Interaction Between Dietary Lipids and Physical Inactivity on Insulin Sensitivity and on Intramyocellular Lipids in Healthy Men

Rodrigue Stettler, MD[1]
Michael Ith, MD, PHD[2]
Kevin J. Acheson, PHD[3]
Jacques Décombaz, PHD[3]
Chris Boesch, MD, PHD[2]
Luc Tappy, MD[1]
Christophe Binnert, PHD[1]

OBJECTIVE — To assess the effect of a possible interaction between dietary fat and physical inactivity on whole-body insulin sensitivity and intramyocellular lipids (IMCLs).

RESEARCH DESIGN AND METHODS — Eight healthy male volunteers were studied on two occasions. After 2 days of an equilibrated diet and moderate physical activity, participants remained inactive (bed rest) for 60 h and consumed either a high–saturated fat (45% fat, of which ~60% was saturated fat [BR-HF]) or a high-carbohydrate (70% carbohydrate [BR-HCHO]) diet. To evaluate the effect of a high-fat diet alone, six of the eight volunteers were restudied after a 2-day equilibrated diet followed by 60 h on a high–saturated fat diet and controlled physical activity (PA-HF). Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp and IMCL concentrations by ^{1}H-magnetic resonance spectroscopy.

RESULTS — Insulin-mediated glucose disposal was decreased by BR-HF condition (−24 ± 6%, $P < 0.05$) but did not change with BR-HCHO (+19 ± 10%, NS). BR-HF and BR-HCHO increased IMCL levels (+32 ± 7%, $P < 0.05$ and +17 ± 8%, $P < 0.0011$, respectively). Although the increase in IMCL levels with PA-HF (+31 ± 19%, $P = 0.12$) was similar to that during BR-HF, insulin-mediated glucose disposal (−7 ± 9%, NS) was not decreased.

CONCLUSIONS — These data indicate that physical inactivity and a high–saturated fat diet may interact to reduce whole-body insulin sensitivity. IMCL content was influenced by dietary lipid and physical inactivity but was not directly associated with insulin resistance.

Diabetes Care **28:1404–1409, 2005**

Insulin resistance is a common disorder in western societies and is thought to play an important role in the pathogenesis of diseases such as diabetes, hypertension, and cardiovascular diseases. Despite a considerable number of studies, the mechanisms are still not completely understood. In addition to genetic background, environmental factors such as high-fat diet, low physical activity, and excess body weight may all be involved in its pathogenesis (1,2).

It has been recognized for many years that lipids exert deleterious effects on insulin sensitivity. Several studies have demonstrated that increased plasma nonesterified fatty acid (NEFA) concentrations interfere with insulin-stimulated glucose uptake in skeletal muscle (3–6). Most studies have used triglyceride emulsion infusions to demonstrate this, but there are few data available about the effects of dietary fat itself on insulin sensitivity. Epidemiological data suggest that dietary fat is involved in insulin resistance (1). However, in long-term studies, it is difficult to differentiate between the effects of the diet alone and those resulting from alterations in body composition. Furthermore, over the last decade, the impact of fat depots (intramyocellular lipids [IMCLs]) in muscle playing a causative role in insulin resistance has been emphasized by studies demonstrating a negative correlation between IMCL content and insulin sensitivity (7–10). It has also been reported that IMCL stores constitute a dynamic cellular triglyceride pool, the level of which can be modulated by dietary fat (11).

A substantial body of data now demonstrates the impact of low physical activity on the development of insulin resistance (12). Furthermore, intervention studies have shown that moderate amounts of daily physical activity are able to ameliorate insulin sensitivity in diabetic or obese subjects (2,13). Several mechanisms have been proposed for this improvement, including enhancement in muscle oxidative metabolism and decreased body fat (14–16).

