The effect of ten days of bed rest on metabolic and vascular insulin action. A study in individuals at risk for type 2 diabetes

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Running title: Ten days of bed rest and insulin sensitivity

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Abstract

Background: Physical inactivity is a known risk factor for type 2 diabetes. We studied whole body and forearm insulin sensitivity in subjects at increased risk for type 2 diabetes (persons with low birth weight (LBW) (n=20) and first degree relatives to type 2 diabetic patients (FDR) (n=13)) and a control group (CON) (n=20) group wise matched for BMI, age, and physical activity level before and after ten days of bed rest.

Methods: The subjects were studied by hyperinsulinemic isoglycemic clamp combined with arterial and deep-venous catheterization of the forearm. Forearm blood flow (FBF) was measured by venous occlusion plethysmography.

Findings: All groups responded with a decrease in whole body insulin sensitivity in response to bed rest (CON: 6.8±0.5 to 4.3±0.3, (P<0.0001), LBW: 6.2±0.5 to 4.3±0.3 (P<0.0001) and FDR: 4.3±0.7 to 3.1±0.3 mg · min⁻¹ · kg⁻¹(P=0.068)). Percent decrease was significantly greater in CON compared with FDR (CON: 34±4; LBW: 27±4; FDR: 10±13 %). Forearm insulin-stimulated glucose clearance decreased significantly in CON and LBW in response to bed rest; in FDR clearance was very low before bed rest and no change was observed. Before bed rest CON and LBW demonstrated a significant increase in FBF during hyperinsulinemia, after bed rest increase in FBF was observed only in CON.

Conclusions: Bed rest induced a pronounced reduction in whole body, skeletal muscle and vascular insulin sensitivity in CON and LBW. The changes were most pronounced in CON. In FDR insulin resistance was already present before bed rest, but even this group displayed a high sensitivity to changes in daily physical activity.
Introduction

Physical activity has beneficial effects on disease prevention (8; 30; 31) and the worldwide decrease in physical activity (work and leisure time) coincides with the increasing incidence and prevalence in non-communicable diseases (3; 5; 14).

Pathophysiological mechanisms related to inactivity are not necessarily the opposite of what is seen with physical training. It is therefore of interest to study the effects of inactivity in the general population and especially in groups at risk for metabolic disease.

In healthy subjects predisposed to type 2 diabetes lack of physical activity could be the potential environmental trigger that unmasks their increased disease susceptibility resulting in more pronounced health effects of physical inactivity than in individuals without the predisposition.

Studies with various lengths of bed rest in healthy individuals have shown that immobilization leads to decreased insulin sensitivity after only 3-5 days (10; 13; 18; 20; 28; 29); however, a common trait of these studies are that only very little or no description and quantification of the habitual physical activity level before the intervention is provided. It is therefore difficult to determine if the findings from these studies are applicable to the lifestyle of today’s population. Furthermore most studies did not control for changes in body weight during the intervention wherefore this could be a confounding parameter.

A family history of type 2 diabetes and low birth weight (LBW) are known risk factors of type 2 diabetes (11; 19) and predisposes to type 2 diabetes via genetically and environmental susceptibility, respectively. Physical inactivity could purposely unmask
their predisposal and reveal a larger vulnerability to physical inactivity than those without
pre-existing risk factors, but this has not been examined previously.

We have now studied the effect of a 10-day bed rest intervention on insulin sensitivity in
healthy but sedentary type 2 diabetes predisposed individuals and appropriate control
subjects in order to compare the health outcome between controls and disease
predisposed groups. We measured whole body and forearm insulin sensitivity and insulin
vascular action in young healthy men with LBW, in young healthy men with first degree
relatives with type 2 diabetes (FDR) and in a matched control group (CON). We
hypothesized that physical inactivity has larger detrimental health effects in the groups
predisposed to type 2 diabetes, and that we therefore would observe a more pronounced
effect of physical inactivity on insulin sensitivity in the exposed groups compared with
CON.
Materials and methods

The data presented in this manuscript are part of a larger study on the influence of physical inactivity in healthy and pre-diabetic subjects. This work is initiated and funded by the European Union Framework VI; EXGENESIS project.

