The effect of 8 days of strict bed rest on the incretin effect in healthy volunteers

Signe Tellerup Nielsen,1 Nina Majlund Harder-Lauridsen,1 Fabiana Braga Benatti,2 Anne-Sophie Wedell-Neergaard,1 Mark Preben Lyngbæk,1 Kirsten Møller,3 Bente Klarlund Pedersen,1 and Rikke Krogh-Madsen1

1Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; 2Rheumatology Division, School of Medicine, University of São Paulo, São Paulo, Brazil; 3Department of Neuroanaesthesiology, Neuroscience Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Submitted 29 September 2015; accepted in final form 14 December 2015

Nielsen ST, Harder-Lauridsen NM, Benatti FB, Wedell-Neergaard A, Lyngbæk MP, Møller K, Pedersen BK, Krogh-Madsen R. The effect of 8 days of strict bed rest on the incretin effect in healthy volunteers. J Appl Physiol 120: 608–614, 2016. First published December 17, 2015; doi:10.1152/japplphysiol.00821.2015.—Bed rest and physical inactivity are the consequences of hospital admission for many patients. Physical inactivity induces changes in muscle metabolism, but its effect on the incretin effect, which is reduced in, e.g., Type 2 diabetes, is unknown. To investigate how 8 days of strict bed rest affects the incretin effect, 10 healthy nonobese male volunteers underwent 8 days of strict bed rest. Before and after the intervention, all volunteers underwent an oral glucose tolerance test (OGTT) followed by an intravenous glucose infusion (IVGI) on the following day to mimic the blood glucose profile from the OGTT. Blood glucose, serum insulin, serum C-peptide, plasma incretin hormones [glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP)], and serum glucagon were measured serially during both the OGTT and the IVGI. The incretin effect is calculated as the relative difference between the area under the curve for the insulin response during the OGTT and that of the corresponding IVGI, respectively. Concentrations of glucose, insulin, C-peptide, and GIP measured during the OGTT were higher after the bed rest intervention (all \( P < 0.05 \)), whereas there was no difference in the levels of GLP-1 and Glucagon. Bed rest led to a mean loss of 2.4 kg of fat-free mass, and induced insulin resistance evaluated by the Matsuda index, but did not affect the incretin effect (\( P = 0.6 \)). In conclusion, 8 days of bed rest induces insulin resistance, but we did not see evidence of an associated change in the incretin effect.

bed rest; incretins; insulin

DESPITE AN INCREASED FOCUS ON MOBILIZATION, hospital admission is associated with bed rest and a clinically important reduction in the level of physical activity. Bed rest as well as physical inactivity induce reduction in maximal oxygen consumption (\( V_{\text{O}_2\text{max}} \)), loss of muscle mass, and an alteration in bone mass (2, 14, 15, 26, 29).

Physical inactivity and bed rest induce changes resembling metabolic syndrome, including increased amounts of visceral fat, insulin resistance, and low-grade systemic inflammation (6, 11, 12). Insulin resistance induced by bed rest is believed to be primarily in skeletal muscle (31). Three weeks of bed rest reduces \( V_{\text{O}_2\text{max}} \) to a greater extent than 30 years of aging (18).

The incretin effect is the relative increment in insulin release to the circulation during an oral glucose tolerance test (OGTT) compared with the insulin release during the same blood glucose profile induced by an intravenous glucose infusion (IVGI) (21), which is responsible for up to 70% of the total insulin secretion related to a glucose stimulus in healthy persons (21, 24). The incretin hormones, glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP), are released from the entero-endocrine L- and K-cells in response to oral intake of glucose or fat (8, 35). The incretin hormones mediate insulin secretion from the pancreatic \( \beta \)-cell, and GLP-1 inhibits glucagon secretion from the pancreatic \( \alpha \)-cell (8, 9, 35). Additionally, several extrapancreatic effects of the hormones have been identified, including delayed gastric emptying, improved function of the left ventricle in ischemic heart disease, and appetite regulation (5, 8, 27, 30). The incretin effect is significantly reduced in patients with T2D (21) and in critically ill patients (25), and incretin hormone-based therapies are widely used in the treatment of T2D.

In the present study, we aimed to investigate if bed rest, as a model of the acute physical inactivity during hospital admission, influences the incretin effect. We hypothesized that 8 days of strict bed rest would reduce the incretin effect in healthy volunteers in parallel with the development of increased insulin resistance.

