrated insulin concentrations were 56% higher after weight gain (Table 3).

The rise of C-peptide following glucose ingestion was similar in both groups except for a significant difference of the maximal increase at 60 min (1.94 ± 0.26 vs. 2.99 ± 0.39 nmol/l, \( P = 0.049 \)). On the other hand, the integrated C-peptide concentration and also that of glucose were not different. The maximal stimulation of insulin secretion after the oral glucose load at 30 min was significantly higher post-weight gain (Fig. 2), whereas the integrated response was not different. Insulin clearance decreased significantly, by 35%.

**iv Glucose infusion.** Before weight gain glucose infusion at a rate of 2.83 ± 0.26 mg·kg\(^{-1}\)·min\(^{-1}\) maintained plasma glucose levels on an average level of 6.4 ± 0.18 mmol/l from 0 to 30 min; thereafter, the higher infusion rate of 5.82 ± 0.31 mg·kg\(^{-1}\)·min\(^{-1}\) established a plateau of 7.98 ± 0.25 mmol/l.

After weight gain plasma glucose during the first plateau (0–30 min) was not different from pre-weight gain glucose, which required an infusion rate of 2.74 ± 0.23 mg·kg\(^{-1}\)·min\(^{-1}\) (\( P = 0.75, \) not significant). The second plateau of 7.86 ± 0.35 mmol/l was reached between 50 and 80 min with a glucose infusion rate of 4.76 ± 0.43 mg·kg\(^{-1}\)·min\(^{-1}\), which was significantly less compared with the pre-weight gain experiment (\( P = 0.037 \)). The total amount of glucose infused during the first clamp was 18.3 ± 0.4 g, and during the second clamp it was 17.3 ± 0.6 g.
With the increase of plasma glucose by 1.38 mmol/l, plasma insulin levels rose to values between 37.7 ± 11.68 at 0 min and 49.08 ± 8.68 pmol/l at 30 min. After weight gain insulin levels increased to concentrations between 69.7 ± 11.9 pmol/l at 0 min and 70.4 ± 10.5 at 30 min, which were significantly different to pre-weight gain at all time points (P < 0.007). With the additional increase of plasma glucose, pre-weight gain insulin rose to levels between 70.4 ± 12.3 and 78.3 ± 14.1 pmol/l. The post-weight gain insulin was significantly higher, with concentrations between 98.4 ± 12.9 and 129.2 ± 18.3 pmol/l (Fig. 3). The AUC of insulin concentrations was significantly higher during both periods of glucose infusion after weight gain (Table 4).

Plasma C-peptide levels increased similarly during both periods, and integrated C-peptide was not different. Similarly, pre- and post-weight gain insulin secretion was not significantly different either during the lower or during the higher glucose infusion rate (Table 4).

The relationship between the mean glucose concentrations during baseline, low, and high glucose infusion rates vs. the respective insulin concentration and insulin secretion was assessed as described previously by Byrne et al. (4) and is shown in Fig. 4. After weight gain the curve for the glucose-insulin concentration shifts to the left. The rise of insulin concentration per millimolar increase of glucose was 20.4 ± 5.1 pmol pre- and 32.9 ± 4.7 pmol post-weight gain (P = 0.031) during low glucose infusion rate. At the high glucose infusion rate the respective values were 22.5 ± 4.4 pmol pre- and 38.5 ± 6.5 pmol post-weight gain (P < 0.005). On the other hand, there was no significant difference between pre- and post-weight gain increases of insulin secretion per milligram rise of the glucose concentration. This is in accord with the assumption that elevated insulin levels are due to clearance. The calculation of the insulin clearance shows that it is 38% less during the lower and 30% less during the higher glucose infusion rate (Table 4).

For the rate of glucose disposal per picomole increase of the insulin concentration, the glucose infusion rate over the 30 min of the respective clamp period was divided by the integrated insulin concentration. It was 3.76 ± 0.9 μmol/kg at the low and 3.21 ± 0.7 μmol/kg at the high rate before weight gain. The respective values post-weight gain were significantly lower with 2.16 ± 0.3 (P = 0.034) and 1.61 ± 0.3 μmol/kg (P = 0.004), which corresponds to reductions of 43 and 50%, respectively (Fig. 5).
strongly with changes of body weight (11), and by BIA (1). The fractional increase of fat in the present study was 3.2 kg, which accounts for 52% of total weight gain. In a previous study (9), 58% of weight gain in normal-weight subjects was due to fat. The relatively high proportion of increased lean body mass could be due to the physical activity of these subjects, since they were told to maintain their activity also during the period of overfeeding. On the other hand, it cannot totally be excluded that, due to BIA measurement in the present study, the change of body fat might have been underestimated.