Subjects

All together 53 young healthy men participated in the study. CON (n=20) and LBW (n=20) were allocated via the Danish National Birth Registry. LBW had birth weight <10 percentile (2548±53 g, P<0.001 for LBW compared with CON). The CON had birth weight between the 50 and 75 percentile (3827±49 g) all with no type 2 diabetic relatives. The FDR (birth weight 3500±150 g) were recruited via their parents who attended the Steno Diabetes Center type 2 diabetes out-patient clinic (n=10) and via local advertisement (n=3). All subjects were from singleton pregnancies born at term (40±0.1, 40± 0.2 and 40±0.3 weeks in CON, LBW and FDR respectively). The inclusion criteria for the FDR were a minimum of one first degree relative and one second degree relative with type 2 diabetes. Matching was done group-wise according to age, body mass index (BMI) and physical fitness. None of the subjects participated in regular structured exercise training activities. All subjects had normal fasting glucose measured before entering the study. The anthropometric details are listed in Table 1.

Before and after bed rest body composition was measured with DEXA scanning (Lunar Prodigy Advance, GE Healthcare, UK) and maximal oxygen consumption (VO2 max) was measured on a bicycle ergometer using a stepwise incremental test using an Oxycon Pro (Jaeger Instruments, Höchberg, Germany) and the leveling off criterion. An
International Physical Activity Questionnaire (IPAQ) (the short 7 last days self-
administered version) was answered by each subject before entering the study.
Two CON, two FDR, and four LBW were smokers (less than 12 cigarettes per day).
The study was approved by the regional ethical committee (ref. # 01-262546) and all
procedures were performed in accordance with the guidelines of the Declaration of
Helsinki. Informed written consent was obtained from all the subjects before
participation.

Control period and intervention
All subjects were provided a dietitian made isocaloric diet (55 E% carbohydrates, 15 E%
protein, and 30 E% fat) four days prior to the before bed rest experimental day (control
period) and during the entire bed rest period. Weight stability during the bed rest period
was ensured to isolate the parameter physical inactivity.
To quantify physical activity level in the control period and to document the decrease in
physical activity level during bed rest a combined accelerometer and heart rate sensor
(Actiheart; Cambridge Neurotechnology, Cambridge, UK) was mounted on the subjects
in the control period (four days) and in the bed rest period. The Actiheart data in the two
periods are given as total number of heart beats pr. day and average activity score based
on the Actiheart activity counts (7). In the control period and in the before bed rest period
(of approximately 3 weeks) the subjects were instructed to continue their daily living
activities and to stay weight stable. During the 10-day bed rest period the subjects were in
bed all day under surveillance and only allowed outside bed for personal hygiene for max
15 min pr. day. They were allowed to use a lab top computer, watch television, read and
Body weight was recorded every morning throughout the bed rest to ensure weight stability.

**Experimental day protocol**

The two experimental days (before and after bed rest) were identical. The before bed rest experimental day was carried out 3 weeks before the bed rest to ensure full recovery before the bed rest, and the after bed rest experimental day was carried out at day ten of the bed rest. All subjects were studied after a 10-hour overnight fast. Electrocardiogram and heart rate were monitored. An arterial catheter was inserted in the brachial artery for blood sampling and blood pressure monitoring. Venous catheters were inserted in the medial antecubital veins of both arms for blood sampling (retro-grade direction) and for infusion of insulin and glucose (antegrade direction). In six subjects (one CON, four FDR and one LBW) it was not possible to insert the arterial line and instead a heated retrograde hand vein was used in replacement. In four CON, four FDR and three LBW, it was not possible to insert the retrograde venous catheter. Therefore the number of subjects analyzed varies and in all figure legends and tables the precise number of subjects is given.

Forearm blood flow (FBF) was measured by venous occlusion mercury-in-Silastic strain-gauge plethysmography (Hokanson EC6 and E20, Bellevue, WA). During the entire experiment the subject was placed in the supine position with the arm rested on a custom made tri-angular cushion. On the upper arm a rapid cuff inflator was set at 40 mmHg to occlude venous outflow from the forearm. Blood flow was measured at cycles of 7 seconds and calculated as an average of 4-6 consecutive readings. Around the wrist a small cuff was positioned and inflated to supra-systolic pressures during
plethysmography recordings and blood sampling, to exclude the circulation of the hand
and the contribution of the arterio-venous (a-v) shunts, respectively.

Before clamp FBF and a-v differences were measured 60 minutes after the arterial
 cannulation to ensure that blood flow had returned to basal level. Clamp FBF
measurements and a-v blood were sampled starting at clamp time 90 minutes. Forearm
fractional glucose extraction was calculated as the glucose a–v difference divided by the
arterial glucose concentration and expressed in percent. Forearm glucose clearance was
calculated as glucose extraction multiplied by the blood flow (expressed as ml ·100 ml⁻¹.
min⁻¹).

