Impact of physical inactivity on subcutaneous adipose tissue metabolism in healthy young male offspring of patients with type 2 diabetes

Højbjerre Lise¹, Sonne Mette Paulli¹, Alibegovic Amra Ciric², Dela Flemming¹, Vaag Allan², Bruun Jens Meldgaard³, Christensen Karl Bang⁴, Stallknecht Bente¹

¹ Center for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.
² Steno Diabetes Centre, Gentofte, Denmark.
³ Department of Medicine and Endocrinology, Aarhus University Hospital, Aarhus, Denmark.
⁴ Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark.

Correspondence:
Lise Højbjerre, M.Sc.
Mail: hojbjerre@sund.ku.dk

Submitted 4 March 2010 and accepted 30 August 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
**Objective:** Physical inactivity is a risk factor for type 2 diabetes (T2D) and may be more detrimental in first degree relative (FDR) subjects unmasking underlying defects of metabolism. Using a positive family history of T2D as a marker of increased genetic risk the aim of this study was to investigate the impact of physical inactivity on adipose tissue (AT) metabolism in FDR subjects.

**Research design and methods:** A total of 13 FDR and 20 control (CON) subjects participated in the study. All were studied before and after 10 days of bed rest (BR) using the glucose clamp technique combined with measurements of glucose uptake, lipolysis and lactate release from subcutaneous abdominal (SCAAT) and femoral (SCFAT) adipose tissue by the microdialysis technique. Additionally, mRNA expression of lipases was determined in biopsies from SCAAT.

**Results:** Before BR FDR subjects revealed significantly increased glucose uptake in SCAAT. Furthermore, mRNA expression of lipases was significantly decreased in SCAAT of FDR subjects. BR significantly decreased lipolysis and tended to increase glucose uptake in SCFAT of both CON and FDR. In response to BR SCAAT glucose uptake significantly increased in CON but not in FDR.

**Conclusion:** FDR subjects exhibit an abnormal adipose tissue metabolism including increased glucose uptake prior to BR. However, the differences between FDR and CON subjects in AT metabolism were attenuated during BR due to relatively more adverse changes in CON compared with FDR subjects. Physical inactivity *per se* is not more deleterious in FDR as compared with CON with respect to derangements in adipose tissue metabolism.

Type 2 diabetes is the product of a complex interplay between genetic susceptibility and environmental factors. The best known environmental modifiable risk factors for T2D are obesity and a low level of habitual physical activity\(^1\). Even though there is substantial evidence that a change towards a healthy lifestyle halts the progression of T2D\(^2\), certain groups, including first degree relatives of patients with type 2 diabetes (FDR), are at increased risk of developing the disease\(^3\). T2D has a major hereditary component \(^4\), and FDR subjects show multiple abnormalities in intermediary metabolism and pancreatic islet cell function displaying insulin resistance despite normal glucose tolerance\(^5\). The metabolic defects include insufficient insulin secretion\(^6\), decreased peripheral glucose uptake\(^7\) and impaired antilipolytic effect of insulin in subcutaneous AT\(^8\).

AT is an active compartment in lipid and glucose metabolism of humans but the role of AT metabolism for development of T2D is not clarified. AT, along with skeletal muscle, is a site of peripheral insulin resistance in T2D\(^9\). The role of AT in the pathophysiology of insulin resistance can partly be attributed to lipolytic activity, resulting in mobilization of free fatty acids (FFA), which are deleterious for glucose utilization and insulin action\(^10\). However, AT may play a more direct role itself being a site of deranged glucose metabolism. Although AT is of minor quantitative importance for whole body glucose disposal, the tissue produces lactate\(^11\) functioning as a gluconeogenic precursor in the liver\(^12\), and previous studies have demonstrated increased plasma lactate\(^13\) and lactate release from adipocytes\(^14\) in FDR subjects.
In this study, we investigated in vivo AT glucose uptake, lipolysis and lactate release as well as SCAAT mRNA expression of lipases and GLUT-4 in FDR and control (CON) subjects prior to and after 10 days of bed rest (BR). We hypothesized that FDR subjects would show abnormalities in baseline AT metabolism and be more sensitive to the unhealthy effects of physical inactivity.

**RESEARCH DESIGN AND METHODS**

The data presented in this manuscript are part of a larger study on the influence of physical inactivity in healthy and prediabetic subjects as initiated and funded within the framework program of the EU EXGENESIS consortium.

**Subjects.** Thirty-three young healthy men participated in the study which included 13 FDR of patients with T2D and 20 CON without any family history of diabetes. All subjects were from singleton pregnancies born at term (FDR: 40±0.3 and CON: 40±0.1 weeks). During inclusion the groups were matched with respect to age, body mass index (BMI) and cardiorespiratory fitness. FDR were recruited via their type 2 diabetic parent attending the outpatient clinic, Steno Diabetes Center, Gentofte and CON subjects were recruited from the Danish National Birth registry. CON and FDR had birth weights between the 50th and 75th percentile (CON: 3827±49 and FDR: 3500±150g, P>0.05). The purpose and potential risk of the study were explained to all subjects before they gave their written informed consent. The study was approved by the Ethical Committee of Copenhagen and Frederiksberg, Denmark (protocol no. (01)-262546) and all procedures used conformed to the “Declaration of Helsinki II”. All subjects had normal fasting glucose measured before entering the study. Subjects were Caucasian, and none took any medication. Subject characteristics are summarized in Table 1.