A high–saturated fat diet and low physical activity often coexist in individuals at risk for obesity. Both are individually associated with insulin resistance; however, to our knowledge, no study has examined the combined effect of inactivity and high-fat diet on insulin sensitivity. The present study was therefore carried out to investigate the effect of a short period of physical inactivity associated with a high–saturated fat (45% fat, of which

From the [1]Department of Physiology, University of Lausanne, Lausanne, Switzerland; the [2]Department of Clinical Research, MR Spectroscopy and Methodology, University of Bern, Bern, Switzerland; and [3]Nestec, Nestlé Research Center, Lausanne, Switzerland.

Address correspondence and reprint requests to Christophe Binnert, Département de Physiologie, 7 rue du Bugnon, 1005 Lausanne, Switzerland. E-mail: christophe.binnert@unil.ch.

Received for publication 29 November 2004 and accepted in revised form 17 February 2005.

Abbreviations: BMR, basal metabolic rate; GIR, glucose infusion rate; ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; IMCL, intramyocellular lipid; NEFA, nonesterified fatty acid.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

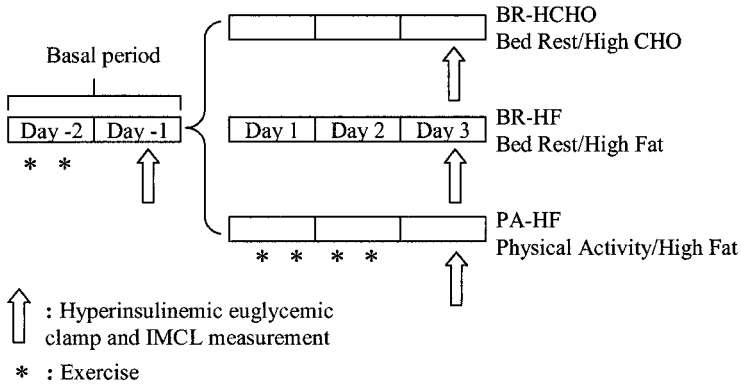

Figure 1—*Study design.*

~60% was saturated fat) or high-carbohydrate (70% carbohydrate) diet on insulin sensitivity in healthy subjects. This was applied over a short period (60 h) to evaluate the effects of metabolic changes independent of significant changes in body composition. IMCLs were quantified by ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) measurements to assess their possible role in modulating insulin sensitivity.

Furthermore, adiponectin has been demonstrated in mice to be related to insulin sensitivity by promoting muscle fatty acid oxidation (17) and in humans to be negatively correlated with IMCL concentrations in the soleus muscle (18). We therefore evaluated whether physical inactivity and dietary fat altered plasma adiponectin concentrations.

RESEARCH DESIGN AND METHODS

— Eight healthy male volunteers (age 23 ± 1 years; weight 67.9 ± 2.9 kg, BMI 21.6 ± 0.8 kg/m^2, body fat 13.4 ± 1.7% [means ± SE]) participated in the study. All subjects were in good health, were nonsmokers, and were not taking any drugs. None had a personal or family history of diabetes or hypertension. The subjects did not regularly take part in strenuous physical activity. Body composition was estimated from subcutaneous skinfold thickness measurements according to Durnin and Womersley (19). The experimental protocol was approved by the ethical committees of the Lausanne University School of Medicine and of the Kanton of Bern. Before participating in the study, each subject was informed in detail about the protocol and signed a written informed consent form.

Subjects were studied on three occasions for 5 consecutive days spent in a metabolic unit (Fig. 1). During baseline (day −2 and day −1), volunteers consumed an isocaloric-equilibrated diet composed of 35% fat (~50% saturated), 50% carbohydrates, and 15% protein corresponding to 1.6 × basal metabolic rate (BMR; previously estimated by indirect calorimetry); besides normal ambulatory activity, subjects performed two exercise bouts of 30 min at 75 W (10:00 A.M. and 2:30 P.M.) on a bicycle ergometer on day −2 . The effect of diet and physical inactivity was tested over the next 60 h (days 1, 2, and 3) with the following protocols: *1*) bed rest associated with an isocaloric (1.2 × BMR) high-carbohydrate diet (BR-HCHO; 70% carbohydrates, 15% fat of which ~35% was saturated fat, 15% protein), 2) bed rest associated with an isocaloric (1.2 × BMR) high-fat diet ([BR-HF]; 40% carbohydrate, 45% fat of which ~60% was saturated fat, 15% protein), and 3) physical activity (2 × 30 min at 75 W on day 1 and 2) associated with an isocaloric (1.6 × BMR) high-fat diet (PA-HF; n = 6). The order of the two bed rest conditions was randomized with >9 days in between each, whereas the physical activity condition was always last after at least a 5-week washout period. Meals were provided at 8:00 A.M. (30% energy), 12:00 P.M. (35% energy), and 6:30 P.M. (35% energy) with no snacks in between. Heart rate was continuously monitored using a Polar cardiometer (Kempele, Finland) to control for physical inactivity compliance (2). To reduce the risk of deep venous thrombosis associated with bed rest, participants were given 100 mg acetylsalicylic acid on day 1 of each protocol.