Hyperinsulinemic isoglycemic clamp

The clamp was performed as previously described (2), using 40 mU · min⁻¹· m² as the
insulin infusion rate and arterial blood samples for measurement of plasma glucose.
Glucose infusion rates were averaged for 10-min periods and whole body insulin-
mediated glucose uptake rates were calculated as the mean of steady-state glucose
infusion rates from t=90 min to t=120 min (M-value).

Biochemical analyses

Plasma concentrations of glucose were analyzed on an automatic analyzer (ABL 735,
Radiometer, Copenhagen, Denmark). Concentrations of brain-derived neurotropic factor
(BDNF), insulin and C-peptide in plasma were measured with ELISA technique (R&D
systems, Oxon, UK and DAKO kits 6219 and 6218, Rodovre, Denmark). Glycosylated
haemoglobin (HbA1C) was measured by high performance liquid chromatography on a
Bio Rad (Bio-Rad Laboratories, CA). Total cholesterol and HDL-cholesterol were
analyzed with an enzymatic colorimetric test (Roche Diagnostic, Mannheim, Germany). LDL-cholesterol was calculated from the Friedewald formula (12) and VLDL-cholesterol was calculated as triglycerides divided by 2.2. Plasma triglyceride concentration was determined with Triglyceride GPO-PAP (Roche Diagnostic, Mannheim, Germany).

Statistics
Statistical analysis was done using Sigma Stat version 3.1. The hypothesis tested in this study allows for testing between differences in bed rest outcome with unpaired student’s t-test when testing single measurements between CON and LBW or FDR, and paired student’s t-test when analyzing bed rest outcome within a group (M-value, anthropometrics, Actiheart counts, lipids). If data not were normally distributed a non-parametric Mann-Whitney was used. Furthermore, two way ANOVA for repeated measurements was applied when comparing basal and insulin-stimulated values between CON and LBW or FDR (forearm glucose clearance, forearm blood flow, glucose extraction fraction). For post-hoc tests the Holm-Sidak Method was used. Correlations were calculated using either Spearman’s (not normally distributed data) or Pearson’s (normally distributed data) correlation coefficient. To analyze differences between correlation equation slopes we used linear regression to calculate the t ratio and Microsoft Office Excel 2003 to calculate the corresponding two-tailed P-value. A P-value of <0.05 was considered significant in two-tailed testing. All data are reported as mean ± SEM.
Results

Anthropometrics and physical activity level

In response to bed rest there was no change in anthropometrics (Table 1). Before and after bed rest FDR and LBW had a higher ratio between trunk and total fat mass compared with CON.

Average physical activity score (Actiheart recordings) during the control period was 28±3, 26±4 and 27±3 in CON, LBW and FDR, respectively, and this decreased significantly in all groups during bed rest (to 3±0.3, 4±0.6 and 4±0.5 in CON, LBW and FDR, respectively), with no significant differences between CON and disease predisposed groups. Total number of heart beats pr. day was in the control period 105467±2802, 102930±6864 and 107856±4906 in CON, LBW and FDR, respectively, with no significant difference between CON and disease predisposed groups. During bed rest total number of heart beats pr. day decreased significantly to 90598±2594, 88522±2560 and 89640±3479 in CON, LBW and FDR, respectively, with no significant differences between CON and disease predisposed groups.

Time spent sitting (from IPAQ questionnaire) was before bed rest not significantly different between groups (CON: 7.2 ± 0.6, LBW: 7.5 ± 1.2, FDR 6.5 ± 0.9 h · day⁻¹).

VO₂ max did not change in response to bed rest in either group (Table 2), but the heart rate at a given work load (100 Watt for 5 min) was increased after bed rest (pooled values: before bed rest 130±2 vs. after bed rest 138±2 beats · min⁻¹ (P<0.001)).
Before and after bed rest FDR had elevated fasting insulin and C-peptide concentrations compared with CON (P<0.05) (Table 2). HDL cholesterol decreased significantly in response to bed rest in all groups, and triglycerides increased significantly in CON and FDR (Table 2). Before bed rest plasma concentrations of BDNF were not different between the groups, but the response to bed rest was mixed. In CON no significant change was seen, in LBW the concentration decreased (P<0.05), while in FDR an increase (P<0.05) was seen (Table 2).