**Pre and post bed rest testing.** Fasting plasma glucose was determined in capillary blood using an ABL 625 (Radiometer, Copenhagen, DK). Body composition was determined by dual-energy X-ray absorptiometry (DEXA) full body scanning (DPX-IQ®, software version 4.7e, Lunar Radiation Corporation, Madison, WI, USA) and abdominal and femoral skin fold thickness were measured using a calliper (Lafayette Instrument Company, Lafayette, Indiana, USA). VO2 max was measured using a bicycle ergometer exercise protocol by means of an Oxycon Pro System (Jaeger, Höchberg, Germany).

**Study design.** All subjects participated in one experimental day approximately 3 weeks before 10 days of strict BR. On the 10th day of the BR period subjects participated in a second and identical experimental day. Four days before the first experimental day subjects were provided a standardized isocaloric diet (i.e. 55 E% of carbohydrates, 15 E% of protein, and 30 E% of fat). Caloric fluids, including alcohol, were prohibited. During these 4 days as well as during the BR period a combined accelerometer and heart rate sensor (Actiheart; Cambridge Neurotechnology, Cambridge, UK) registered movements and heart rate and the energy expenditure was calculated. The Actiheart device was placed on the subject's upper left chest clipped on two standard ECG electrodes. The participants wore the Actiheart device continuously during the 4 days prior to the first experiment and throughout the entire bed rest period. The set time resolution for measurements was 30 sec during the 4 days of “free living” and 1 min during the bed rest period. The data recorded from the Actiheart monitors were downloaded into a database via the Actiheart.
reader/charger interface, for assessment of activity, heart rate, and calculated energy expenditure and analyzed using the Actiheart commercial program provided with the device (Actiheart® version 2.2 software). Data are edited with full traceability and without compromising the integrity of the original recording. Subjects were instructed to continue their daily living activities including physical activity habits during the 4 days and in the 3 weeks prior to the BR period and to refrain from vigorous physical activity 24 h prior to the first experimental day. During the BR period subjects were in bed all day (a maximum of 60° upper body elevation) under surveillance. A standardized isocaloric diet was provided during BR with adjusted reduced amounts to ensure weight stability.

**Experimental day protocol.** The experimental day consisted of a 210-min baseline period followed by a 180-min hyperinsulinemic euglycemic clamp. Microdialysis catheters were inserted and dialysate was sampled during the last 60 min of baseline and the last 60 min of the clamp period.

An arterial catheter (Becton-Dickinson, Great Britain) was inserted in the brachial artery and blood was sampled at time 180 and 210 min (basal) and at time 360 and 390 min (clamp). A venous catheter (18G Venflon, Medex Medicine, Great Britain) was inserted in the medial antecubital vein for infusion of insulin and glucose. Subcutaneous adipose tissue blood flow (ATBF) was measured by the local 133Xenon wash-out method.

**Microdialysis.** Prior to insertion of the microdialysis catheters (CMA60, CMA Microdialysis AB, Solna, Sweden) the skin was anaesthetized with 0.2 ml lidocaine (Lidokain SAD, 5 mg · ml⁻¹, Sygehus Apotekerne, Denmark). One catheter was inserted in SCAAT approximately 4 cm lateral to the umbilicus and another catheter was inserted in SCFAT of the leg adjacent to the vastus lateralis part of the quadriceps muscle 30 cm above the patella. The catheters were perfused with Ringer acetate containing 2 mM glucose (Skanderborg Apotek, Skanderborg, Denmark) at 1 µl · min⁻¹ using a high precision syringe pump (CMA100, CMA Microdialysis AB, Solna, Sweden). Dialysate was immediately frozen and stored at -20°C. The relative recovery (RR) over the membrane was determined using internal reference calibration and used for calculation of interstitial metabolite concentrations. For internal reference calibration 12 µl D-1[¹⁴C]-glucose (PerkinElmer, Boston, MA, USA) and 1 µl 2-[³H]-glycerol (PerkinElmer, Boston, MA, USA) was added to 10 ml of perfusate in order to achieve a specific activity of 5 kBq · ml⁻¹ for each isotope. Mean RR for glucose and glycerol were approximately 45% and 55%. RR of lactate was assumed to equal RR of glucose as found previously. Data were excluded if RR was less than 20%. The absolute rate of exchange of glucose, glycerol and lactate was calculated using Fick’s equation, as described previously.

**Biochemical analysis.** Blood samples for analysis of glucose, glycerol, lactate, FFA and triglyceride were distributed in iced tubes containing 1.5 mg ethylene-diamine-tetraacetic acid per ml blood. For analysis of insulin the tubes contained 500 KIE Trasylol and 1.5 mg EDTA per ml blood. The tubes were centrifuged (Hettich Labinstrument ApS, Hvidovre, Denmark) and stored at -80°C. Plasma and microdialysate glucose, glycerol and lactate were determined by a CMA600 Microdialysis Analyser (CMA Microdialysis AB, 2001 Solna, Sweden). Plasma FFA was determined using a non-esterified fatty acids (NEFA) C kit (Wako Chemicals, Neuss, Germany). Plasma insulin analysis was determined by ELISA technique (DAKO ELISA, United Kingdom) and plasma triglyceride with Triglyceride GPO-PAP (Roche Diagnostic, Mannheim, Germany).
Adipose tissue blood flow. ATBF was measured by the local $^{133}$Xe wash-out technique$^{15}$. Briefly, 0.5-1 MBq gaseous $^{133}$Xe (Amersham Health, Amersham, UK) was injected into the SCAAT and SCFAT contra lateral to the regions in which microdialysis was performed. Washout of $^{133}$Xe was registered by a Mediscint system (Oakfield Instruments, Oxford, UK) and ATBF was calculated as described previously$^{19}$.

