

# Effects of supplemented isoenergetic diets differing in cereal fiber and protein content on insulin sensitivity in overweight humans<sup>1–3</sup>

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## ABSTRACT

**Background:** Despite their beneficial effects on weight loss and blood lipids, high-protein (HP) diets have been shown to increase insulin resistance and diabetes risk, whereas high-cereal-fiber (HCF) diets have shown the opposite effects on these outcomes.

**Objective:** We compared the effects of isoenergetic HP and HCF diets and a diet with moderate increases in both cereal fibers and dietary protein (Mix diet) on insulin sensitivity, as measured by using euglycemic-hyperinsulinemic clamps with infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose.

**Design:** We randomly assigned 111 overweight adults with features of the metabolic syndrome to 1 of 4 two-phased, 18-wk isoenergetic diets by group-matching. Per 3-d food protocols, the percentages of energy derived from protein and carbohydrates and the intake of cereal fiber per day, respectively, were as follows—after 6 wk: 17%, 52%, and 14 g (control); 17%, 52%, and 43 g (HCF); 28%, 43%, and 13 g (HP); 23%, 44%, and 26 g (Mix); after 18 wk: 17%, 51%, and 15 g (control); 17%, 51%, and 41 g (HCF); 26%, 45%, and 14 g (HP); and 22%, 46%, and 26 g (Mix). Eighty-four participants completed the study successfully and were included in the final analyses. Adherence was supported by the provision of tailored dietary supplements twice daily in all groups.

**Results:** Insulin sensitivity expressed as an M value was 25% higher after 6 wk of the HCF diet than after 6 wk of the HP diet (subgroup analysis:  $4.61 \pm 0.38$  compared with  $3.71 \pm 0.36 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P = 0.008$ ; treatment  $\times$  time interaction:  $P = 0.005$ ). Effects were attenuated after 18 wk (treatment  $\times$  time interaction:  $P = 0.054$ ), which was likely explained by lower adherence to the HP diet. HP intake was associated with a tendency to increased protein expression in adipose tissue of the translation initiation factor serine-kinase-6-1, which is known to mediate amino acid-induced insulin resistance. Biomarkers of protein intake indicated interference of cereal fibers with dietary protein absorption.

**Conclusion:** Greater changes in insulin sensitivity after intake of an isoenergetic HCF than after intake of an HP diet might help to explain the diverse effects of these diets on diabetes risk. This trial is registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT00579657. *Am J Clin Nutr* 2011;94:459–71.

## INTRODUCTION

High energy intake, a sedentary lifestyle, and genetic predisposition can lead to weight gain and an increased risk of the

development of insulin resistance and type 2 diabetes (1). It is less clear whether the macronutrient composition of diets per se also plays a major role in this context (2). High-protein (HP) diets have a beneficial effect on weight loss, body composition, and blood lipids (3). However, a high protein intake in past decades (4) has also been paralleled by an epidemic of type 2 diabetes (5), and a recent prospective cohort study in 38,084 participants with a 10-y follow-up suggests that consumption of 5% of energy from both red meat or total protein at the expense of carbohydrates or fat increases diabetes risk as much as 30% (6). Moreover, HP diets are typically low in cereal fiber, whereas high-fiber diets and particularly diets high in cereal fiber (HCF) are consistently associated with a marked 20–30% reduction in diabetes risk (7, 8) after correction for confounders. Although it is likely that various

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other factors are involved, the observation that HP and HCF diets may diversely influence diabetes risk (9) deserves further investigation.

Impaired glucose uptake by peripheral tissues, resulting from insulin resistance, is one of the earliest defects responsible for type 2 diabetes development (10, 11). Several cross-sectional studies have shown improved surrogate estimates of insulin sensitivity with HCF diets (12, 13) and reduced insulin sensitivity with HP diets (14). However, no causal relations can be derived from epidemiologic observations, and most interventions investigating the metabolic effects of HCF or HP diets have reported estimates of glucose metabolism, such as fasting glucose and insulin concentrations, glycated hemoglobin, or indexes of insulin sensitivity (2, 15–19), rather than measuring insulin sensitivity by using gold-standard methods. Of the few relatively small and/or short-term interventions using euglycemic-hyperinsulinemic clamps and/or stable-isotope techniques for the measurement of whole-body and hepatic insulin sensitivity, we have shown greater whole-body insulin sensitivity after an HCF than after a control diet, with a difference in cereal fiber intake of  $>30$  g/d (20); two 6-wk interventions using smaller differences of 10 to 12 g cereal fiber/d reported diverse outcomes (21, 22); one study showed better whole-body insulin sensitivity after a diet high mainly in cereal fiber than after a diet low in fiber (control), but differences in the fat contents of the diets may have influenced the results (23); and one study in 6 participants per group showed no significant effects of an HP diet on insulin sensitivity and improved insulin sensitivity with a high-carbohydrate diet (24). Importantly, in none of the previous intervention studies were the metabolic effects of HCF and HP diets compared directly. Furthermore, failure to achieve dietary targets is a very common phenomenon in longer-term nutritional interventions (2, 25). Thus, we designed a trial to investigate whether supplemented isoenergetic diets varying in protein and cereal fiber but with comparable fat contents affect whole-body and hepatic insulin sensitivity, using various instruments that increased the likelihood that dietary targets were reached.

## SUBJECTS AND METHODS

### Participants

Participants were recruited from the ongoing Metabolic-Syndrome-Berlin-Potsdam Study cohort (currently  $n = 2700$ ) (26). The Ethics Committee of the University of Potsdam approved the study (BMBF FKZ 0313826). All participants had given written informed consent. Absolute values of the baseline variables of the entire study population are given in **Table 1**.