On the morning of the clamp at 8:00 A.M., subjects received a breakfast containing 1.63 g carbohydrate/kg body wt (day −1), 1.77 g carbohydrate/kg (day 3 of high-carbohydrate diet), or 1.10 g carbohydrate/kg (day 3 of high-fat diet). Glucose infusion rate (GIR) was measured at least 5 h after breakfast (~1:00–1:30 P.M.). On completion of the clamp procedure, subjects were transferred to the Magnetic Resonance Center at the University and Inselspital Bern, where IMCL concentrations were determined by localized ^{1}H-MRS of the vastus intermedius muscle of the right quadriceps. The IMCL measurements were realized 3 h after the completion of the clamp studies; the interval was kept constant between volunteers.

Hyperinsulinemic-euglycemic clamp

A 90-min hyperinsulinemic (1 mU insulin · kg^{-1} · min^{-1})-euglycemic (5.6 mmol/l) clamp (20) was performed between 12:00 P.M. and 1:30 P.M. Blood was obtained every 30 min for insulin and NEFA determination. During the last 30 min of the clamp, the GIR was used to evaluate insulin-mediated glucose disposal.

^{1}H-MRS. Magnetic resonance examinations of the right thigh included image acquisition and ^{1}H-MRS on a 1.5 Tesla whole-body system (SIGNA; General Electrics, Milwaukee, WI), using a ^{13}C/^{1}H double-tuned flexible extremity coil (^{1}H-coil, Helmholtz design; Medical Advance, Milwaukee, WI). Inside the magnet, the volunteers were placed on a specially designed positioning aid to guarantee a reproducible fixation of the right leg for all six magnetic resonance sessions (four in two cases). Repositioning of the volunteer and placement of the coil were monitored on the localizer images.

It has been shown that the signals from IMCLs and extramyocellular lipids can be separated by single-voxel ^{1}H-MRS (21,22). The sensitive volume (18 × 11 × 12 mm^3, inferior/superior × right/left × anterior/posterior) was positioned in the m. vastus intermedius close to the femoral bone. Care was taken that no sign of fatty infiltration was visible inside the sensitive volume, which could lead to contamination by the large extramyocellular lipid signals. Positioning as well as repositioning (sessions 2–6) of the sensitive volume was done in the localizer image series (fast spin echo sequence with a repetition time of t_r = 1,000 ms and an echo time of t_e = 17 ms). ^{1}H-MRSs were recorded using an optimized PRESS-sequence (t_r = 3 s, t_e = 20 ms, 128 acquisitions, 16 phase-rotating steps, 2,000 Hz, 1,024 pts) with