Whole body glucose uptake. Whole body glucose disposal was determined using the hyperinsulinemic (40 mU min$^{-1}$ m$^{-2}$, Actrapid, Novo Nordisk, Copenhagen, Denmark), euglycemic clamp technique as described previously$^{20}$. Whole body insulin-mediated glucose uptake rates were averaged for 10 min and calculated as the mean of steady-state glucose infusion rates from t=360 to t=390 (M-value).

Adipose tissue biopsy. Subcutaneous abdominal AT biopsies were collected in the basal state and in the end of a 180-min hyperinsulinemic (80 mU min$^{-1}$ m$^{-2}$), euglycemic clamp before BR and on the 9th day of the BR intervention using the Bergstrom biopsy needle technique with suction under local anesthesia (1% lidocaine). Biopsies were snap frozen in liquid nitrogen and stored at -80°C.

Determination of mRNA levels. Total RNA was isolated using Trizol reagent (Gibco BRL, Life Technologies, Roskilde, Denmark) and cDNA was made with random hexamer primers using the GeneAmp PCR kit (Applied Biosystems, CA, USA). GADPH was chosen as housekeeping gene for HSL, LPL and ATGL and β-actin as housekeeping gene for GLUT4. Quantification was performed with a SYBR-Green real time PCR assay using an iCycler PCR machine (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as previously described$^{21}$.

Statistical analysis. The computer program SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Data were analyzed using a linear mixed model. The model estimates differences between mean values. Repeated measures data, as in the present study, requires special attention to the covariance structure due to the sequential nature of the data on each individual. The covariance structure refers to variances at individual times and to correlation between measures at different times on the same subject. In the mixed model the variation between subjects is specified by the RANDOM statement, and covariation within subjects is specified by the REPEATED statement. In the present study we assumed compound symmetry in the model, i.e. that all measurements on the same subject was equally correlated and had the same variance. The implication is that the only aspect of the covariance between repeated measures is due to the subject contribution, irrespective of proximity of time. Compound symmetric structure was fitted with the RANDOM statement The fixed effects parameters: group, experimental day and time was included in the MODEL statement”.

The figures presented (Fig. 1–3) are absolute means, while the differences and confidence intervals (CI) described in the results section, are from the linear mixed model. If data were not normally distributed or homogeneous as assessed by residual plots of each dependent variable, log transformation data were used and significant effects described as relative (%) differences. We evaluated the effect of groups (CON and FDR), effect of BR (before versus after) and time (baseline or insulin-stimulated) and tested an eventual differential effect of BR between FDR and CON by including interaction between groups and BR as well as the marginal effects in the model. Threshold for significance was $P \leq 0.05$. The Bonferroni corrected $p$-value that is equivalent to an uncorrected $p$-value of 0.05 in the present study is $P=0.0028$, although this
is likely overly conservative. However in the interpretation of results the risk of type I errors is acknowledged. Data are presented as absolute means ± S.E (figures) or differences and confidence interval (CI).

RESULTS
FDR was characterized by increased body fat percentage and abdominal adiposity compared with CON but similar BMI and waist/hip ratio (Table 1). In response to BR no changes in anthropometrics were observed. Total, resting and activity-related energy expenditure as well as the physical activity score (PAL) (Actiheart® recordings) did not differ between groups during daily living but decreased significantly in both groups during BR with no difference between groups (Table 2). VO2 max expressed in absolute units (ml/min) was slightly lower in FDR compared to CON before BR (P=0.035), but when expressing VO2 max per kg body weight there was no difference between groups. VO2 max did not change significantly in response to BR in FDR or in CON (Table 1).

Subcutaneous adipose tissue glucose uptake. Abdominal - The interstitial glucose concentration in SCAAT was significantly lower than plasma water glucose indicating that glucose is taken up in SCAAT in both groups (Table 3). Before BR basal and insulin-stimulated SCAAT glucose uptake was higher in FDR compared to CON (difference basal: 1.21 nmol · 100 g⁻¹ · min⁻¹, 95% confidence interval (CI) 0.39 – 2.02, P=0.005; difference insulin-stimulated: 0.94 nmol · 100 g⁻¹ · min⁻¹, 95% CI 0.17 – 1.72, P=0.02), with no differences between groups after BR (Fig. 1). The difference disappeared as glucose uptake increased only in CON in response to BR (difference basal: 1.1 nmol · 100 g⁻¹ · min⁻¹, 95% CI 0.3 – 1.9 , P=0.01; difference insulin-stimulated: 0.7 nmol · 100 g⁻¹ · min⁻¹, 95% CI 0.1 – 1.4, P=0.02). Test for interaction between groups and BR was significant in the basal state (P=0.02) and during insulin stimulation (P=0.047). Insulin did not stimulate SCAAT glucose uptake in any of the groups (Fig. 1).

Subcutaneous adipose tissue glucose uptake. Femoral - Also in SCFAT the interstitial glucose concentration was significantly lower than the arterial plasma water concentration indicating that glucose is taken up in SCFAT in both groups (Table 3). SCFAT glucose uptake did not differ between groups before or after BR. There was a non-significant tendency to increased SCFAT glucose uptake in both groups after BR (P=0.06). Insulin did not stimulate SCFAT glucose uptake in any of the groups (Fig. 1).

Subcutaneous adipose tissue lipolysis. Abdominal - The concentration of glycerol was significantly higher in the interstitial fluid than in arterial plasma water indicating glycerol release from and thus lipolysis in SCAAT in both groups (Table 3). SCAAT lipolysis did not differ between groups before or after BR, and did not influence SCAAT lipolysis in any of the groups (Fig.2). Insulin inhibited SCAAT lipolysis in both groups before (FDR P=0.006, CON P=0.01) and after BR (FDR P=0.004, CON P=0.0009).