Insulin concentrations during the hyperinsulinemic clamp were not different between before and after bed rest in either group (pooled values: 296 ± 10 and 309 ± 10 pm · l⁻¹ before and after bed rest respectively, P>0.05). Before bed rest, M-values in CON, LWB and FDR were 6.8±0.5, 6.2±0.5, and 4.3±0.7 mg · min⁻¹ · kg⁻¹, respectively (Fig. 1), (P<0.05 CON compared with FDR and P>0.05 CON compared with LBW). M-value expressed pr. lean body mass (LBM) revealed the same pattern (CON: 8.7±0.6, LBW: 7.9±0.6, and FDR: 6.0±0.8 mg · min⁻¹ · kg LBM⁻¹, P<0.05 CON compared with FDR and P>0.05 CON compared with LBW). With ten days of inactivity (bed rest) M-value decreased in all groups (CON: 4.3±0.3, (P<0.0001); LBW: 4.3±0.3 (P<0.0001); FDR: 3.1±0.3 mg · min⁻¹ · kg⁻¹ (P=0.068)) (Fig. 1) as did the M-value pr. LBM (CON: 5.6±0.4, (P<0.0001); LBW: 5.4±0.4 (P<0.0001); FDR: 4.3±0.2 mg · min⁻¹ · kg LBM⁻¹(P=0.065)).

After bed rest the FDR had lower M-value compared with CON (P<0.05). The percent decrease in M-value in response to bed rest was higher in CON (P<0.05) than in FDR, but there was no difference (P>0.05) between CON and LBW (CON: 34±4; LBW: 27±4; FDR: 10±13 %) (Fig. 1). It appeared that the higher initial M-value the larger the change...
in M-value in response to bed rest, hence a positive correlation between before bed rest M-value and delta M-value (before minus after bed rest M-value) (Fig. 2). Moreover the slope of the relationship in FDR was different from that in CON (P<0.05) (Fig. 2). There was no significant difference between the slopes of CON and LBW.

**Forearm glucose clearance**

Before bed rest baseline forearm glucose clearance was significantly higher in CON compared with FDR (CON: 0.18±0.02, LBW: 0.14±0.03, FDR: 0.10±0.02 ml ·100 ml⁻¹· min⁻¹). Bed rest did not change baseline forearm glucose clearance in any group (data not shown). Before bed rest insulin-stimulated forearm glucose clearance (insulin-stimulated minus baseline) tended to be higher in CON compared with LBW (P=0.077) and FDR (P=0.071) (CON: 1.2±0.2, LBW: 0.6±0.1, FDR: 0.4±0.1 ml ·100 ml⁻¹· min⁻¹) (Fig. 3).

After bed rest the insulin effect was significantly diminished in CON (0.3±0.1 ml ·100 ml⁻¹· min⁻¹ (P<0.05)) and LBW (0.3±0.1 ml ·100 ml⁻¹· min⁻¹ (P<0.05)) compared with before bed rest, whereas in FDR (0.4±0.1 ml ·100 ml⁻¹· min⁻¹) no significant change was observed (P>0.05) (Fig. 3).

Before bed rest baseline glucose extraction fraction was significantly higher in CON compared with FDR (data not shown) but did not change in response to bed rest in any group. Insulin-stimulated glucose extraction (insulin-stimulated minus baseline) did not differ significantly between groups before bed rest, and decreased in response to bed rest significantly in LBW but not in CON (P=0.09) and FDR (P=0.08) (Fig. 4).

Whole body insulin-stimulated glucose uptake rates (M-value) correlated significantly (r²=0.11; P<0.005 (Fig. 5)) with insulin-stimulated forearm glucose uptake rates.
**Insulin-stimulated forearm blood flow**

Baseline FBF (i.e. before insulin-stimulation) was significantly higher in CON compared with LBW and FDR before bed rest (3.2 ±0.3, 2.3±0.1 and 1.9±0.2 ml ·100 ml⁻¹ · min⁻¹, respectively). After bed rest basal FBF tended to be higher in CON compared with LBW (P=0.069) and FDR (P=0.068) (3.8 ±0.4, 2.9±0.3 and 2.7±0.3 ml ·100 ml⁻¹ · min⁻¹, respectively).

The effect of insulin to stimulate FBF (insulin-stimulated FBF minus baseline) before bed rest was significantly higher in CON compared with LBW and FDR (1.9±0.5, 0.6±0.2, 0.2±0.2 ml ·100 ml⁻¹ · min⁻¹ in CON, LBW and FDR, respectively) (Fig. 6). After bed rest, the effect of insulin on FBF decreased in CON (to 0.9±0.3, P<0.05). In LBW FBF decreased to values that were not significantly different from zero, and in FDR no significant effect of insulin on FBF could be detected, neither before nor after bed rest (Fig. 6).