Table 1—*Plasma glucose, insulin, NEFA, and adiponectin concentrations*

	Baseline (day −1)			Intervention (day 3)		
	BR-HCHO	BR-HF	PA-HF	BR-HCHO	BR-HF	PA-HF
Pre-clamp values						
Glucose (mmol/l)	5.30 ± 0.30	5.40 ± 0.20	5.60 ± 0.3	4.96 ± 0.07 (P = 0.211)	5.01 ± 0.14 (P = 0.043)	5.54 ± 0.19 (P = 0.756)
Insulin (pmol/l)	119.1 ± 38.3	69.2 ± 15.0	61.3 ± 20.4	61.2 ± 15.4 (P = 0.257)	52.4 ± 9.7 (P = 0.121)	45.7 ± 7.9 (P = 0.367)
NEFA (μmol/l)	475 ± 105	292 ± 52	270 ± 49	245 ± 55 (P = 0.021)	408 ± 51* (P = 0.078)	279 ± 39 (P = 0.0811)
Adiponectin (μg/ml)	6.08 ± 1.05	5.70 ± 0.89	5.33 ± 1.30	5.75 ± 1.36 (P = 0.848)	6.10 ± 1.02 (P = 0.721)	5.92 ± 1.49 (P = 0.183)
Clamp values						
Glucose (mmol/l)	5.47 ± 0.04	5.48 ± 0.03	5.35 ± 0.04	5.42 ± 0.03 (P = 0.413)	5.50 ± 0.03 (P = 0.668)	5.54 ± 0.02 (P = 0.032)
Insulin (pmol/l)	510.9 ± 15.9	509.4 ± 13.8	487.2 ± 13.8	512.5 ± 14.4 (P = 0.770)	519.3 ± 10.0 (P = 0.257)	484.0 ± 26.5 (P = 0.878)
NEFA (μmol/l)	95 ± 17	99 ± 18	74 ± 16	64 ± 7 (P = 0.026)	102 ± 12 (P = 0.847)	87 ± 6 (P = 0.253)
GIR (mg · kg^{-1} · min^{-1})	7.33 ± 0.83	7.88 ± 0.81	8.51 ± 0.56	8.26 ± 0.47 (P = 0.130)	5.82 ± 0.53* (P = 0.007)	7.71 ± 0.63 (P = 0.353)
IMCL (mmol/kg wet wt)	2.95 ± 0.36	3.33 ± 0.64	3.34 ± 0.60	3.36 ± 0.33 (P = 0.037)	4.17 ± 0.68 (P < 0.0001)	4.22 ± 0.82 (P = 0.121)

Data are means ± SE. Values in parentheses correspond to P value compared with day −1 of the same experiment condition (paired t test). *Different from BR-HCHO and from PA-HF values (P < 0.05; post hoc Fisher's test).

water and additional outer-volume suppression. The spectra were quantified using the fully relaxed, unsuppressed water signal as an internal concentration standard. IMCL concentrations (in millimoles per kilogram wet weight) were evaluated as previously described (23).

Analytical procedures

Plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). NEFA concentrations were measured with a commercial colorimetric method (NEFA C; Wako Chemicals, Freiburg, Germany). Radioimmunoassay kits were used for determination of plasma insulin and adiponectin (Linco Research, St. Charles, MO).

Statistical analysis

Data are expressed as means ± SE. Statistical significance was assessed using ANOVA with repeated measures and a post hoc Fisher's test for comparison of the three test conditions or a paired t test for comparison between day 3 and day −1 within each condition.

RESULTS — Plasma glucose, insulin, NEFAs, and adiponectin concentrations are shown in Table 1. At baseline (day −1), there were no differences in plasma hormones, substrate concentrations, GIR, or IMCL levels between groups (Table 1 and Fig. 2).

On day 3, preclamp glucose concentrations were less after BR-HF (P < 0.05) and NEFA concentrations decreased after BR-HCHO (P = 0.021). Preclamp-insulin and -adiponectin concentrations remained unchanged. During the clamp (60–90 min), GIR decreased only in the BR-HF condition (−24 ± 6%, P = 0.007) (Fig. 2*A*). Compared with day −1, IMCL concentrations on day 3 increased significantly by 17 ± 8% with BR-HCHO (P = 0.037) and 32 ± 7% with BR-HF (P < 0.0001). After PA-HF, a 31 ± 19% increase in IMCL was observed but failed to reach statistical significance (P = 0.12) (Fig. 2*B*).