Subcutaneous adipose tissue lipolysis. Femoral - Also in SCFAT the concentration of glycerol was significantly higher in the interstitial fluid than in arterial plasma water indicating lipolysis in the tissue in both groups (Table 3). Lipolysis did not differ between groups before or after BR (Fig. 2). However, after BR basal lipolysis was decreased in both FDR (difference before vs. after BR: 43%, 95% CI 29 - 64, P=0.0006) and CON (difference before vs. after BR: 62%, 95% CI 42 - 91, P=0.02). In FDR insulin inhibited lipolysis was also decreased after BR (difference before vs. after BR: 50%, 95% CI 31 - 81, P=0.02). Insulin inhibited SCFAT lipolysis in both groups before (FDR: P=0.0004 and CON: P<0.0001) and after BR (FDR: P=0.02 and CON: P=0.02).

Subcutaneous adipose tissue lactate release. Abdominal - Lactate concentrations were
significantly higher in the interstitial space than in arterial plasma water indicating that lactate is released from SCAAT in both groups (Table 3). SCAAT lactate release did not differ between the groups before or after BR, and did not change in response to BR or insulin stimulation in any of the groups (Fig.3).

**Femoral**: Also in SCFAT, lactate concentrations were significantly higher in the interstitial space than in arterial plasma water indicating lactate release in both groups (Table 3). Before BR there was a non-significant tendency to lower SCFAT lactate release in FDR compared to CON (P=0.06), while lactate release was similar after BR (Fig.3). BR and insulin did not influence SCFAT lactate release in any of the groups.

### Whole body insulin sensitivity.
Before BR, whole body insulin sensitivity (M-value) was lower (P<0.05) in FDR compared with CON (4.3±0.5 versus 6.8±0.5 mg · min⁻¹ · kg⁻¹), and BR decreased the M-value in both FDR (P=0.007) and CON (P=0.0001) although less in FDR compared with CON (28±1% versus 37±4%, P<0.05). After BR, the M-value was still lower in FDR compared to CON (3.1±0.3 versus 4.3±0.3 mg · min⁻¹ · kg⁻¹, P<0.05) as described previously²⁹. Test for interaction between groups and BR was non-significant (P=0.32), and thus do not support a differential effect of BR between the groups but a main effect. Thus plasma triglyceride concentrations increased in both groups in response to BR (P=0.04) Insulin inhibited plasma triglyceride concentrations in both groups (P<0.02). Fasting plasma insulin was elevated in FDR compared with CON before (P=0.02) and after BR (P<0.04), and did not change in response to BR in any of the groups.

### Subcutaneous, adipose tissue blood flow.
SCAAT blood flow did not differ between groups before or after BR and did not change in response to BR or insulin stimulation in any of the groups (data not shown). Before BR SCFAT blood flow was lower in FDR compared to CON (1.03 ml · 100 g⁻¹ · min⁻¹, 95% CI 0.39 – 1.69, P=0.003). SCFAT blood flow did not change in response to BR or insulin stimulation in any of the groups (data not shown).

### Arterial plasma glucose, glycerol, lactate, FFA, triglyceride and insulin.
Fasting plasma glucose was higher (P=0.002) in FDR compared to CON before and after BR and decreased in both groups after BR (P<0.0001) (Table 4). Fasting plasma glycerol did not differ between CON and FDR before or after BR and did not change in response to BR in any of the groups but decreased (P<0.0001) during insulin stimulation in both groups. Plasma lactate was similar between the groups before BR but lower in FDR compared to CON after BR during insulin stimulation (P=0.03). Plasma lactate increased during insulin stimulation in CON before BR (P=0.007), and decreased in FDR (P=0.03) after BR. Before BR, fasting (P=0.045) as well as insulin-stimulated (P=0.008) plasma FFA were higher in FDR, but after BR only insulin-stimulated concentrations remained different between groups (P=0.03). BR did not influence plasma FFA in any of the groups while plasma FFA decreased during insulin stimulation in both groups (P<0.01). Basal plasma triglyceride concentrations were higher in FDR before (P=0.04) and after BR (P=0.03) and increased in response to BR in FDR (P=0.03) but not in CON (P=0.7).
did not change in response to BR in any of the groups (Table 5). In FDR, HSL expression increased in response to insulin stimulation before \(P=0.01\) and after BR \(P=0.006\). Basal LPL expression was lower \(P<0.0001\) in FDR compared to CON before and after BR. In CON, but not in FDR, basal LPL expression increased \(P=0.02\) markedly in response to BR and the interaction between group and BR \(P=0.017\) supported this. During insulin stimulation LPL expression increased in FDR before \(P=0.03\) and after BR \(P=0.006\). GLUT-4 expression did not differ between CON and FDR and did not change in response to BR in any of the groups. In both groups, insulin stimulation increased GLUT-4 expression after BR (FDR: \(P=0.03\); CON: \(P=0.02\), but not before BR.

**DISCUSSION**

The major finding was that individuals with a family history of T2D (FDR) have a significantly higher glucose uptake in subcutaneous abdominal adipose tissue (SCAAT) compared to individuals without predisposition (CON), and that physical inactivity in non-predisposed individuals increases SCAAT glucose uptake to the same level as in predisposed individuals (Fig. 1). These differences were not found in the femoral adipose tissue (SCFAT). However, in SCFAT but not in SCAAT lipolysis decreased in response to physical inactivity in both predisposed and non-predisposed individuals (Fig. 2). SCAAT mRNA expression of ATGL, HSL and LPL was consistently lower in FDR compared to CON (Table 5) despite similar SCAAT lipolysis in the 2 groups.