### Protocol

This was a randomized, controlled, parallel-group 18-wk nutritional intervention in 111 group-matched overweight participants with features of the metabolic syndrome. The protocol was designed to investigate whether supplemented isoenergetic HP and HCF diets affect insulin sensitivity if adhered to and after exclusion of known confounders, such as changes in body weight, physical activity, or intake of drugs known to affect insulin sensitivity. The study was divided into 2 phases: an initial 6-wk isoenergetic period with intense dietary advice, followed by an additional 12-wk period during which participants were en-

couraged to continue with their respective diets, including the intake of tailored dietary supplements, but no further dietary advice. We further included a control group with a diet as typically emphasized in current nutritional recommendations (27) and a group with more moderate increases in both cereal fibers and dietary protein (Mix). Participants in all dietary groups received tailored dietary supplements (drink powders and baking mixes; compositions are outlined below and in **Table 2**) twice daily in an effort to increase adherence to the respective dietary targets. The study was conducted between August 2007 and March 2010.

### Outcome measures

Primary outcomes were the effects of isoenergetic HP compared with HCF diets on insulin sensitivity after 6 and 18 wk. Secondary outcomes were changes in factors that may contribute to diet-induced alterations in insulin sensitivity, such as changes in protein expression in adipose tissue, body fat distribution and liver fat, adipokines, and inflammatory markers.

### Inclusion and exclusion criteria

To be eligible, participants had to be 24–70 y of age, be overweight [body mass index (BMI; in  $\text{kg}/\text{m}^2$ )  $>25$ ;  $n = 45$ ] or obese (BMI  $>30$ ;  $n = 66$ ), have a waist circumference  $>80$  cm in females and  $>94$  cm in males, and have at least one more feature of the metabolic syndrome according to International Diabetes Federation criteria (28); 101 of the participants fulfilled the criteria for the metabolic syndrome. All participants were characterized by using oral-glucose-tolerance tests. The major exclusion criteria were diabetes; pregnancy; diseases of the heart, liver, or kidneys; allergies, including food allergies; and metal implants. According to predefined criteria (clinicaltrials.gov number NCT00579657), participants that had a relevant change in body weight of  $>3$  kg during the first 6 wk ( $n = 3$ : control,  $n = 1$ ; HCF,  $n = 1$ ; HP,  $n = 0$ ; Mix,  $n = 1$ ), a significant deviation from dietary targets during the 6-wk isoenergetic period ( $n = 6$ : control,  $n = 0$ ; HCF,  $n = 2$ ; HP,  $n = 2$ ; Mix,  $n = 2$ ), or an intake of drugs with known effects on insulin sensitivity (eg, cortisone, acetylsalicylic acid, or antibiotics) ( $n = 5$ : control,  $n = 1$ ; HCF,  $n = 2$ ; HP,  $n = 2$ ; Mix,  $n = 0$ ) were excluded from the analyses. Seven participants did not attend the study day after 6 wk ( $n = 7$ : control,  $n = 2$ ; HCF,  $n = 3$ ; HP,  $n = 1$ ; Mix,  $n = 1$ ), and 1 participant provided no data from food protocols and food-frequency questionnaires (FFQs) ( $n = 1$ : control,  $n = 0$ ; HCF,  $n = 1$ ; HP,  $n = 0$ ; Mix,  $n = 0$ ). Thus, 89 participants were eligible for analysis after 6 wk (overweight,  $n = 39$ , obese,  $n = 50$ ; metabolic syndrome,  $n = 81$ ). Only 5 participants dropped out between 6 and 18 wk (3 participants did not attend after 18 wk ( $n = 3$ : control,  $n = 1$ ; HCF,  $n = 1$ ; HP,  $n = 1$ ; Mix,  $n = 0$ ), 1 participant (control group) performed relevant exercise directly before the third study day, and 1 participant (Mix group) was started on metformin treatment by his general practitioner, although no diabetes was diagnosed. The flow of participants throughout the trial is depicted in **Figure 1**.

### Blinding

Participants were randomly allocated to 1 of the 4 diets and were not told which diet they would consume. All 4 diets were