MATERIALS AND METHODS

Study Design

Ten healthy male volunteers were included in the study (Fig. 1). As a part of the original study design, these volunteers will act as a control group in another yet unpublished study. Exclusion criteria were body mass index (BMI) >25, diabetes in a close relative, a history of bariatric surgery or of surgery on the small intestine, risk of deep venous thrombosis, tobacco smoking, and alcohol abuse. The volunteers underwent a medical examination and were screened for diabetes and thyroid, liver, kidney, respiratory, cardiovascular, and hematologic disease.

All volunteers underwent 8 days of strict bed rest and were only allowed to stand up during transfer to and from a wheel chair. Bed rest was initiated on day 1 in the morning and terminated on day 9 at noon after IVGI and dual-energy X-ray absorptiometry (DXA) scan. The volunteers were under constant observation throughout the whole bed rest period. They received three meals per day representing an isocaloric diet containing 15% protein, 32% fat, and 53% carbohydrates. Energy expenditure was calculated by the Mifflin equation (20). During the intervention, physical activity and 24-h blood glucose profiles were controlled with an accelerometer (ActiHeart System,
Camtec, United Kingdom) and a continuous glucose monitoring system (CGM) (Medtronic Diabetes, Northridge). All volunteers wore compression stockings to prevent deep venous thrombosis.

Before and after the bed rest intervention, body composition was measured by DXA scans (Lunar Prodigy Advance, GE Health Care, United Kingdom), and VO$_2$$_{max}$ (Cosmed Quark, Italy) was measured with an ergometer bike (Monark, Sweden). An OGTT (83 g dextrose monohydrate in 293 ml of water) and a corresponding IVGI (glucose, 200 mg/ml; Fresenius Kabi, Uppsala, Sweden) mimicking the glucose infusion indicated by a radioimmunoassay (RIA) (Electra-box, Copenhagen, Denmark).

**Incretin hormones.** Blood for measurement of GLP-1 and GIP during OGTT/IVGI was collected at baseline, 20, 40, 60, 120, and 180 min in chilled EDTA tubes containing aprotinin (50KIU/ml blood; Becton Dickinson, Plymouth, United Kingdom). Samples were kept on ice and centrifuged immediately at 3,500 rpm and 4°C for 15 min, following which the supernatant was stored at −80°C until analysis. Plasma GIP and GLP-1 were measured by enzyme-linked immunosorbant assays (ELISA) (Millipore and Meso Scale Discovery, respectively).

**Markers of inflammation.** Fasting blood samples were collected on the morning of days 1 and 9 of the bed rest intervention in EDTA tubes. Samples were kept on ice and then centrifuged at 3,500 rpm and 4°C for 15 min, following which the supernatant was stored at −80°C until analysis. Plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured by ELISA (Meso Scale Discovery).

**Cortisol.** Fasting blood samples for analysis of cortisol were collected on the morning of days 1, 3, 5, 7, 8, and 9 of the bed rest intervention in lithium heparin tubes (Greiner Bio-One). Plasma was analyzed using sandwich electrochemiluminescence immunoassay (ECLIA) (Roche) at the Department of Clinical Biochemistry, Righshospitalet, Denmark.

**Calculations**

The incretin effect was calculated as [incremental area under the curve (iAUC) Insulin$_{OGTT}$ − iAUC Insulin$_{IVGI}$]/iAUC Insulin$_{OGTT}$ (21).

### Table 1. Anthropometry

<table>
<thead>
<tr>
<th>Time point (days)</th>
<th>Screening</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bed rest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OGTT (180 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVGI (180 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA and VO$<em>2$$</em>{max}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accelerometer and CGM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Anthropometry