At the whole body level FDR subjects in the present and several other studies\(^5,22\) are clearly insulin resistant. Whole body insulin sensitivity, as determined by the hyperinsulinemic, euglycemic clamp technique, is primarily a measure of insulin-stimulated glucose uptake in skeletal muscle, and we previously found insulin resistance in skeletal muscle of FDR subjects as evidenced by decreased insulin-stimulated forearm blood flow and glucose clearance\(^23\). In another study we found increased glucose production in FDR subjects indicating hepatic insulin resistance in these individuals\(^24\). In a recent \(^{25}\) as well as in the present study we found higher glucose uptake in SCAAT in FDR indicating redirection of glucose uptake to AT promoted by a combination of muscle insulin resistance as well as an increased rate of hepatic glucose production as shown previously\(^24\). Mice with muscle-specific inactivation of the insulin receptor gene (MIRKO), and as a consequence insulin resistance in skeletal muscle, likewise show increased glucose uptake in AT\(^26\).

Furthermore, MIRKO mice exhibit increased fat mass, serum triglyceride and FFA levels in face of normoglycemia and normal body weight as also seen in the FDR subjects of the present study. Thus, in the prediabetic state insulin resistance confined to skeletal muscle can induce dyslipidemia, increased AT glucose uptake and increased fat mass. We speculate that insulin resistance will develop also in AT during the further pathophysiological development towards T2D, possibly in response to a further increase in fat mass. Interestingly, the shunting of glucose seems to be site specific as glucose uptake was increased in SCAAT but not in SCFAT, which might explain the preferential localization of fat in abdominal depots of the FDR subjects.

Glucose may be used for reesterification of FFA via glycerol-3-phosphate or oxidized, stored as triglyceride or degraded to lactate. Lactate release in SCAAT was not different between groups indicating that the extra glucose taken up in SCAAT of FDR subjects takes other metabolic pathways. A likely pathway is conversion to glycerol-3-phosphate, which is a key substrate for lipid storage in adipocytes. Also the tendency to decreased lactate release in SCFAT of FDR
combined with normal SCFAT glucose uptake is leaving extra glucose for lipid storage.

Our study revealed that lipolysis in subcutaneous AT was similar in predisposed and non-predisposed individuals. Despite normal lipolysis in FDR subjects we found increased plasma FFA concentrations, which probably reflect an increased amount of AT in FDR subjects. The reduced expression of ATGL and HSL found in FDR subjects could contribute to the relatively high abdominal fat mass. The low expression of LPL in FDR could potentially increase plasma triglyceride, which we indeed found in the FDR subjects underpinning a metabolic abnormal condition.

AT lipolysis is very sensitive to the action of insulin, and failure to adequately turn off lipolysis could induce skeletal muscle and liver insulin resistance through excessive availability of FFA. The coexistence of normal SCAAT lipolysis and low mRNA expression of lipases in FDR subjects could be explained by the increased fasting plasma insulin levels in FDR subjects and indicates a state of insulin resistance seen as failure to turn off lipolysis in AT of FDR subjects. The low mRNA expression of AT lipases suggests that a primary defect in the development of insulin resistance relates to impaired ability to regulate the activity of AT lipases, important for the balance of triglyceride storage and mobilization of FFA.

CON subjects responded to BR by increasing SCAAT glucose uptake to the same level as seen in FDR subjects prior to BR. BR induced whole body insulin resistance in both FDR and CON, although the decrease in insulin sensitivity was relatively less in FDR subjects. When skeletal muscle reduces its utilization of glucose as a consequence of physical inactivity, glucose is shunted to as also seen in the insulin resistant FDR subjects before BR. Also, SCAAT LPL mRNA expression was up regulated after BR in CON but not in FDR subjects. Increased FFA and glucose uptake in SCAAT would tend to increase fat mass after BR, which we did not find. This may relate to the short time frame and the fact that we aimed at a constant body weight during the BR period. However, a high AT substrate uptake caused by a low level of physical activity would in the long term contribute to increased fat mass. The fact that CON and FDR subjects had similar AT glucose uptake after BR does to some extent reject our a priori hypothesis that FDR subjects are more sensitive to the unhealthy effects of physical inactivity than CON subjects. However, it is worth noting that the abnormalities of whole body and AT metabolism seen in FDR subjects in their “native” normal activity state are comparable to the metabolic abnormalities induced by 10 days of BR in healthy CON subjects without any family history of T2D. Shifting to a sedentary lifestyle causes less need for energy supply especially to skeletal muscle wherefore lipolysis is expected to decrease which we indeed found in SCFAT of both groups.

Interestingly, SCAAT GLUT-4 mRNA expression was markedly up regulated by insulin in both groups after BR indicating that physical inactivity increases the capacity of AT to respond to insulin stimulation supporting the finding of increased AT glucose uptake after BR. Chronic hyperinsulinemia in rats induces insulin resistance in skeletal muscle but a hyper-response to insulin in AT, which is associated with an increase in AT GLUT-4 mRNA and protein, and our study seem to confirm that hyperinsulinemia per se can produce divergent effects in AT and skeletal muscle.

In the present study, increased plasma insulin (~300 pM) during the hyperinsulinemic, euglycemic clamp did not stimulate AT glucose uptake, lactate release or blood flow. In support of our findings, previous studies have revealed that even supraphysiological insulin concentrations (~1600 pM) barely
stimulate glucose uptake in AT$^{17,25}$ and that physiological concentrations of insulin do not stimulate ATBF$^{17,25}$.