Correlations

No correlations were observed between IMCL content and GIR on day 3 or between increments of IMCL and changes in GIR between day −1 and day 3. Similarly, no correlations were observed between

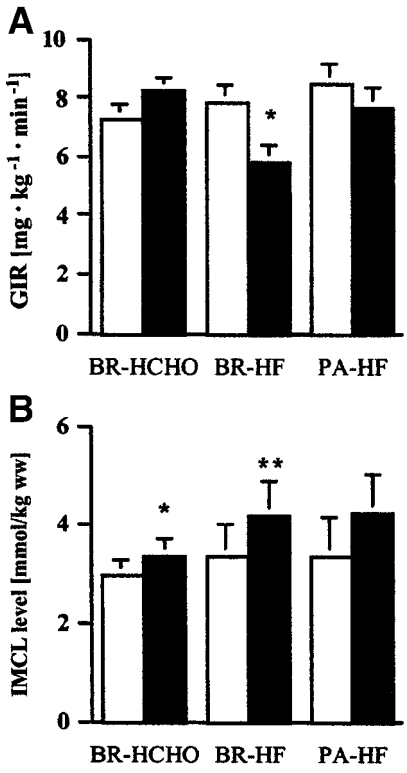

Figure 2—A: *GIR on day −1 (□) and day 3 (■) in each condition. GIR values were determined as the mean GIR level between 60 and 90 min.* B: *IMCL concentrations on day −1 (□) and day 3 (■) in each condition. IMCL levels were measured in the vastus intermedius muscle of the right thigh after the hyperinsulinemic-euglycemic clamp.* *$P < 0.05$, *different from day −1;* **$P < 0.001$, *different from day −1. Data are means ± SE.*

IMCL and adiponectin concentrations or between GIR and adiponectin concentrations on day 3.

Heart rate

Compared with day −1, diurnal heart rate decreased during both bed rest conditions from 74 ± 3 to 64 ± 1 bpm ($P < 0.001$) and from 74 ± 2 to 64 ± 2 bpm ($P = 0.003$) during BR-HCHO and BR-HF, respectively. During the PA-HF condition, heart rate was not modified (77 ± 4 vs. 78 ± 3 bpm, NS).

CONCLUSIONS — The major observations in this prospective study are that *1*) whole-body insulin sensitivity was decreased only in response to the combination of physical inactivity and high-fat diet; *2*) IMCL concentrations increased with bed rest, whether associated with a high-carbohydrate or a high-fat diet, and a similar trend was observed with high-fat diet associated with physical activity; and *3*) no correlations were observed during any of these three conditions between whole-body insulin sensitivity and IMCL concentration in the vastus intermedius muscle of the quadriceps; moreover, no correlations were noted between increments of IMCL levels and changes in whole-body insulin sensitivity between day −1 and day 3.

Insulin sensitivity was assessed as the average GIR during the last 30 min of the clamp. Since insulin concentrations achieved during the clamps are known to completely suppress hepatic glucose output (24), the difference most likely reflects extrahepatic (muscle) insulin resistance (25). These differences in insulin sensitivity were observed during hyperinsulinemic clamps performed between 12:00 P.M. and 1:30 P.M., 5 h after the last meal, i.e., breakfast at 8:00 A.M. Although one cannot completely exclude that dietary carbohydrate absorption was finished at this time, previous studies indicate that the bulk of carbohydrates are absorbed within 4 h of a liquid meal (26). Although absorption of a solid meal may take somewhat longer, it is very unlikely that differences in gastric emptying or rates of dietary carbohydrate absorption induced by high-fat feeding or physical inactivity were of sufficient magnitude to account for the reduction in GIR observed after BR-HF. We therefore feel confident that our observation reflects a decreased insulin sensitivity after BR-HF.