<table>
<thead>
<tr>
<th></th>
<th>Before Bed Rest</th>
<th>95% CI</th>
<th>After Bed Rest</th>
<th>95% CI</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>83.6</td>
<td>78.3–88.8</td>
<td></td>
<td>80.8</td>
<td>75.6–86.0</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>24.5</td>
<td>23.2–25.8</td>
<td></td>
<td>23.7</td>
<td>22.4–25.0</td>
</tr>
<tr>
<td>Total lean body mass, kg</td>
<td>63.3</td>
<td>58.3–68.4</td>
<td></td>
<td>60.9</td>
<td>55.9–65.9</td>
</tr>
<tr>
<td>Lean body mass legs, kg</td>
<td>21.7</td>
<td>19.7–23.7</td>
<td></td>
<td>20.5</td>
<td>18.6–22.5</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>16.3</td>
<td>12.8–19.9</td>
<td></td>
<td>16.2</td>
<td>12.5–19.8</td>
</tr>
<tr>
<td>Total fat, %</td>
<td>20.6</td>
<td>16.3–25.0</td>
<td></td>
<td>20.9</td>
<td>16.5–25.4</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>96.6</td>
<td>92.6–100.5</td>
<td></td>
<td>96.0</td>
<td>93.0–99.0</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>85.6</td>
<td>83.0–88.3</td>
<td></td>
<td>85.8</td>
<td>83.5–88.0</td>
</tr>
<tr>
<td>VO$<em>2$$</em>{max}$, ml·min$^{-1}$·kg$^{-1}$, lean body mass, n = 6</td>
<td>56.3</td>
<td>49.0–63.5</td>
<td></td>
<td>48.2</td>
<td>44.1–52.2</td>
</tr>
</tbody>
</table>

All values are means ± SE. BMI, body mass index; VO$_2$$_{max}$, maximal oxygen consumption.
As a marker of stimulated insulin sensitivity, the Matsuda index was calculated as $10000/\sqrt{[\text{baseline glucose} \times \text{mean glucose}] \times [\text{baseline insulin} \times \text{mean insulin}]}$ (16).

As a marker of fasting insulin resistance, Homeostatic Model Assessment (HOMA-IR) was calculated as $\text{HOMA-IR} = \left( \frac{\text{fasting glucose} \times \text{fasting insulin}}{22.5} \right)$ (17).

As a marker of stimulated β-cell function, C-peptide derived insulinogenic index was calculated as $\text{iAUC C-peptide } 0\text{–}40\text{ min OGTT/iAUC glucose } 0\text{–}40\text{ min OGTT}$ (32).

**Statistics**

Statistical analyses of data obtained during the OGTT and the IVGI were based on iAUC. iAUC was calculated by the trapezoidal rule. iAUCs from the OGTT/IVGI and pre-bed rest and post-bed rest measurements were compared by paired t-tests. Only a few data were missing. Missing data were imputed by using the average of the value recorded one time point before and one time point after the missing value. If data were missing in the beginning or at the end of the OGTT or IVGI, the last available value was multiplied by the average percentage of change in the other volunteers at that time point. Daily measurements of blood glucose and plasma cortisol were compared by a mixed model of repeated measurements.

$P < 0.05$ was considered statistically significant. Distribution of data was evaluated by a histogram before the statistical analysis was performed. Data are presented in the text as mean with 95% CI unless otherwise indicated. The statistical analyses were performed with SAS 9.3 (SAS Institute, North Carolina).

**RESULTS**

**Anthropometry and Physical Capacity**

All 10 healthy male volunteers, mean age 23 yr (range: 18–29 yr), mean HbA1c 30 mmol/mol (range: 26–34 mmol/mol), completed the intervention (Table 1). Data from the CGM-system and the accelerometer ($n = 8$) confirmed that the study protocol was followed (data not shown).

Body weight was significantly reduced after the bed rest period. This was mainly accounted for by a severe reduction in lean body mass (average loss of 2.4 kg), as fat mass remained unchanged. There was no change in hip or waist circumference. $V_o_2\max$ was lower after bed rest. The difference between the OGTT and the IVGI, median (range), was 5.3% (1.5–12.1%) before bed rest and 4.2% (2.1–12.9%) after bed rest.
Insulin and C-Peptide

Serum insulin and C-peptide were higher during the OGTT than during the corresponding IVGI both before and after bed rest (insulin before: \( P = 0.0004 \); insulin after: \( P = 0.0002 \); C-peptide before: \( P = 0.0001 \); C-peptide after: \( P < 0.0001 \)), thus confirming the incretin effect (Fig. 2). Furthermore, levels were higher after bed rest during both the OGTT and the IVGI (insulin OGTT: \( P = 0.013 \); insulin IVGI: \( P = 0.0009 \); C-peptide OGTT: \( P = 0.0057 \); C-peptide IVGI: \( P = 0.0011 \)), indicating increasing insulin resistance.