A multiple comparisons procedure was applied in the present study which introduce a risk of type I errors wherefore caution is needed in interpretation of the results revealing variance heterogeneity. Furthermore, non-inherited factors as e.g. dietary habits and habitual physical activity level might differ between FDR and CON, which potentially could explain some of the between-group differences before as well as in response to BR.

In conclusion, FDR subjects show abnormalities in subcutaneous, AT metabolism prior to BR displaying increased glucose uptake in SCAAT and decreased SCAAT mRNA expression of lipases. Ten days of BR decreases lipolysis and tends to increase glucose uptake in SCFAT in both FDR and CON subjects, and in SCAAT glucose uptake is increased by BR in CON to the same level as in FDR subjects. Thus, with respect to AT metabolism FDR subjects are not more sensitive than CON subjects to the unhealthy effects of physical inactivity, but the abnormalities of whole body and AT metabolism seen in FDR subjects prior to BR are comparable to the metabolic abnormalities induced by 10 days of BR in healthy CON subjects without any family history of T2D.

**Author contributions.** L.H. wrote manuscript, researched data M.P.S. researched data, reviewed/edited manuscript A.C.A. researched data, reviewed/edited manuscript F.D. contributed to discussion, reviewed/edited manuscript. A.V. contributed to discussion, reviewed/edited manuscript. J.MB. reviewed/edited manuscript. K.B.C. researched data, reviewed/edited manuscript. B.S. wrote manuscript.

**ACKNOWLEDGEMENTS**
We thank Regitze Kraunsøe, Jeppe Bach, Thomas Beck, Lenette Pedersen and Pia Hornbek as well as the laboratory at Steno Diabetes Center for technical assistance. The metabolic kitchen at Steno Diabetes Hospital is thanked for providing and managing the diet. Steno Diabetes Center is thanked for providing location for the BR. Financial support from a European Union grant (6th framework LSHM-CT-2004-005272, EXGENESIS) and the Nordea-foundation is gratefully acknowledged. L. Højbjerg was granted a PhD scholarship from the Ministry of Science, Technology and Innovation, Copenhagen, Denmark.

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**FIGURE LEGENDS**

**Figure 1** Abdominal and femoral subcutaneous adipose tissue glucose uptake in the basal state (no pattern) and the insulin-stimulated state (patterned) before (grey) and after (black) bed rest (BR) in controls (CON) and first degree relatives (FDR) of type 2 diabetic subjects.

*basal* (p=0.005) and insulin-stimulated state (p=0.02) before BR, CON vs. FDR.

**basal** (p=0.01) and insulin-stimulated state (p=0.02) before vs. after BR in CON.

**Figure 2** Abdominal and femoral subcutaneous adipose tissue lipolysis in the basal state (no pattern) and the insulin-stimulated state (patterned) before (grey) and after (black) bed rest (BR) in controls (CON) and first degree relatives (FDR) of type 2 diabetic subjects.

(*)=abdominal adipose tissue, (‘)=femoral adipose tissue

*Abdominal adipose tissue:* *basal* vs. insulin-stimulated state before BR in CON (p=0.01) and FDR (p=0.006). **basal** vs. insulin-stimulated state after BR in CON (p=0.0009) and FDR (p=0.004).

*Femoral adipose tissue:* + basal state before vs after BR in CON (p=0.02) and in FDR (p=0.0006). ++insulin-stimulated state before vs after BR in FDR (p=0.009). +++basal vs
insulin-stimulated state before BR in CON (p=0.0001) and in FDR (p=0.0004). ++++basal vs insulin-stimulated state after BR in CON (p=0.02) and in FDR (p=0.02).

Figure 3
Abdominal and femoral subcutaneous adipose tissue lactate release in the basal state (no pattern) and the insulin-stimulated state (patterned) before (grey) and after (black) bed rest (BR) in controls (CON) and first degree relatives (FDR) of type 2 diabetic subjects.

Fig. 1
Fig. 2

Glycerol release (nmol/100 g/min)

- basal
- insulin

Abdominal adipose tissue
- CON
- FDR

Femoral adipose tissue
- CON
- FDR

* * * * * * * *

+ ++ +++ +++
Fig. 3

Lactate release (µmol/100 g/min)