Numerous studies in humans and animals have investigated the acute effects of high concentrations of fatty acids on insulin sensitivity using intravenous triglyceride emulsion infusions. Increasing fatty acid concentrations has been shown on several occasions to reduce insulin-stimulated glucose uptake (4,6,27), but few experiments have focused on the effect of increased dietary fat on insulin sensitivity. Bachmann et al. (28) compared the effect of a high-fat diet (60% of energy from fat, predominantly saturated fat) with that of an acute intravenous lipid infusion. These authors reported that the decrease in GIR was less after 3 days of high-fat diet (−20%) than after triglyceride infusion (−40%). In our study, we did not observe a significant decrease in insulin sensitivity with the high-fat diet associated with physical activity. This discrepancy probably results from the higher proportion of fat (55–60 vs. 45%) and larger energy intake consumed by the subjects in Bachmann's study or possibly by the smaller number of subjects in our study. In both our study and Bachmann et al.'s studies, the effect of short-term high-fat feeding on insulin sensitivity was small. This may be due to the short duration of dietary changes, since after extended exposure to dietary fat, rodents have been shown to develop larger changes in whole-body and muscle insulin sensitivity (29).

Even though some studies have also investigated the effect of physical inactivity on insulin sensitivity (30–35), none have combined physical inactivity and a high-fat diet. In 1972, Lipman et al. (31) reported a deleterious effect of 3 days of bed rest on glucose tolerance in healthy adults. However, in this study, no proper distinction could be made between the effects of the diet and physical inactivity on insulin sensitivity since the relative proportions of the macronutrients were not specified. More recently, Blanc et al. (34) reported a decrease in insulin sensitivity after 7 days of head-down bed rest in healthy men and women on a conventional diet. The authors suggested that this impairment occurred mainly in skeletal muscle and mostly in men. However, fluid redistribution during head-down tilt may cause insulin resistance by reducing muscle blood flow. Stuart et al. (35) studied the effect of 7 days of bed rest in healthy subjects on a controlled diet and showed that whole-body insulin-stimulated glucose uptake was impaired whereas hepatic glucose production was adequately inhibited by insulin. In the present study, 60 h of bed rest combined with a high-carbohydrate diet was not sufficient to alter insulin sensitivity. This difference might be explained by the shorter duration and lower proportion of fat in our study (15 vs. 31%).

To our knowledge, this is the first time that a relatively moderate increase in dietary fat (45% of total energy from fat, mainly saturated fat) and a short period of physical inactivity (60 h) have been shown to interact to decrease insulin sensitivity. The short duration of intervention was done to focus on acute metabolic effects rather than changes in body composition. Our experiment suggests an interaction between short-term bed rest and saturated fat, causing a significant ~25% reduction in insulin sensitivity. In con-

trast, physical inactivity associated with high-carbohydrate diet or physical activity associated with high–saturated fat diet failed to lead to a similar deleterious effect. This indicates a protective effect of even a moderate amount of physical activity on fat-induced insulin resistance.

In the past decade, several studies have focused on the relation between IMCL content and insulin resistance (7–10). Most have shown, both in humans and in animals, a negative correlation between IMCL content and insulin sensitivity. Increased IMCL concentrations have been demonstrated in obesity and type 2 diabetes, and weight loss has been shown to decrease IMCL concentrations (36,37). In the current study, we observed an increase in IMCL concentrations of similar magnitude in the three conditions tested (although the increase after PA-HF fell short of statistical significance). Moreover, the increases observed after high-fat diet (with or without physical inactivity) tended to be larger than those observed after physical inactivity with high-carbohydrate intake. This appears to be consistent with the observation of Décombaz et al. (11,38) that IMCL stores are rapidly replenished by dietary fat after exercise-mediated depletion. However, the increased IMCL content after the BR-HCHO condition indicates that inactivity also exerts an effect on IMCL metabolism, presumably by decreasing muscle lipid oxidation.

Surprisingly, there was no correlation between IMCL content of the vastus intermedius muscle and insulin sensitivity in healthy subjects in these short-term conditions. It is not the first time that such a dissociation has been observed; it is well documented that endurance athletes have high insulin sensitivity despite increased IMCL levels (39). Along the same line, insulin sensitivity is improved in rats overexpressing hepatic malonyl-CoA decarboxylase by a mechanism that does not require lowering of muscle triglycerides or long-chain fatty acyl-CoA despite increased IMCL content (40). Furthermore, in a recent study, Pruchnic et al. (41) reported significant increases in both IMCL contents and muscle oxidative capacity in elderly volunteers who took part in a 12-week endurance training program. Together, these observations clearly indicate that high IMCL contents alone are not directly responsible for insulin resistance, but may rather indicate concomitant changes in intracellular lipid metabolites (42). It can be speculated that an imbalance between the release of fatty acid from IMCL and muscle fatty-acid oxidation during physical inactivity reduce insulin sensitivity through an accumulation of lipid metabolites but that such an imbalance is not reflected by IMCL concentrations.