Apart from the comparisons of the effect of insulin (i.e. insulin-stimulated FBF minus baseline) between the groups (Fig. 6), it is also important to focus on the effect of insulin to stimulate blood flow within the groups. Before bed rest, CON and LBW demonstrated a significant increase in FBF with insulin. After bed rest this insulin-mediated effect was only observed in CON (Fig 6). In FDR a significant effect of insulin on FBF could be detected neither before nor after bed rest.
Discussion
A major finding of this study is that all groups responded to the 10-day bed rest with a
decrease in whole body insulin sensitivity, which in CON and LBW was statistically
significant. In line with the findings on the whole body level, insulin sensitivity of the
forearm (forearm insulin-stimulated glucose clearance) also decreased in CON and LBW
in response to bed rest whereas in FDR no change was observed. Vascular insulin action
was also affected by inactivity in CON and LBW whereas FDR demonstrated impairment
already before bed rest.

Insulin sensitivity
Physical inactivity reduced insulin sensitivity. However, in contrast to our hypothesis
CON demonstrated the largest decrease in whole body insulin sensitivity. A priori, we
had expected that the disease predisposed groups (LBW and FDR) would be more prone
to inactivity than the non-predisposed group (CON). Even though the decrease in whole
body and forearm insulin sensitivity in the FDR group was not statistically significant
(probably due to a low pre-intervention insulin sensitivity, see below), the data does
indicate that the FDR demonstrated the greatest sensitivity to physical inactivity, because
this group demonstrated the steepest slope in the relation between initial M-value and
degree of change in M-value due to bed rest (Fig. 2).
The FDR already had decreased insulin sensitivity compared with CON before bed rest
but even so eight out of thirteen FDR subjects demonstrated further decrement in whole
body insulin sensitivity after bed rest. It is noteworthy that those five FDR subjects who
increased their M-value in response to inactivity, were those who displayed the lowest
pre-intervention M-value. The molecular basis of the further reduction in insulin
sensitivity in the remaining eight FDR subjects remains to be explored. Studies have
provided evidence for distinct defects in skeletal muscle glucose transport and insulin
signalling in FDR (15; 22) all of which could contribute to the very low set point of
whole body insulin sensitivity in this group.

The three groups had similar VO2 max, similar time spent sitting before bed rest and
similar reduction in physical activity level due to bed rest. The finding of the largest
reduction in insulin sensitivity in CON can therefore not be explained by differences in
exercise habits and non-structured physical activity prior to bed rest.

Others have found reduced insulin sensitivity after bed rest (10; 13; 18; 20; 28; 29) and
after detraining (1; 21; 26). However, insulin sensitivity after bed rest has not previously
been studied in such well characterized individuals or in subjects at increased risk of type
2 diabetes.

The forearm insulin sensitivity, representing primarily skeletal muscle, was impaired
beforehand in LBW and FDR and did only decrease marginally in response to bed rest.
This suggests that insulin sensitivity of the forearm muscle in those groups may already
have reached a threshold, at which a further impairment of muscular insulin sensitivity
requires the presence of a stronger stimulus, for example a more strict bed rest regimen
without allowance for the use of arm musculature in reading, eating etc. Moreover,
forearm muscle insulin sensitivity is not necessarily representative of all skeletal muscle
(23) and forearm skeletal muscle represents only a very small fraction of the entire
skeletal muscle mass. Further, the fiber type composition in the forearm muscles may be
different from e.g. the vastus lateralis muscle, and the arm is further characterized by a
significantly smaller fat mass/muscle mass ratio than other regions in the body. In light of
these differences between whole body and forearm musculature, it is not surprising that
the correlation of glucose uptake rates was weak, albeit significant (Fig. 5). At the used
plasma insulin concentrations, the relationship is, however, in accordance with previous
findings covering a wider range of insulin (and glucose) concentrations (32).

In CON we found a similar reduction in systemic and forearm insulin sensitivity. In a
study with seven days of bed rest in six healthy young individuals (20) the insulin
resistance found in the leg after bed rest was more pronounced than that of the whole
body. The differences in findings could be due to extremity examined, the amount of
muscle examined, study group size and different insulin concentrations during the clamps
in the present study and in the study by Mikines et al (20).

Collectively whole body and forearm insulin sensitivity data suggests that CON, with the
superior health condition beforehand, experienced a greater impact of physical inactivity
than the groups with pre-existing risk factors. This is against our hypothesis that physical
inactivity is more harmful in the disease predisposed groups. In spite of the predisposed
individuals’ reduced insulin sensitivity a further reduction in insulin sensitivity was
observed although it was of a relatively smaller degree than in CON.