**TABLE 1**  
Baseline characteristics of the study participants<sup>1</sup>

Characteristics of participants	Control	HCF	HP	Mix	P value <sup>2</sup>
Matched variables					
Age (y)	54.6 ± 8.4 <sup>3</sup>	52.8 ± 11.8	55.3 ± 10.4	55.2 ± 7.7	0.74
Sex (n)					0.98
Female	17	17	18	16	
Male	11	11	10	11	
Waist circumference (cm)	102.1 ± 8.7	99.0 ± 8.3	101.2 ± 11.5	100.0 ± 12.7	0.70
BMI (kg/m <sup>2</sup> )	31.4 ± 3.3	31.4 ± 2.9	31.5 ± 4.0	31.0 ± 3.7	0.96
Use of lipid-lowering and/or antihypertensive drugs (n)	14	13	14	14	0.72
Additional characteristics					
Height (m)	1.68 ± 0.09	1.69 ± 0.10	1.68 ± 0.11	1.68 ± 0.08	0.93
Weight (kg)	88.4 ± 11.8	90.1 ± 12.7	89.1 ± 16.6	87.9 ± 15.1	0.95
Fat mass (kg)	36.2 ± 8.1	36.8 ± 7.6	34.8 ± 9.4	34.9 ± 11.7	0.82
Lean mass (kg)	52.1 ± 12.0	52.2 ± 10.7	54.2 ± 12.3	52.9 ± 9.5	0.92
VAT (L)	4.4 ± 1.7	3.7 ± 2.0	4.4 ± 2.0	4.6 ± 2.8	0.51
SCAT <sub>a</sub> (L)	16.2 ± 4.5	16.8 ± 3.6	15.8 ± 5.8	15.2 ± 6.7	0.74
Liver fat (%)	8.7 ± 10.7	7.1 ± 9.0	5.9 ± 5.7	9.4 ± 11.4	0.77
Insulin sensitivity					
M value (mg · kg <sup>-1</sup> · min <sup>-1</sup> )	4.38 ± 1.68	4.42 ± 1.65	4.21 ± 1.70	4.49 ± 1.79	0.95
Fasting EGP (mg · kg <sup>-1</sup> · min <sup>-1</sup> )	1.62 ± 0.14	1.61 ± 0.16	1.59 ± 0.18	1.56 ± 0.18	0.76
Glucose metabolism <sup>4</sup> (n)					0.40
NGM	14	17	11	15	
IFG	9	9	10	6	
IGT	0	0	3	2	
IFG+IGT	5	2	4	4	
Blood pressure (mm Hg)					
Systolic	140 ± 14	143 ± 20	134 ± 18	137 ± 14	0.27
Diastolic	92 ± 11	93 ± 14	90 ± 10	89 ± 11	0.69
RQ	0.80 ± 0.10	0.76 ± 0.06	0.79 ± 0.07	0.79 ± 0.07	0.34
REE (kcal/d)	1462 ± 237	1488 ± 278	1520 ± 319	1428 ± 248	0.64
Cholesterol (mmol/L)					
Total	5.2 ± 1.1	5.4 ± 1.4	5.2 ± 1.1	5.5 ± 0.8	0.65
HDL	1.3 ± 0.3	1.4 ± 0.4	1.2 ± 0.3	1.4 ± 0.3	0.10
LDL	3.3 ± 0.9	3.6 ± 1.2	3.3 ± 0.8	3.5 ± 0.7	0.64
Triglycerides (mmol/L)	1.1 ± 0.4	1.1 ± 0.4	1.5 ± 1.0	1.2 ± 0.8	0.92
Free fatty acids (mmol/L)	0.6 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.6 ± 0.2	0.55
Hb A <sub>1c</sub> (%)	5.0 ± 0.3	5.1 ± 0.4	5.1 ± 0.3	5.2 ± 0.3	0.45
Plasma glucose (mg/dL)	87.9 ± 8.4	83.6 ± 7.3	84.9 ± 6.9	85.5 ± 6.5	0.18
Plasma insulin (mU/L)	8.9 ± 4.0	8.8 ± 4.1	9.3 ± 3.5	10.0 ± 7.7	0.83
Serum adiponectin (μg/mL)	12.5 ± 6.8	14.4 ± 10.0	13.3 ± 6.2	11.4 ± 5.6	0.51
Serum leptin (ng/mL)	18.7 ± 9.9	20.2 ± 13.6	17.1 ± 10.5	19.4 ± 15.9	0.82
Biomarkers for adherence					
Urinary N:C ratio	8.4 ± 0.5	8.0 ± 0.4	8.2 ± 0.9	7.8 ± 0.5	0.88
Fecal isovalerate (mmol/L)	3.5 ± 0.6	2.7 ± 0.3	3.3 ± 0.3	2.9 ± 0.3	0.57
Breath hydrogen (ppm)	8.6 ± 1.5	14.1 ± 3.3	7.5 ± 0.9	11.3 ± 2.6	0.16

<sup>1</sup> n = 111 participants. HCF, diet high in cereal fiber; HP, diet high in protein; Mix, diet moderately high in both cereal fiber and protein; VAT, visceral adipose tissue; SCAT<sub>a</sub>, subcutaneous abdominal adipose tissue; Hb A<sub>1c</sub>, glycated hemoglobin; EGP, endogenous glucose production; NGM, normal glucose metabolism; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; M value, insulin-mediated glucose uptake as a measurement of whole-body insulin sensitivity; RQ, respiratory quotient; REE, resting energy expenditure; N:C, nitrogen to creatinine. Differences between groups were analyzed by using one-factor ANOVA.

<sup>2</sup> P value = treatment × time interaction.

<sup>3</sup> Mean of absolute values ± SD (all such values).

<sup>4</sup> Data derived from an oral-glucose-tolerance test.

based on foods derived from plants and vegetables. In the HP groups, legumes and dairy products were emphasized, and high-fat animal-protein sources were restricted. Tailored dietary supplements for consumption twice daily in all groups were provided in counted prepackaged single portions for all groups, at weeks 0, 3, 6, and 12. Supplements were not distinguishable regarding taste, consistency, and visual appearance (*see* Online

Supporting Material under “Supplemental data” in the online issue). Although participants with some nutritional knowledge might have been aware that the protein content was of importance, distinguishing between diets was difficult. Investigators and staff who measured metabolic outcomes and performed analyses on biomarkers were unaware of the diet assignment of the participants.