It has also been proposed that both insulin sensitivity and IMCL concentrations are modulated by adiponectin. We therefore considered the possibility that adiponectin may mediate the effects of bed rest with a high-fat diet on insulin sensitivity. We did not, however, find any differences in preclamp adiponectinemia in the three studies. Moreover, no correlation was observed between preclamp adiponectinemia and IMCL or GIR. This is in accordance with a previous report by Thamer et al. (18) that indicated that adiponectinemia was not related to IMCL concentrations in soleus muscle of humans fed a high-fat diet for 3 days.

In summary, the present study demonstrated that high–saturated fat intake associated with physical inactivity after a period as short as 60 h is sufficient to significantly impair insulin sensitivity in healthy subjects. However, physical inactivity with a high-carbohydrate diet or physical activity with a high–saturated fat diet did not significantly impair insulin sensitivity. Thus, our results suggest that even mild physical activity may confer protection against the deleterious effects of a high-fat diet. The insulin resistance induced by short-term high-fat feeding and physical inactivity does not, however, appear to be directly related to changes in IMCLs.

Acknowledgments— This work was supported by grants 32-67787.02 (to L.T.) and 3100-065315.01 (to C.B.) from the Swiss National Science Foundation, and from la Fondation romande pour la recherché sur le diabète (to L.T).

We thank the staff of the Département de Physiologie de Lausanne and of the Magnetic Resonance Spectroscopy Unit of Bern for their excellent assistance. The authors are very indebted to Eric Ravussin for his critical review of this manuscript.

This study was presented as an abstract at the annual scientific meeting of the North American Association for the Study of Obesity, Las Vegas, Nevada, 14–18 November 2004.

References

1. Marshall JA, Hoag S, Shetterly S, Hamman RF: Dietary fat predicts conversion from impaired glucose tolerance to NIDDM: the San Luis Valley Diabetes Study. *Diabetes Care* 17:50–56, 1994
2. Wareham NJ, Wong MY, Day NE: Glucose intolerance and physical inactivity: the relative importance of low habitual energy expenditure and cardiorespiratory fitness. *Am J Epidemiol* 152:132–139, 2000
3. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997
4. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
5. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97:2859–2865, 1996
6. Thiebaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jequier E, Felber JP: Effect of long chain triglyceride infusion on glucose metabolism in man. *Metabolism* 31:1128–1136, 1982
7. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46:983–988, 1997
8. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42:113–116, 1999
9. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU: Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48:1113–1119, 1999
10. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H–13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606, 1999
11. Décombaz J, Fleith M, Hoppeler H, Kreis R, Boesch C: Effect of diet on the replenishment of intramyocellular lipids after exercise. *Eur J Nutr* 39:244–247, 2000
12. Helmrich SP, Ragland DR, Leung RW, Paffenbarger RS Jr: Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. *N Engl J Med*