The lipid profile after bed rest (i.e. reduced HDL-cholesterol and increased triglycerides)
(Table 2) is in accordance with findings in very insulin resistant but otherwise healthy
subjects (25). The changes in lipid profiles in this study are not marked; however, it is
noteworthy that the changes are solely the result of a short time of inactivity. The diet
was strictly controlled (prepared and provided) four days before the first experimental
day and during the bed rest eliminating dietary confounders.
Concentrations of BDNF in plasma have previously been shown to be inversely correlated with insulin resistance and fasting plasma glucose concentrations, and hyperglycemia seems to inhibit cerebral BDNF output (16). The suggestion of a regulatory role of hyperglycemia is not supported by the present study, in which a 10-37% decrease in insulin-mediated whole body glucose uptake was seen in the face of plasma glucose levels maintained in the normo-glycemic range, but unchanged (CON), decreased (LBW) and increased (FDR) BDNF concentrations. It has also been suggested that habitual exercise training is associated with lower BDNF concentrations (9), but with the present data it can be concluded that a short-term marked decrease in daily physical activity level does not influence BDNF concentration in plasma in a predictable manner.

Vascular insulin action

The effect of insulin on stimulation of skeletal muscle bulk flow is, in accordance with our findings in LBW and FDR, impaired in type 2 diabetes susceptible groups (6; 17; 24). After bed rest no insulin-mediated increase in blood flow in LBW and FDR was found, but the insulin-mediated blood flow increase was preserved in CON, albeit the magnitude of the increase was significantly diminished after bed rest. The findings in CON are in accordance with a study of seven days of bed rest in healthy young individuals (20) in which leg blood flow measured during hyperinsulinemia was reduced in response to bed rest. The novel finding in LBW of deterioration of vascular insulin action after bed rest indicates an endothelial dysfunction that was unmasked by inactivity, emphasizing the vulnerability of the this disease predisposed group and extending the harmful health effects of physical inactivity. FDR vascular insulin action was severely impaired even
before the bed rest intervention, which probably explains why a further impairment did not occur.

Bed rest did not change the anthropometric parameters; hence, our observations are due to physical inactivity alone. Notably, the total lean and fat mass, including regional distribution, remained the same in all three groups after bed rest.

Strengths and weaknesses

Bed rest as a model of inactivity has advantages and disadvantages. Bed rest allows supervision of the subjects eliminating non-compliance, it is easy to standardize and diet is easily controlled, but bed rest is an exaggerated stimulus and whether the outcome of bed rest resembles real life inactivity is difficult to address. Bed rest eliminates the daily non-exercise activities (14), and therefore provides a unique model to study severe inactivity in a population like our study group, who are at risk of becoming even more inactive (5). Our study is characterized by a less strict bed rest regimen than in most other bed rest studies (10; 18; 20; 28; 29). We believe this is a better mimic of a real life situation which broadens the conclusions and center the health problems of tomorrow.

The before bed rest study day and the bed rest were not directly followed by each other. This may be an advantage because of insurance of full recovery of the subjects after the before bed rest experiment but it was also necessary for logistic reasons.

Only arms were examined. Skeletal muscle tissue is not uniform (23) and has been shown to demonstrate regional variation in insulin sensitivity. The impact of bed rest is more pronounced on the legs than the arms, and naturally it seems obvious to examine the most effected extremity. This was however not possible due logistic and technical problems.
However it seems advantageous to examine the upper extremities due to their less strict bed rest regimen which enhances the generalization to real life inactivity. A decrease in insulin sensitivity due to bed rest is shown to be detectable within few days in healthy individuals (13). Moreover hyperinsulinemic isoglycemic clamp has been demonstrated to have intra-subject reproducibility with a coefficient of variation (CV) of 6-14% (4; 27). FBF measured with venous occlusion plethysmography and forearm glucose clearance has however not been examined in these populations after bed rest before. Reproducibility of plethysmography resting FBF has in our laboratory been tested on seven subjects on two separate days and demonstrated an inter-subject CV of 12% and an intra-subject CV of 14%.

Conclusion

Ten days of bed rest had marked impact on health parameters in all the groups. Inactivity alone resulted in a pronounced reduction in whole body insulin sensitivity and vascular insulin sensitivity in CON and LBW. The changes were most pronounced in CON. In FDR whole body insulin resistance was already present before bed rest, but even this group displayed a high sensitivity to changes in daily physical activity. If genetic predisposal to type 2 diabetes combined with an environmental stressor (i.e. physical inactivity) is the most devastating combination or just the environmental stressor alone (as in CON) is really not answered by this study. Rather the results indicate that the initial health status (i.e. the degree of insulin sensitivity) determined the bed rest outcome, as the largest decrease in whole body insulin sensitivity was seen in the most insulin sensitive group (CON).
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Table 1. Characteristics of CON, LBW and FDR before and after bed rest.