**TABLE 2**Example of diets in the 4 intervention groups<sup>1</sup>

	Control <sup>2</sup>	HCF	HP	Mix
Macronutrient content (% of energy)				
Carbohydrates	55	55	40–45	45–50
Protein	15	15	25–30	20–25
Fat	30	30	30	30
Cereal fiber	Not emphasized	Emphasized	Not emphasized	Emphasized
Emphasized foods <sup>3</sup>	Bread (both refined and whole-meal bread products), fruit, legumes, pasta, potatoes, rice (both polished and brown rice products), vegetables	Brown rice, fruit, legumes, potatoes (preferably unpeeled boiled), vegetables, whole-meal bread, whole-meal cereal, whole-meal pasta	Legumes, low-fat dairy products (low-fat milk, cheese, yogurt, low-fat curd cheese), low-fat meat (especially poultry) and fish, low-fat sausages (eg, ham, chicken breast), vegetables	Fruit, legumes, low-fat dairy products (low-fat milk, cheese, yogurt, curd cheese), pasta, polished rice, potatoes, vegetables, whole-meal bread, wheat and rye bread, whole-meal cereal
Moderate intake	Low-fat dairy products (milk, cheese, yogurt, curd cheese), low-fat sausages (eg, ham, chicken breast), low-fat meat (poultry, fish)	Low-fat dairy products (milk, cheese, yogurt, curd cheese), low-fat sausages (eg, ham, chicken breast)	Fruit, pasta, polished rice, potatoes, wheat and rye bread	Low-fat meat (poultry, fish), low-fat sausages (eg, ham, chicken breast)
Restricted	High-fat meat and sausages (eg, salami, pork sausage, roast pork), jam, honey, spread (butter, margarine), sweets, snacks	High-fat meat and sausages (eg, salami, pork sausage, roast pork), low-fat meat (poultry, fish), spread (butter, margarine), sweets, snacks	High-fat meat and sausages (eg, salami, pork sausage, roast pork), jam, honey, spread (butter, margarine), sweets, snacks	High-fat meat and sausages (eg, salami, pork sausage, roast pork), jam, honey, spread (butter, margarine), sweets, snacks
Emphasized beverages	Water, tea, coffee	Water, tea, coffee	Water, tea, coffee	Water, tea, coffee
Calculated energy intake from 3-d food protocols after 6 wk (kcal/d) <sup>4</sup>	2007 ± 101	1963 ± 108	1971 ± 112	2037 ± 77
Supplements	Basic supplement (carbohydrates, protein, fat, and cereal fiber): 2 × 28, 8, 3, and 1 g	Basic supplement enriched with 2 × 15 g cereal fiber extract (carbohydrates, protein, fat, and cereal fiber): 2 × 25, 8, 3, and 15 g	Basic supplement enriched with 2 × 29 g protein isolates of whey and peas (carbohydrates, protein, fat, and cereal fiber): 2 × 25, 29, 3, and 1 g	Basic supplement enriched with 2 × 8 g from cereal fiber and 2 × 19 g protein isolates of whey and peas (carbohydrates, protein, fat, and cereal fiber): 2 × 24, 19, 3, and 8 g

<sup>1</sup> Participants in all dietary groups received individual one-on-one dietary advice in the metabolic unit at weeks 0, 3, and 6 of the intervention, supported by additional telephone recalls at week 1. Daily food-frequency questionnaires were provided for the first 42 d, with direct analysis and feedback provided to the participants at weeks 3 and 6. Three-day food protocols were analyzed at weeks 0, 6, 12, and 18. Body weight and analyses of food protocols were used as markers for energy intake with direct analysis and feedback provided to the participants at weeks 3 and 6. Biomarkers of dietary adherence were used for protein and fermentable fiber intakes. Tailored dietary supplements were used in all groups as an additional tool to support the achievement of dietary targets. HCF, diet high in cereal fiber; HP, diet high in protein; Mix, diet moderately high in both cereal fiber and protein.

<sup>2</sup> Participants in the control group were instructed to consume a healthy low-fat, moderate-protein, and high-carbohydrate diet, as usually emphasized in current nutritional recommendations (27). Fiber-rich foods in the control group were not restricted, although the intake of foods high in insoluble cereal fiber was not particularly emphasized.

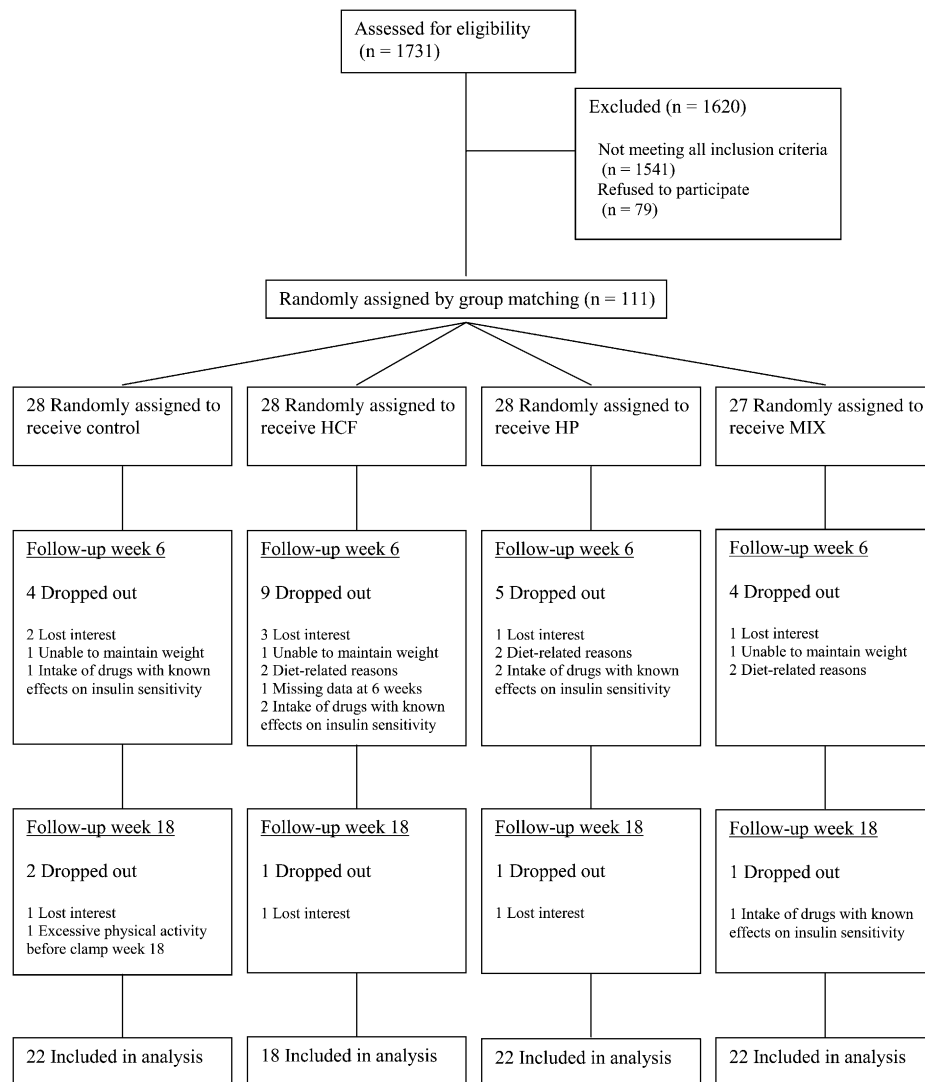
<sup>3</sup> Given in alphabetical order.