Basal hyperinsulinemia of obese subjects has been attributed to an increase of insulin secretion in most studies (3, 13, 14, 31). An additional contribution of clearance was reported by Rossell et al. (38). The present data support a role of both mechanisms with a greater contribution of insulin clearance relative to hypersecretion. The physiological increase of plasma insulin concentrations following meal ingestion is due to both the stimulation of pancreatic insulin secretion and reduced hepatic insulin clearance (29, 39). Hyperinsulinemia of obese subjects in response to ingested or injected substrates can be attributed to augmented insulin secretion but also decreased insulin clearance (3, 13, 17, 18, 27, 28, 31, 37, 41, 42). In most of these studies, normal-weight subjects were compared with obese groups of substantially higher body weight. A few cross-sectional studies with normal and overweight subjects demonstrated a positive correlation between insulin secretion and BMI, although no clear discrimination between different weight groups was possible (6, 16, 41), most likely because of the substantial interindividual variation and overlap of the data. The present study demonstrates for the first time that the initially developing postprandial hyperinsulinemia following modest weight gain is most likely due to reduced insulin clearance, whereas postprandial changes of insulin secretion were not observed. It should be kept in mind, however, that for the calculation of insulin secretion in the present study no individual decay curves of C-peptide were available as used previously by Polonsky et al. (31) or Balent et al. (2). Since we are not able to perform such studies, we have employed previously reported rate constants obtained in normal-weight subjects. Since the error generated by this approach is the same for the pre- and post-weight gain calculations, it is rather unlikely to challenge the observed differences.

The present data favor the concept that at this early stage of weight gain changes of secretory products from fat cells, incretin hormones of the intestinal tract, or metabolic factors such as fatty acids (2) have a substantial influence on basal and
postprandial hepatic insulin metabolism, whereas increased β-cell function plays a minor role and is primarily restricted to the basal state.

The elevation in both basal insulin levels and the insulin response to ingested or injected substrates is an indication of insulin resistance when glucose levels are unchanged or the elevation of insulin is disproportionally higher than that of glucose (30).

The reduction of insulin sensitivity with increasing body weight above the normal range is well documented (6, 12, 14, 16, 20, 23, 24, 41). Due to the wide interindividual variation of β-cell function and insulin action, cross-sectional studies of even large populations do not permit a robust discrimination between groups with small differences in body weight. The intraindividual comparison of the present study demonstrates for the first time that insulin resistance, indicated by increased HOMA-IR, higher postprandial insulin concentrations associated with an unchanged rise of glucose, and the reduced amount of iv glucose required to maintain a defined elevation of plasma glucose levels in the presence of increased insulin concentrations, is an early phenomenon in the course of weight gain, and it starts already within the normal range of body weight. Moreover, it should be noted that the glucose disposal rates shown in Fig. 5 were at different insulin levels. This difference was even greater when a comparison had been made at similar insulin levels.

In the basal state the increase of insulin secretion together with augmented clearance was not able to compensate for the loss of sensitivity since basal plasma glucose levels were unchanged by weight gain (Table 4).

### Table 4. Integrated postprandial changes before and after weight gain after intravenous glucose

<table>
<thead>
<tr>
<th></th>
<th>Before Weight Gain</th>
<th>After Weight Gain</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol·l⁻¹·30 min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>1.287±0.303 (691.4–1,882.6)</td>
<td>2.148±0.385 (1,392.8–2,904.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>50–80 min</td>
<td>2.846±0.468 (1,928.6–3,765.2)</td>
<td>4.017±0.548 (2,941.4–5,093.0)</td>
<td>0.014</td>
</tr>
<tr>
<td>C-peptide, nmol·l⁻¹·30 min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>19.0±2.13 (14.8–23.2)</td>
<td>22.5±2.89 (16.8–28.2)</td>
<td>0.390</td>
</tr>
<tr>
<td>50–80 min</td>
<td>42.4±5.21 (32.2–52.6)</td>
<td>46.5±9.41 (36.9–56.1)</td>
<td>0.526</td>
</tr>
<tr>
<td>Insulin secretion, pmol/30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>1.258±0.1189 (1,025.7–1,491.7)</td>
<td>1.434±0.141 (1,155.9–1,712.1)</td>
<td>0.379</td>
</tr>
<tr>
<td>50–80 min</td>
<td>2.087±0.330 (1,440.2–2,733.8)</td>
<td>2.434±0.417 (1,627.1–3,240.9)</td>
<td>0.292</td>
</tr>
<tr>
<td>Clearance, l/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>1.94±0.37 (1.2–2.7)</td>
<td>1.21±0.21 (0.8–1.6)</td>
<td>0.036</td>
</tr>
<tr>
<td>50–80 min</td>
<td>1.26±0.18 (0.9–1.6)</td>
<td>0.90±0.13 (0.6–1.2)</td>
<td>0.023</td>
</tr>
<tr>
<td>C-peptide/insulin ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>34.4±5.34 (23.9–44.9)</td>
<td>21.6±3.04 (15.6–27.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>50–80 min</td>
<td>25.6±3.04 (19.6–31.6)</td>
<td>17.7±1.91 (14.0–21.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>Glucose infusion rate, mg·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30 min</td>
<td>1.23±0.26 (2.3–3.3)</td>
<td>2.74±0.3 (2.3–3.2)</td>
<td>0.750</td>
</tr>
<tr>
<td>50–80 min</td>
<td>5.82±0.31 (5.2–6.4)</td>
<td>4.76±0.43 (3.9–5.6)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 (95% confidence intervals are in parantheses).
significantly higher after weight gain. This in turn can favor further stimulation of β-cell function.

Following a meal or glucose-induced stimulation of insulin secretion the glucose response above baseline remained unchanged after weight gain. This indicates that in the postprandial state the augmented insulin resistance can be counterbalanced, most likely via an alteration of hepatic insulin metabolism so that postprandial glucose homeostasis remains unchanged. When changes of body weight and the degree of insulin resistance become greater, the previously demonstrated augmentation of postprandial insulin secretion apart from clearance is additionally required for maintenance of glucose homeostasis in overweight and obese subjects (33).

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