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<td>23.3 ± 1</td>
<td>24.9 ± 0.9</td>
<td>465</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 0.5</td>
<td>23.3 ± 1</td>
<td>24.9 ± 0.9</td>
<td></td>
<td>466</td>
</tr>
<tr>
<td></td>
<td>467</td>
<td>24.1 ± 0.5</td>
<td>23.3 ± 1</td>
<td>24.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Total fat mass</td>
<td>14.3 ± 1.6</td>
<td>16.4 ± 1</td>
<td>21.6 ± 2.5</td>
<td>468</td>
<td></td>
</tr>
<tr>
<td>(kg)</td>
<td>469</td>
<td>14.3 ± 1.6</td>
<td>16.4 ± 1</td>
<td>21.6 ± 2.5</td>
<td>469</td>
</tr>
<tr>
<td>Total lean mass</td>
<td>63.8 ± 1.1</td>
<td>57.1 ± 1.4</td>
<td>58.6 ± 1.5</td>
<td>470</td>
<td></td>
</tr>
<tr>
<td>(kg)</td>
<td>471</td>
<td>63.8 ± 1.1</td>
<td>57.1 ± 1.4</td>
<td>58.6 ± 1.5</td>
<td>471</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>17.4 ± 1.7</td>
<td>19.2 ± 2.0</td>
<td>25.0 ± 2.3</td>
<td>472</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>473</td>
<td>17.4 ± 1.7</td>
<td>19.2 ± 2.0</td>
<td>25.0 ± 2.3</td>
<td>472</td>
</tr>
<tr>
<td>Trunk fat mass/total</td>
<td>0.48 ± 0.01</td>
<td>0.52 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>473</td>
<td></td>
</tr>
<tr>
<td>fat mass</td>
<td>474</td>
<td>0.48 ± 0.01</td>
<td>0.52 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>474</td>
</tr>
<tr>
<td>Leg fat mass/total</td>
<td>0.37 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>475</td>
<td></td>
</tr>
<tr>
<td>fat mass</td>
<td>476</td>
<td>0.37 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.29 ± 0.01</td>
<td>476</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>84.9 ± 1.7</td>
<td>83.6 ± 2.7</td>
<td>89.7 ± 2.9</td>
<td>477</td>
<td></td>
</tr>
<tr>
<td></td>
<td>478</td>
<td>85.5 ± 1.6</td>
<td>81.8 ± 2.6</td>
<td>88.1 ± 2.8</td>
<td>479</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.85 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>0.88 ± 0.02</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td></td>
<td>481</td>
<td>0.86 ± 0.01</td>
<td>0.86 ± 0.02</td>
<td>0.87 ± 0.02</td>
<td>481</td>
</tr>
</tbody>
</table>

- LBW compared with CON
- FDR compared with CON
- NM: not measured, NS: non significant
Table 2. Characteristics of CON, FDR and LBW before and after bed rest.

<table>
<thead>
<tr>
<th></th>
<th>BEFORE</th>
<th></th>
<th>AFTER</th>
<th></th>
<th>P (effect of bed rest within each group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON, n=20</td>
<td>LBW, n=20</td>
<td>FDR, n=13</td>
<td>CON, n=20</td>
<td>LBW, n=20</td>
</tr>
<tr>
<td><strong>VO₂ max (ml O₂/kg)</strong></td>
<td>43.5 ± 1.3</td>
<td>43.5 ± 2.7</td>
<td>39.1 ± 1.4</td>
<td>42.8 ± 1.1</td>
<td>41.2 ± 2.1</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>128 ± 2</td>
<td>128 ± 3</td>
<td>126 ± 3.1</td>
<td>127 ± 2</td>
<td>124 ± 3</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>68 ± 2</td>
<td>71 ± 3</td>
<td>71 ± 3</td>
<td>70 ± 2</td>
<td>70 ± 3</td>
</tr>
<tr>
<td><strong>Fasting arterial glucose (mmol/L)</strong></td>
<td>5.3 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>NS</td>
<td>NM</td>
</tr>
<tr>
<td><strong>Fasting insulin (nmol/L)</strong></td>
<td>33 ± 3</td>
<td>40 ± 6</td>
<td>50 ± 8</td>
<td>† P&lt;0.05</td>
<td>35 ± 3</td>
</tr>
<tr>
<td><strong>C-peptide (pmol/L)</strong></td>
<td>508 ± 26</td>
<td>531 ± 41</td>
<td>663 ± 51</td>
<td>† P&lt;0.05</td>
<td>507 ± 32</td>
</tr>
<tr>
<td><strong>BDNF (pg/ml)</strong></td>
<td>195 ± 44</td>
<td>189 ± 43</td>
<td>207 ± 44</td>
<td>NS</td>
<td>221 ± 48</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4.5 ± 0.3</td>
<td>† P&lt;0.05</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td><strong>LDL (mmol/L)</strong></td>
<td>2.2 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>† P&lt;0.05</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>NS</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td><strong>VLDL (mmol/L)</strong></td>
<td>0.4 ± 0.03</td>
<td>0.5 ± 0.09</td>
<td>0.5 ± 0.05</td>
<td>NS</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>† P&lt;0.05</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