<sup>4</sup> Calculated energy intake includes the consumption of dietary supplements twice daily in all 4 groups. There were no differences in energy intake between groups (ANOVA, treatment × time interaction:  $P = 0.96$ ).

## Physical activity

The individual physical activity of the participants was quantified by a translated questionnaire, as described previously (26). None of the overweight or obese participants of this study were involved in major exercise. The mean age of the participants was  $54.5 \pm 9.6$  y (women:  $53.9 \pm 9.4$  y). Because physical activity is known to exert significant effects on whole-body insulin sensitivity, even in the short-term, participants were instructed to continue their usual level of exercise throughout the study and that it was crucial to inform the dietitians if there were any relevant change in their exercise level during the study (29). Details of any

uncommon physical activities or exercising habits were ascertained from each participant at telephone recalls and at each of the individual sessions in the metabolic unit, particularly before each measurement of insulin sensitivity. Only 2 of the participants needed to be excluded for involvement in relevant physical activity, with 1 (HCF group) having performed bicycling (8 km) directly before the third study day (see inclusion and exclusion criteria). Another participant (HCF group), who was unemployed at the time of inclusion, commenced work as a builder during the study and had a weight loss of  $>3$  kg ( $-8.4$  kg), thus meeting 2 exclusion criteria.



**FIGURE 1.** Flow of participants throughout the trial. HCF, diet high in cereal fiber; HP, diet high in protein; MIX, diet moderately high in both cereal fiber and protein.

### Dietary interventions

Participants were randomly allocated to 1 of 4 isoenergetic diets using a computerized group-matching algorithm ensuring homogeneity of the main variables, such as age, sex, waist circumference, BMI, and drug intake. (For details, *see* Online Supporting Material under “Supplemental data” in the online issue.) All diets were based on assumed healthy foods, such as vegetables, legumes, cereal fiber, fruit, and dairy products. The targeted percentages of energy derived from protein and carbohydrates and intake of cereal fiber per 1000 kcal/d were set at 15%, 55%, and <15 g (control); 15%, 55%, and >20 g (HCF); 25–30%, 40–45%, and <15 g (HP); and 20–25%, 45–50%, and 15–20 g (Mix). The goal for fat intake was 30% of energy in all groups.

Participants completed food records for 3 consecutive days, including 1 weekend day before the study and after 6, 12, and 18 wk. During the first 6 wk of the intervention, participants weighed all their foods whenever possible and/or supplied information on brand names, cooking, processing, and household measures. FFQs offering a choice of 84 commonly consumed food items suitable for achieving the respective targets

were provided daily for the first 42 d of the intervention, and the participants received feedback at weeks 3 and 6. All foods noted were coded to foods listed in country-specific food databases. Dietary records were analyzed by using PRODI-4.5-expert software (Nutriscience, Stuttgart, Germany), based on Bundeslebensmittelschlüssel (BLS version II3), which includes nutrient details of ≈11,400 foods and food preparations (30). FFQs and 3-d food protocols showed high correlations (carbohydrate intake:  $R = 0.836$ ,  $P < 0.0001$ ; protein-intake:  $R = 0.851$ ,  $P < 0.0001$ ).

Each participant's energy needs for isoenergetic conditions were calculated from the person's resting energy expenditure (REE) and his or her physical activity level (PAL). Participants of all dietary groups received individual dietary advice in both one-on-one and group sessions in the metabolic unit at weeks 0, 3, and 6, which was supported by additional telephone recalls at week 1. Participants were encouraged to contact the nutritionists at any time for further advice. Recommended macronutrient and energy intakes were adjusted after 3 wk by using the information gained from food protocols and from weighing the participants. Typical foods emphasized are shown in Table 2.