GLP-1, GIP, and Glucagon

Plasma GLP-1 was higher during the OGTT than during the corresponding IVGI before and after bed rest (before \( P < 0.0001 \), after \( P < 0.0001 \)), whereas there was no difference between OGTTs or between IVGIs before and after bed rest (Fig. 3). Plasma GIP was also higher during the OGTT than during the corresponding IVGI, both before and after bed rest (before \( P < 0.0001 \), after \( P < 0.0001 \); Fig. 3). Furthermore, and in contrast to GLP-1, GIP was higher during the OGTT after compared with before bed rest (\( P = 0.0021 \)), whereas there was no difference between levels during IVGIs. No difference in serum glucagon before and during bed rest or between the OGTTs and the IVGIs was observed (Fig. 4).

Glucose

The iAUC for blood glucose during the OGTT was higher after compared with before bed rest (\( P = 0.0103 \)), indicating impaired glycemic control (Fig. 2). Furthermore, blood glucose during the IVGI was higher than during the corresponding OGTT both before and after bed rest (before bed rest: \( P = 0.0017 \); after bed rest: \( P = 0.0049 \)). Daily fasting blood glucose did not change during the bed rest period.

Incretin Effect

There was no difference in the incretin effect before and after bed rest (\( P = 0.5804 \); Fig. 5).

Insulin Resistance and \( \beta \)-Cell Function

Insulin resistance developed during the bed rest period: Matsuda index before bed rest 6.97 (5.40–8.53); after bed rest 5.63 (4.44–6.82), \( (P = 0.0174) \), whereas fasting insulin resistance was unchanged; HOMA-IR before bed rest 1.23 (0.94–1.52), after bed rest 1.29 (0.97–1.61) \( (P = 0.4) \). There was no difference in secretory \( \beta \)-cell function: C-peptide derived insulinogenic index before bed rest 162 (123–201), after bed rest 183 (121–244) \( (P = 0.3795) \).

**Fig. 3.** Glucose and glucagon during OGTT and IVGI. Panels at left indicate time course, whereas panels at right reflect iAUC. All values are means ± SE. *Significant difference between OGTT and IVGI; #significant difference between pre- and post-bed rest levels. A and B: plasma glucagon-like peptide (GLP-1); C and D: glucose-dependent insulinotropic peptide plasma (GIP).
Markers of Inflammation and Cortisol

There were no differences in plasma levels of the inflammatory markers TNF-α and IL-6 before compared with after bed rest (TNF-α: \( P = 0.2521 \); IL-6: \( P = 0.6294 \)). Plasma cortisol concentrations were significantly higher on days 3, 7, and 8 than on day 1 (data not shown).

DISCUSSION

The main finding of this study was that 8 days of bed rest had no effect on the incretin effect in healthy volunteers, despite the development of insulin resistance and significant reductions in \( \text{VO}_2\text{max} \) and lean body mass.

After the bed rest intervention, the volunteers showed reduced body weight mainly due to loss of lean body mass, as fat mass remained unchanged. This indicates that the diet provided was sufficient to maintain the caloric needs and that the loss of body weight represents loss of skeletal muscle induced by bed rest. \( \text{VO}_2\text{max} \) was reduced after bed rest as demonstrated in previous studies (29), with the largest reduction in those with the highest \( \text{VO}_2\text{max} \) pre-bed rest. Even though volunteers lost lean body mass, this may not fully explain this response. A previous study showed that decreased stroke volume, and reduced muscle oxidative capacity, induced by a decrease in mitochondria volume and oxidative enzyme activities, were the major determinants limiting \( \text{VO}_2\text{max} \) after bed rest (4).

Hyperglycemia seems to reduce the magnitude of the incretin effect in T2D (19), but not in healthy volunteers (28). Fasting blood glucose level was unaffected by the bed rest intervention. Despite a significant increase in both insulin and GIP secretion during the OGTT after bed rest, the incretin effect remained unchanged. The mechanism of this increase is not obvious but could, in theory, be induced by insulin resistance and/or by \( \beta \)-cell GIP resistance, similar to the underlying mechanism of T2D (23).

The \( \beta \)-cell function, as measured by the C-peptide derived insulinogenic index, was unaffected by the bed rest intervention. Maximal secretory capacity could potentially affect the
incretin effect (19), but was not investigated in the present study.