Abdominal adipose tissue

Femoral adipose tissue

CON CONF DR FDR

Basal
Insulin
Table 1. Subject Characteristics

|                              | Before bed rest |        | After bed rest |        |
|------------------------------|-----------------|--------|----------------|--------|
|                              | CON             | FDR    | aP             | CON    | FDR    | bP   | cP |
| Age (years)                  | 25 ± 0.2        | 26.1± 1| ns             |        |        |      |    |
| VO₂ max (ml O₂/kg/min)       | 43.5 ± 1.5      | 39.1± 1.9 | ns         | 42.8 ± 1.1 | 37.5 ± 2.0 | ns  | ns |
| VO₂ max (ml/min)             | 3659 ± 125      | 3405 ± 162 | p<0.05   | 3527 ± 101 | 3190 ± 160 | ns  | ns |
| Fasting arterial glucose (mmol/L) | 5.3 ± 0.1   | 5.5 ± 0.1 | ns         | 5.1 ± 0.1 | 5.2 ± 0.1 | p<0.05 | p<0.05 |
| Weight (kg)                  | 82.5 ± 2.3      | 84.0 ± 3.3 | ns         | 82.2 ± 2.3 | 83.6 ± 3.2 | ns  | ns |
| Height (m)                   | 1.85 ± 0.01     | 1.84 ± 0.02 | ns         | -        | -      | nm  | nm |
| BMI (kg/m²)                  | 24.1 ± 0.5      | 24.9± 0.9 | ns         | 23.9 ± 0.5 | 24.8 ± 0.9 | ns  | ns |
| Total fat mass (kg)          | 14.3 ± 1.6      | 21.6 ± 2.5 | p<0.05   | 14.7 ± 1.8 | 21.5 ± 2.7 | ns  | ns |
| Total lean mass (kg)         | 63.8 ± 1.1      | 58.6 ± 1.5 | p<0.05   | 63.6 ± 1.1 | 58.4 ± 1.5 | ns  | ns |
| Whole body fat percentage (%)| 17.4 ± 1.7      | 25.0 ± 2.3 | p<0.05   | 17.1 ± 1.6 | 24.9 ± 2.5 | ns  | ns |
| Trunk fat mass/total fat mass | 0.48 ± 0.1      | 0.58 ± 0.001 | p<0.05  | 0.50 ± 0.01 | 0.58 ± 0.01 | ns  | ns |
| Leg fat mass/total fat mass  | 0.37 ± 0.1      | 0.29 ± 0.01 | p<0.05  | 0.37 ± 0.01 | 0.29 ± 0.01 | ns  | ns |
| Waist/hip ratio              | 0.85 ± 0.01     | 0.88 ± 0.02 | ns       | 0.86 ± 0.01 | 0.87 ± 0.02 | ns  | ns |
| Abdominal skinfold (mm)      | 21 ± 1.8        | 25 ± 3.0 | ns          | 21 ± 1.6 | 23 ± 2.0 | ns  | ns |
| Femoral skinfold (mm)        | 16 ± 1.5        | 16 ± 2.0 | ns          | 15 ± 1.4 | 16 ± 2.0 | ns  | ns |

Data are presented as mean ± SE. aP Significant difference between the groups before bed rest, (P<0.05). bP: Significant difference before vs. after bed rest within CON, (P<0.05). cP: Significant difference before vs. after bed rest within FDR, (P<0.05). ns: non significant; nm: not measured
Table 2. Energy expenditure and physical activity level before and during bed rest

|                          | Daily living |       | During bed rest |       |       |       |
|--------------------------|--------------|-------|-----------------|-------|-------|-------|
|                          |              | CON   | FDR             | CON   | FDR   |       |
| Resting energy expenditure (kJ/day/kg) (REE) | 99 ± 1.0     | 96 ± 2.7 | ns              | 101 ± 1.4 | 97 ± 2.4 | ns   |
| Activity energy expenditure (kJ/day/kg) (AEE) | 83 ± 7       | 102 ± 8 | ns              | 26 ± 4    | 27 ± 3   | p<0.0001 |
| Total energy expenditure (kJ/day/kg) (TEE)     | 207 ± 8      | 218 ±12 | ns              | 146 ± 5   | 138 ± 4   | p<0.0001 |
| PAL (daily physical activity level) (TEE/REE)   | 2.1 ± 0.1    | 2.3 ± 0.1 | ns              | 1.5 ± 0.05 | 1.4 ± 0.03 | p<0.0001 |
| Sitting (hours/day)       | 7.2 ± 0.6    | 6.5 ± 0.9 | ns              | -         | -        | -     |

Data are presented as mean ± SE. 

*aP* Significant difference between the groups before bed rest.

*bP*: Significant difference before vs. after bed rest within CON.

*cP*: Significant difference before vs. after bed rest within FDR. ns: non significant. REE, AEE, TEE and PAL based on Actiheart® recordings during 4 days of daily living 3 weeks before bed rest. Hours sitting per day based on IPAQ questionnaire.
Table 3. Basal subcutaneous adipose tissue interstitial and plasma water concentrations of metabolites

|                                      | Control subjects | First degree relatives |
|--------------------------------------|------------------|------------------------|
|                                      | Before BR        | After BR               | Before BR        | After BR               |
| Interstitial glucose abdominal (µM)  | 4.4 ± 0.2 (n=19) | 3.6 ± 0.4 (n=18)      | 4.0 ± 0.5 (n=11) | 4.5 ± 0.3 (n=13)      |
| Interstitial glucose femoral (µM)    | 4.7 ± 0.2 (n=19) | 4.0 ± 0.2 (n=19)      | 5.7 ± 0.4 (n=11) | 4.3 ± 0.3 (n=11)      |
| Arterial plasma water glucose (µM)   | 5.3 ± 0.1 (n=20) | 5.1 ± 0.1 (n=20)      | 5.9 ± 0.1 (n=13) | 5.6 ± 0.1 (n=13)      |
| Interstitial glycerol abdominal (µM) | 235 ± 29 (n=20)  | 172 ± 13 (n=20)       | 228 ± 40 (n=13)  | 241 ± 39 (n=12)       |
| Interstitial glycerol femoral (µM)   | 309 ± 38 (n=20)  | 232 ± 34 (n=19)       | 355 ± 49 (n=11)  | 205 ± 29 (n=12)       |
| Arterial plasma water glycerol (µM)  | 36 ± 3 (n=20)    | 33 ± 2 (n=20)         | 42 ± 4 (n=13)    | 36 ± 3 (n=13)         |
| Interstitial lactate abdominal (µM)  | 2.7 ± 0.2 (n=19) | 3.0 ± 0.3 (n=18)      | 3.1 ± 0.5 (n=10) | 3.0 ± 0.3 (n=13)      |
| Interstitial lactate femoral (µM)    | 2.8 ± 0.3 (n=19) | 3.1 ± 0.3 (n=19)      | 2.8 ± 0.2 (n=10) | 2.6 ± 0.3 (n=12)      |
| Arterial plasma water lactate (µM)   | 0.8 ± 0.06 (n=20) | 0.8 ± 0.05 (n=20)    | 0.8 ± 0.08 (n=13) | 0.8 ± 0.05 (n=13)    |