## Dietary supplements

As an additional tool to support the achievement of dietary targets, all participants were provided with supplements for consumption twice daily over 18 wk, which were given in 4 lots (week 0, week 3, week 6, and week 12). The supplements were counted, and the exact amount needed was given until the next scheduled appointment in the metabolic unit. The supplement provided to the control group was based on a low-fiber grain mixture, which also served as carrier for the protein- and/or cereal fiber-enriched supplements in the other intervention groups. The HCF dietary supplements were enriched with  $2 \times 15$ -g insoluble cereal fiber extracts, the HP dietary supplements contained  $2 \times 29$ -g isolates from whey and pea proteins, and the supplements provided to the Mix groups contained  $2 \times 8$ -g cereal fiber extracts and  $2 \times 19$ -g protein isolates from whey and peas. Details about the cereal fiber and protein extracts are given below. All supplements were produced in one batch, as coordinated by the Institute for Cereal Processing (Potsdam, Germany), which also performed an analysis of the macro- and micronutrient contents of the supplements and sensory tests. Anona N  hrmittel Inc (Colditz, Germany) was responsible for the production and packaging of the drinking powders. Kathi Rainer Thiele Inc (Halle, Germany) was responsible for the production and packaging of the baking mixes. All supplements were provided in prepackaged single-portion sachets, both for drinking powders and baking mixes. Portions were mixed with 200 mL cold low-fat milk (1.5%) in a purpose-made shaker that was provided for all participants. For the drinking powders produced for the dietary intervention, participants in all groups had a choice between 5 flavors (banana, caramel, chocolate, vanilla, and white coffee). For preparing the pancakes, 120 mL low-fat milk (fat content: 1.5%; energy and macronutrient contents per 100 mL: 48 kcal, 4.9 g carbohydrates, 3.4 g protein, and 1.6 g fat) was used. Participants were provided instructions on how to use both drinking powders and baking mixes throughout the intervention, ideally in a 1:1 ratio. The visual appearance of the drinking powders and baking products is shown elsewhere (*see* Online Supporting Material under “Supplemental data” in the online issue).

### *Cereal fiber extracts for the HCF and Mix diets*

For the enrichment of supplements with cereal fibers, we used a purified fiber extract derived from oat hulls, which contained 70% cellulose, 25% hemicelluloses, and 3–5% lignin (Vitacel HF101; Rettenmayr & Soehne Inc, Holzm  hle, Germany), as used in previous interventions studies both in humans and mouse models (20, 31, 32). Fiber extracted from oat hulls made up  $\approx 60\%$  of the total fiber intake in the HCF group and  $\approx 50\%$  of the total fiber intake in the Mix group. Most of the soluble fiber content, including  $\beta$ -glucans, starch, proteins, and lipids, was removed during the preparation of these products. Details about the processing steps were published previously (32).

### *Protein isolates for the HP and Mix diets*

For the enrichment of the HP and Mix supplements with protein, we used a mixture of 70% whey protein isolate (arla biolac Inc, Harnbarnsen, Germany) and 30% pea protein isolates (Pisane F9; Breuer GmbH, K  nigstein, Germany), with an amino acid composition emphasizing leucine and isoleucine and

restricting methionine, which was assumed to have beneficial metabolic effects (33–36). Details about the analysis of the proteinogenic amino acid contents in the dietary supplements and of the amino acid compositions of the supplements in the 4 dietary intervention groups are shown elsewhere (*see* Online Supporting Material under “Supplemental data” in the online issue).

## Analyses

Apart from the baseline characteristics of the entire study population (Table 1) and the intention-to-treat (ITT) analyses, all results refer to the 84 participants who completed the study successfully and were included in the final analyses. All measurements were performed at weeks 0, 6, and 18. Biomarkers and anthropometric markers were additionally measured after 3 and 12 wk. Magnet resonance imaging and H1 spectroscopy imaging studies were performed  $\pm 1$  wk before the respective study days at weeks 0, 6, and 18. Euglycemic-hyperinsulinemic clamp results, measures of body composition, and biomarkers were analyzed in all participants on the respective study days at weeks 0, 6, and 18 ( $n = 259$  experiments,  $n = 18$ –22 participants/group and study day). Adipose tissue biopsy samples ( $n = 81$  samples,  $n = 6$ –7 participants/group and study day) and stable-isotope experiments for the measurement of hepatic insulin sensitivity ( $n = 153$  experiments,  $n = 12$ –14 participants/group and study day) were analyzed in matched subsets of participants, who agreed to these procedures on all study days. Adipose tissue biopsy samples were collected from periumbilical subcutaneous adipose tissue 120 min before the euglycemic hyperinsulinemic clamp studies began. Blood and urine samples were collected on the respective study days at weeks 0, 3, 6, 12, and 18, immediately after the participants arrived in the metabolic unit. Freshly voided fecal samples were collected  $\pm 1$  d before the respective study days, transported in air-tight plastic boxes under anaerobic conditions (AnaeroGen; Oxoid, Basingstoke, United Kingdom), and processed within 3 h after defecation.

## Metabolic markers of dietary adherence

Urinary ratios of nitrogen to creatinine (37) and fecal concentrations of isovaleric acid (38) were used as markers for protein intake. Fecal butyrate and hydrogen breath tests (20) were used as markers for fermentable fiber intake. Details about these methods are given elsewhere (*see* Online Supporting Material under “Supplemental data” in the online issue). REE was estimated by using indirect calorimetry (20).

## Measurements of insulin sensitivity

### *Euglycemic hyperinsulinemic clamps for the measurement of whole-body insulin sensitivity*

Subjects arrived at the metabolic unit between 0715 and 0830 after fasting overnight for 10 h. Consumption of any meals or drinks other than tap water was not allowed within the 12 h preceding the studies. After arrival of the subjects, 2 intravenous catheters were inserted into their contralateral forearm veins. The arm in which blood samples were drawn was kept in a warming box (65  C) throughout the studies. After administration of an insulin bolus at  $\sim 10$  min (individually adjusted according to the body surface area of the participants), euglycemic-hyperinsulinemic clamps were

performed at a constant insulin infusion rate of  $40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Clamps were performed for  $\geq 2 \text{ h}$  until steady state conditions were achieved, defined as stable glucose infusion rates (GIRs) over  $\geq 30 \text{ min}$  plus stable plasma glucose concentrations (range:  $4.4 \pm 0.4 \text{ mmol/L}$ ). Whole-body glucose disposal [expressed as insulin-mediated glucose uptake (M value)] was calculated from the glucose infusion rate, which was constant during the last 30 min of the respective clamp periods. Blood samples were drawn at timed intervals during the clamps, immediately chilled, and centrifuged, and the supernatant fluid was stored at  $-80^\circ\text{C}$  until analyzed.