† FDR compared with CON
NS: not significant, NM: not measured
Average glucose infusion rate (M-value) during hyperinsulinemic isoglycemic clamp in 20 CON, 20 low birth weight (LBW) and 13 first degree relatives (FDR) before and after 10 days of bed rest. Thick square indicates the average within each group before and after bed rest. All groups had lower M-value after bed rest compared with before bed rest (CON: P<0.001; LBW: P<0.001, FDR: P=0.068). % change in M-value in response to bed rest was significantly larger (P<0.05) in CON compared with FDR as indicated by + in the insert.

Before bed rest average glucose infusion rate during hyperinsulinemic clamp (M-value) vs. ΔM-value (before bed rest M-value minus after bed rest M-value) in 20 CON (squares), 20 low birth weight (LBW) (circles) and 13 first degree relatives (FDR) (triangles). In all groups there was a significant positive correlation between the parameters (r²=0.40, P=0.003; r²=0.52, P=0.003; r²=0.78, P=0.00007 in CON, LBW and FDR, respectively). The slope of CON was different from the slope of FDR (P<0.05), whereas there was no difference between the slopes of CON and LBW (P>0.05).
Insulin-stimulated forearm glucose clearance (insulin stimulated – basal) in 15 CON and 16 low birth weight (LBW) and 9 first degree relatives (FDR) before (black bars) and after (white bars) 10 days of bed rest. * denotes P<0.05 for before bed rest compared with after bed rest, # denotes P=0.077 CON compared with LBW and P= 0.071 CON compared with FDR.

Insulin-stimulated forearm fractional glucose extraction (insulin stimulated – basal) in 15 CON, 16 low birth weight (LBW) and 9 first degree relatives (FDR) before (black bars) and after (white bars) 10 days of bed rest. * denotes P<0.05 for before bed rest compared with after bed rest.

Insulin-stimulated forearm vs. whole body glucose uptake rates before (black symbols) and after bed rest (white symbols) in 14 CON (square), 16 low birth weight (LBW) (circles) and 9 first degree relatives (FDR) (triangles). Data are expressed relative to lean forearm/body mass, primarily representing skeletal muscle. Insulin-stimulated forearm glucose uptake and M-value was positively correlated (all groups r²=0.11, P<0.005).

Insulin-stimulated forearm blood flow measured with venous occlusion plethysmography in 20 CON, 20 low birth weight (LBW) and 13 first degree relatives (FDR) before (black
bars) and after (white bars) 10 days of bed rest. * denotes P<0.05 for before bed rest
compared with after bed rest. # denotes P<0.05 for CON compared with LBW and FDR.
+ denotes not significantly different from zero.
Fig. 1

M-value (mg · min⁻¹ · kg⁻¹)

CON LBW FDR

% decrease in M-value

0 10 20 30 40 50

CON LBW FDR

Fig. 2

Before bed rest M-value (ml · 100 ml⁻¹ · min⁻¹)

Δ M-value (mg · min⁻¹ · kg⁻¹)

FDR, r²=0.78, P=0.00007
LBW, r²=0.52, P=0.003
CON, r²=0.40, P=0.003
Fig. 3: Delta forearm glucose clearance (insulin-stimulated - basal) (ml · 100 ml⁻¹ · min⁻¹)

- CON
- LBW
- FDR

Values are mean ± SE.

Fig. 4: Delta forearm glucose extraction fraction (insulin-stimulated - basal) (%)

- CON
- LBW
- FDR

Values are mean ± SE.

* indicates a significant difference from basal (FDR, LBW, CON).

# indicates a significant difference from CON.
Fig. 5

Forearm glucose uptake (mg · min⁻¹ · kg lean forearm weight⁻¹)

M-value (mg · min⁻¹ · kg lean body weight⁻¹)

$r^2 = 0.11$, $P < 0.005$
Fig. 6

Delta forearm blood flow (insulin-stimulated - basal) (ml · 100 ml⁻¹ · min⁻¹)

FDR  LBW  CON

*  +  +

#


Reference List