325:147–152, 1991
13. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
14. Howald H, Hoppeler H, Claassen H, Mathieu O, Straub R: Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflugers Arch* 403:369–376, 1985
15. Suter E, Hoppeler H, Claassen H, Billeter R, Aebi U, Horber F, Jaeger P, Marti B: Ultrastructural modification of human skeletal muscle tissue with 6-month moderate-intensity exercise training. *Int J Sports Med* 16:160–166, 1995
16. Tremblay A, Simoneau JA, Bouchard C: Impact of exercise intensity on body fatness and skeletal muscle metabolism. *Metabolism* 43:814–818, 1994
17. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
18. Thamer C, Machann J, Tschritter O, Haap M, Wietek B, Dahl D, Bachmann O, Fritsche A, Jacob S, Stumvoll M, Schick F, Haring HU: Relationship between serum adiponectin concentration and intramyocellular lipid stores in humans. *Horm Metab Res* 34:646–649, 2002
19. Durnin JV, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32:77–97, 1974
20. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
21. Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O: Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 29:158–167, 1993
22. Boesch C, Slotboom J, Hoppeler H, Kreis R: In vivo determination of intra-myocellular lipids in human muscle by means of localized 1H-MR-spectroscopy. *Magn Reson Med* 37:484–493, 1997
23. Boesch C, Décombaz J, Slotboom J, Kreis R: Observation of intramyocellular lipids by means of 1H magnetic resonance spectroscopy. *Proc Nutr Soc* 58:841–850, 1999
24. Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630–E639, 1981
25. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG: Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322:223–228, 1990
26. Radziuk J, McDonald TJ, Rubenstein D, Dupre J: Initial splanchnic extraction of ingested glucose in normal man. *Metabolism* 27:657–669, 1978
27. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA: Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 72:1737–1747, 1983
28. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T, Loviscach M, Stumvoll M, Claussen CD, Schick F, Haring HU, Jacob S: Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 50:2579–2584, 2001
29. Yoshioka S, Uemura K, Tamaya N, Tamagawa T, Miura H, Iguchi A, Nakamura J, Hotta N: Dietary fat-induced increase in blood pressure and insulin resistance in rats. *J Hypertens* 18:1857–1864, 2000
30. Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq FR, Schnure JJ: Glucose intolerance during decreased physical activity in man. *Diabetes* 21:101–107, 1972
31. Lipman RL Schnure JJ, Bradley EM, Lecocq FR: Impairment of peripheral glucose utilization in normal subjects by prolonged bed rest. *J Lab Clin Med* 76:221–230, 1970
32. Mikines KJ, Dela F, Tronier B, Galbo H: Effect of 7 days of bed rest on dose-response relation between plasma glucose and insulin secretion. *Am J Physiol* 257: E43–E48, 1989
33. Mikines KJ, Richter EA, Dela F, Galbo H: Seven days of bed rest decrease insulin action on glucose uptake in leg and whole body. *J Appl Physiol* 70:1245–1254, 1991
34. Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C: Fuel homeostasis during physical inactivity induced by bed rest. *J Clin Endocrinol Metab* 85:2223–2233, 2000
35. Stuart CA Shangraw RE, Prince MJ, Peters EJ, Wolfe RR.: Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism* 37:802–806, 1988
36. Perseghin G, Lattuada G, Danna M, Sereni LP, Maffi P, De Cobelli F, Battezzati A, Secchi A, Del Maschio A, Luzi L: Insulin resistance, intramyocellular lipid content, and plasma adiponectin in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab* 285:E1174–E1181, 2003
37. Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E: Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* 51:144–151, 2002
38. Décombaz J, Schmitt B, Ith M, Decarli B, Diem P, Kreis R, Hoppeler H, Boesch C: Postexercise fat intake repletes intramyocellular lipids but no faster in trained than in sedentary subjects. *Am J Physiol Regul Integr Comp Physiol* 281:R760–R769, 2001
39. Goodpaster BH, He J, Watkins S, Kelley DE: Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86:5755–5761, 2001
40. An J, Muoio DM, Shiota M, Fujimoto Y, Cline GW, Shulman GI, Koves TR, Stevens R, Millington D, Newgard CB: Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. *Nat Med* 10:268–274, 2004
41. Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C, Goodpaster BH: Exercise training increases intramyocellular lipid and oxidative capacity in older adults. *Am J Physiol Endocrinol Metab* 287:E857–E862, 2004
42. Kim JK, Gimeno RE, Higashimori T, Kim HJ, Choi H, Punreddy S, Mozell RL, Tan G, Stricker-Krongrad A, Hirsch DJ, Fillmore JJ, Liu ZX, Dong J, Cline G, Stahl A, Lodish HF, Shulman GI: Inactivation of fatty acid transport protein 1 prevents fat-induced insulin resistance in skeletal muscle. *J Clin Invest* 113:756–763, 2004