#### *Stable-isotope studies for the measurement of hepatic insulin sensitivity*

For calculation of hepatic endogenous glucose production (EGP; in  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), a primed  $[0.06 \text{ (mg)} \times \text{body weight (kg)} \times \text{fasting plasma glucose (mg/dL)}, \text{ from } -120 \text{ to } -115 \text{ min}]$ , continuous  $[0.27 \text{ (mg)} \times \text{body weight (kg)}, \text{ from } -115 \text{ to } 320 \text{ min}]$  infusion of  $[6,6\text{-}^2\text{H}_2]\text{glucose } 99\%$  (Euriso-Top, Saarbrücken, Germany) was administered. A basal period of 100 min was allowed for tracer equilibration, as described previously (39). The priming dose was adjusted to fasting glucose concentrations to avoid overestimation of glucose production rates. Rates of EGP were determined from the tracer infusion rate of  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$ , and its enrichment to the hydrogen bound to carbon 6 was divided by the mean percentage enrichment of plasma  $\text{D-}[6,6\text{-}^2\text{H}_2]\text{glucose}$ . Because both GIRs and plasma glucose concentrations were held constant during the steady state phase of the clamps, steady state equations were appropriate for the calculation of EGP (39).

#### **Other biomarkers in plasma and serum**

Routine laboratory markers were measured by using standard methods in the research laboratories of the German Institute of Human Nutrition. Glucose concentrations were measured in venous blood (ABX Pentra 400; ABX Diagnostics, Montpellier, France), and additionally, for the performance of clamp studies, in arterialized blood samples. Arterialized plasma glucose concentrations were measured immediately by using the glucose oxidase method (Super-GL glucose analyzer; Dr. Müller, Freital, Germany). Serum adiponectin was measured by using a human adiponectin enzyme-linked immunosorbent assay (Biovendor, Heidelberg, Germany; intraassay CV: 5.5%; interassay CV: 8.6%). Serum leptin was measured by using a quantitative sandwich enzyme immunoassay (Quantikine, Wiesbaden, Germany; intraassay CV: 3.2%; interassay CV: 3.5%). Plasma plasminogen activator inhibitor-1 was measured by using an enzyme-linked immunosorbent assay (IBL, Hamburg, Germany; intraassay CV: 4.7%; interassay CV: 5%). Serum interleukin-10 (IL-10) was measured by using a highly sensitive immune assay (Quantikine, Wiesbaden, Germany; intraassay CV: 6.6%; interassay CV: 8.1%). C-reactive protein was measured by using turbidimetric immunoprecipitation on an ABX Pentra 400 (ABX Diagnostics; intraassay CV: 1.6%; interassay CV: 4.3%).

#### **Measurement of nutrient-dependent signaling cascades in adipose tissue**

Adipose tissue biopsy samples were collected from abdominal subcutaneous fat by using a percutaneous needle-biopsy tech-

nique with suction, preceded by subcutaneous injection of 5 mL lidocaine 2% (Braun, Melsungen, Germany). Samples (an average of 1 to 1.5 g subcutaneous fat tissue/sample) were rinsed carefully with a sterile saline solution, immediately frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$  until analyzed.

#### **SDS-PAGE and Western blot analysis**

Adipose tissue protein extracts were prepared in RIPA-lysis buffer (Roche, Mannheim, Germany). Each 50- $\mu\text{g}$  protein sample was separated by SDS-PAGE. Western blotting was performed as described previously (40). Primary and secondary antibodies were purchased from Cell Signaling (Frankfurt, Germany): rabbit-anti human m-TOR antibody, rabbit anti-human p70S6Kinase antibody, rabbit anti-human 4E-BP1 antibody, rabbit anti-human phospho-4E-BP1 (Ser65) antibody, GAPDH(14C10) rabbit anti-human monoclonal antibody, goat anti-rabbit IgG HRP-linked antibody, and anti-biotin horseradish peroxidase-linked antibody. Immunoreactive bands were visualized by using the luminol chemiluminescent substrate Lumi-GLO (Cell Signaling). The chemiluminescence signal was quantified by using a CCD camera, and densitometric analysis was performed with Image Reader LAS-1000 ProV2.1 software (Fujifilm, Tokyo, Japan) and AIDA 2.11 Image Analyzer (Raytest, Straubenhardt, Germany). Relative protein concentrations were calculated by normalizing target protein concentrations with GAPDH signals.

#### **Measurement of body composition**

For the measurement of visceral and subcutaneous adipose fat distribution, magnetic resonance imaging was performed on a 1.5 T whole-body imager (Magnetom-Avanto, Siemens-Health Care, Germany). The lipid content in the liver (%; fat/water) was measured by localized proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ). Total body fat and lean mass were measured by using air-displacement plethysmography. The use of this method in overweight and obese persons has been validated (41). For details, see Online Supporting Material under "Supplemental data" in the online issue.

#### **Power calculation**

The power calculation was based on a predicted difference in the end-of-treatment M value between the HP and HCF groups of  $0.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , with an SD of effect of  $1.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $\alpha = 0.05$ ,  $1 - \beta = 0.80$ ) and an assumed dropout rate of 30%. We required 26 participants for each treatment.