Corticosteroid reduces the incretin effect in healthy volunteers (10) as does the combination of corticosteroid and reduced physical activity level (7). The present findings indicate that the impairment in the incretin effect caused by the combination of corticosteroid and reduced physical activity level may predominately be related to the effects of corticosteroid. Corticosteroids affect several different direct and indirect pathways mediating insulin synthesis and β-cell signaling which leads to an increase in β-cell mass (1, 33, 34); in addition, corticosteroids may induce peripheral insulin resistance (3). Although plasma levels of cortisol were higher on days 7 and 8 than at the beginning of the bed rest period, this increased endogenous production did not lead to a change in the incretin effect, but may have influenced the β-cell mass and contributed to the development of insulin resistance. In glucose intolerant humans, blood glucose during OGTT is inversely related to lean body mass, independent of insulin resistance (13); thus lean body mass consists primarily of skeletal muscle, which is a major site of glucose uptake. Thus the loss of lean body mass may have contributed to the higher blood glucose levels during the OGTT after bed rest.

Limitations. Eight days of strict bed rest did not change fasting blood glucose level (19) or the incretin effect. However, it is possible that a longer bed rest intervention potentially could affect both. In this study we did not observe any effect of bed rest on the incretin effect in healthy young male volunteers. However, it cannot be ruled out that bed rest could have a potential effect on patients admitted to a hospital. The mean blood glucose level during the IVGI was unintentionally higher than during the OGTT before and after bed rest. This may have led to higher serum levels of insulin during the IVGI and a reduction in the incretin effect. However, since this is the case both before and after the intervention, it is unlikely that it influenced the comparison of the data. Moreover, the lack of use of glucose tracers makes it impossible to quantify hepatic glucose production. Thus although the glucagon level during the OGTT did not differ before and after bed rest, we cannot further substantiate that the increased glucose level during the OGTT post-bed rest was caused by peripheral insulin resistance and was not a consequence of increased hepatic glucose production.

In conclusion, 8 days of bed rest did not change the incretin effect in the healthy volunteers despite increased circulating levels of glucose, insulin, and GIP during the OGTT after bed rest and the development of insulin resistance. Furthermore, as previously shown, 8 days of strict bed rest reduced lean body mass and maximal oxygen consumption, emphasizing that physical activity during hospital admission needs to be prioritized.

ACKNOWLEDGMENTS

We thank the staff at the Department of Infectious Diseases 8642, Rigshospitalet, and Department Heads Aase Bengaard Andersen and Eva Westphal for housing the project. We thank Louise Seier Hansen, Katja Steenborg Kofoed, Dorte Eriksen, Rune Djurup, Naja Zenius Jespersen, Lone Pejls, Morten Zacho, Kristian Karstoft, Louise Lehrskov-Schmidt, and Marie Kivistgaard, all Centre of Inflammation and Metabolism/Centre for Physical Activity Research, Rigshospitalet, and Jessica Velasquez, Brown University, for the kind assistance with execution of the studies. Senior Lecturer, PhD, Thomas P. Solomon, University of Birmingham, United Kingdom, is acknowledged for designing the computer program for the IVGI and Hanne Villumsen and Ruth Rovsing, Centre of Inflammation and Metabolism/Centre for Physical Activity Research, Rigshospitalet, for providing technical assistance. Last but not least, we are grateful to the volunteers who participated in the study.

GRANTS

The Centre for Physical Activity Research (CFAS) is supported by a grant from TrygFonden. During the study period, the Centre of Inflammation and Metabolism (CIM) was supported by a grant from the Danish National Research Foundation (DNRF55). This study was further supported by grants from The Augustinus Foundation and Aase and Ejnar Danielsen’s Foundation. CIM/CFAS is a member of DD2, the Danish Center for Strategic Research in Type 2 Diabetes (the Danish Council for Strategic Research, Grant No. 09-067009 and 09-075724).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.T.N., N.M.H.-L., F.B.B., K.M., B.K.P., and R.K.-M. conceived and designed the computer program for the IVGI and Hanne Villumsen and Ruth Rovsing, Centre of Inflammation and Metabolism/Centre for Physical Activity Research, Rigshospitalet, for providing technical assistance. Last but not least, we are grateful to the volunteers who participated in the study.

REFERENCES


J Appl Physiol • doi:10.1152/japplphysiol.00821.2015 • www.jappl.org