Data are presented as mean ± SE. *Significant difference interstitial vs. arterial plasma water concentration, (P<0.05)
Table 4 Plasma glucose, glycerol, lactate, FFA, triglyceride and insulin

|                       | Control subjects | First degree relatives |
|-----------------------|------------------|------------------------|
|                       | Before bed rest  | After bed rest         | Before bed rest  | After bed rest |
|                       | (n=20)           | (n=20)                 | (n=13)           | (n=13)         |
| **P-glucose (mM)**    | Basal            | 5.1 ± 0.06 (*) (***)   | 4.8 ± 0.05 (*) (***) | 5.4 ± 0.1 (*) (**) |
|                       | Ins.stim         | 4.9 ± 0.07 (*)         | 4.8 ± 0.07 (**)   | 5.3 ± 0.1 (*) (**) |
| **P-glucose (mM)**    | Basal            | 5.1 ± 0.06 (*) (***)   | 4.8 ± 0.05 (*) (***) | 5.4 ± 0.1 (*) (**) |
|                       | Ins.stim         | 4.9 ± 0.07 (*)         | 4.8 ± 0.07 (**)   | 5.3 ± 0.1 (*) (**) |
| **P-glycerol (µM)**   | Basal            | 34.6 ± 2.7 (*** )      | 31.2 ± 2.1 (*** ) | 38.5 ± 3.7 (*** ) |
|                       | Ins.stim         | 14.5 ± 0.9             | 13.9 ± 0.9        | 14.7 ± 1.2 |
| **P-lactate (mM)**    | Basal            | 0.7 ± 0.05 (*** )      | 0.7 ± 0.05        | 0.7 ± 0.07 |
|                       | Ins.stim         | 0.9 ± 0.05             | 0.8 ± 0.04(*)     | 0.7 ± 0.06 |
| **P-FFA (µM)**        | Basal            | 264 ± 30 (*) (***)     | 282 ± 30 (*** )   | 369 ± 46 (*) (*** ) |
|                       | Ins.stim         | 13 ± 2 (*)             | 11 ± 2            | 26 ± 5 (*) |
| **P-triglyceride (mM)** | Basal          | 0.9 ± 0.1 (*** )       | 1.0 ± 0.1 (*** )  | 1.1 ± 0.1 (*** ) |
|                       | Ins.stim         | 0.5 ± 0.05             | 0.6 ± 0.1 (**)    | 0.7 ± 0.1 |
| **P-insulin (pM)**    | Basal            | 35 ± 3 (*)             | 35 ± 3 (*)        | 50 ± 8 (*) |
|                       | Ins.stim         | 294 ± 10               | 272 ± 13          | 287 ± 26 |

Data are presented as mean ± SE. * Significant difference CON vs. FDR, P<0.05; ** Significant difference before vs. after BR within the group; P<0.05; *** Significant difference basal vs. insulin-stimulated state within the group on the specified day, P<0.05.

Data were log transformed before statistical test.
Table 5. ATGL, HSL, LPL mRNA expression relative to GADPH and GLUT-4 mRNA expression relative to β-actin in subcutaneous, abdominal adipose tissue

|                      | Control subjects       | First degree relatives         |
|----------------------|------------------------|--------------------------------|
|                      | Before bed             | After BR                       | Before bed rest                   | After bed rest                   |
|                      | Rest (basal n=16)      | (basal n=16)                   | (basal n=10)                      | (basal n=10)                     |
|                      | Ins.stim. n=14)        | Ins.stim. n=16)                | Ins.stim. n=11)                   | Ins.stim. n=11)                  |
| ATGL⁺ Basal          | 0.10 ± 0.02 (*)        | 0.10 ± 0.01                    | 0.04 ± 0.02 (**)(***             | 0.06 ± 0.04                      |
| ATGL⁺ Ins.stim.      | 0.12 ± 0.03            | 0.11 ± 0.02                    | 0.08 ± 0.02                       | 0.07 ± 0.01                      |
| HSL⁺ Basal           | 0.10 ± 0.02 (*)        | 0.15 ± 0.04 (*)                | 0.02 ± 0.01 (**)(***             | 0.01 ± 0.00 (**)(***             |
| HSL⁺ Ins.stim.       | 0.08 ± 0.01            | 0.09 ± 0.02                    | 0.09 ± 0.03                       | 0.06 ± 0.02                      |
| LPL⁺ Basal           | 1.2 ± 0.2 (**)(***)    | 2.3 ± 0.4 (**)(***)            | 0.4 ± 0.1 (**)(***)               | 0.3 ± 0.2 (**)(***)              |
| LPL⁺ Ins.stim.       | 1.8 ± 0.4              | 1.7 ± 0.2                      | 1.0 ± 0.3                         | 1.1 ± 0.3                        |
| GLUT 4⁺ Basal        | 0.9 ± 0.3              | 0.6 ± 0.1 (***)                | 1.0 ± 0.3                         | 1.0 ± 0.3 (***)                  |
| GLUT 4⁺ Ins.stim.    | 0.8 ± 0.2              | 1.2 ± 0.2                      | 0.6 ± 0.2                         | 2.3 ± 0.8                        |

Data are presented as mean ± SE. * Significant difference CON vs. FDR, P<0.05;
** Significant difference before vs. after BR within the group; P<0.05;
*** Significant difference basal vs. insulin-stimulated state within the group on the specified day, P<0.05,
+ Data were log transformed before statistical test.