#### **Statistical analysis**

The characteristics of the participants are given as means  $\pm$  SDs; all other data are given as means  $\pm$  SEs. Analyses of this proof-of-principle nutritional intervention were performed according to the study protocol (clinicaltrials.gov number NCT00579657) in an attempt to exclude confounding factors with known effects on insulin sensitivity that are likely to obscure diet-induced effects. Participants who reported that they were not willing or able to adhere to the respective diets were also excluded. Although not planned according to the study protocol, an additional ITT analysis was performed for the



primary outcome measures. The ITT analysis included all 111 randomized participants, with the baseline observation carried forward for all participants who did not complete the study.

For the investigation of treatment effects from 0 to 6 wk (intervention phase with intense dietary advice) and from 6 to 18 wk (intervention phase with no further dietary advice) of the dietary intervention, normally distributed data were set relative to the baseline value (week 0), and treatment  $\times$  time interactions are separately given for effects after 6 and 18 wk [one-factor analysis of variance (ANOVA) with Bonferroni adjustment for post hoc comparisons]. Data from all time points and dietary groups were included in one model. For investigation of the full model (4 dietary treatments, weeks 0–18), a mixed-model analysis for repeated measures was additionally performed for the main outcome measure. Non-normally distributed data were analyzed by using a Kruskal-Wallis test, and Mann-Whitney  $U$  tests were used for comparisons in subgroups. Longitudinal changes within groups were addressed by using a 2-tailed Student's  $t$  test for paired samples.  $P < 0.05$  was considered significant. The analyses were performed by using SPSS version 16 (SPSS Inc, Chicago, IL).

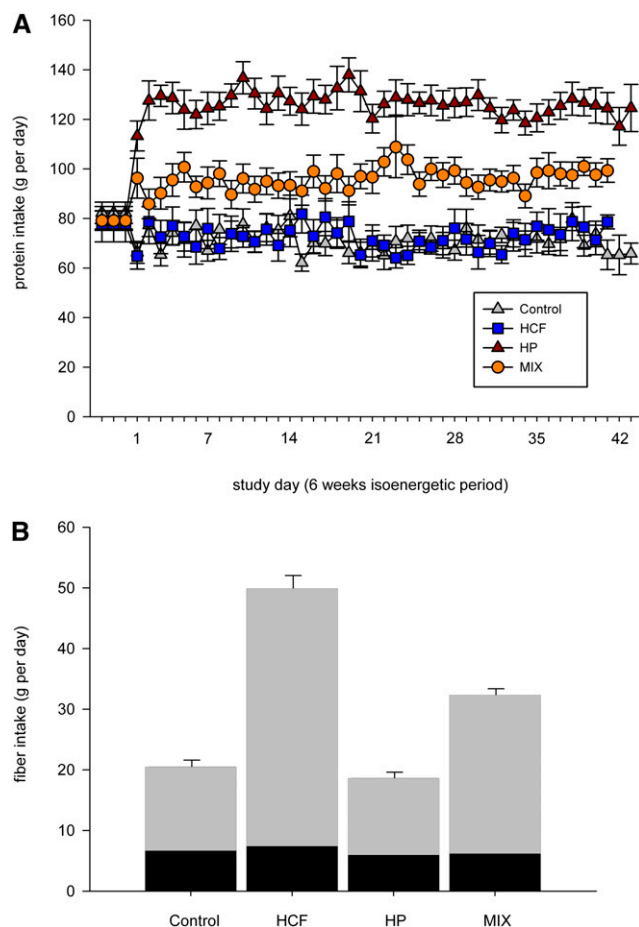
## RESULTS

### Adherence and diet acceptability

The flow of participants throughout the trial is presented in Figure 1. Energy intake (ANOVA, treatment  $\times$  time interaction:  $P = 0.96$ ), satisfaction with the diets, and dietary adherence were comparable between groups. The dropout rate was 20% (22 of 111 participants; control,  $n = 4$ ; HCF,  $n = 9$ ; HP,  $n = 5$ ; Mix,  $n = 4$ ) after 6 wk and 24% (total dropouts weeks 0–18; control,  $n = 6$ ; HCF,  $n = 10$ ; HP,  $n = 6$ ; Mix,  $n = 5$ ) after 18 wk. No serious adverse effects were observed.

Analyses of FFQs and 3-d food protocols indicated that participants were largely successful in reaching dietary targets. Data from the analysis of 3738 FFQs (daily food diaries from  $n = 89$  participants during the first 6 wk of dietary intervention) are shown in Figure 2A. Differences in protein intake between groups were balanced by modulating carbohydrate intake (control and HCF, 50–60% of energy intake; HP, 40–45% of energy intake; and Mix, 45–50% of energy intake), whereas the goal of fat intake was 30% of energy intake in all groups. No differences in fat intake were observed between groups (ANOVA, treatment  $\times$  time interaction: week 0,  $P = 0.44$ ; week 6,  $P = 0.53$ , and week 18,  $P = 0.26$ ).

Protein intake was significantly different between diets (Figure 2A; ANOVA, treatment  $\times$  time interaction,  $P < 0.0001$ , both after 6 and 18 wk). When the diets were compared separately, protein intake was comparable at week 0 and was significantly lower in the control group than in either the HP or the Mix group, both after 6 and 18 wk (subgroup analyses, after Bonferroni correction:  $P < 0.0001$  and  $P = 0.001$ , respectively). Protein intake in the control and HCF groups was comparable ( $P = 1.00$ ). Protein intake was significantly higher in the HP group than in the Mix group (6 wk:  $131 \pm 5$  compared with  $109 \pm 4$  g/d,  $P = 0.009$ ; 18 wk:  $122 \pm 4$  compared with  $94 \pm 4$  g/d,  $P = 0.001$ ). Protein intake was significantly higher in the Mix group than in either the control or the HCF group ( $P = 0.001$  for both) after 6 wk, but not after 18 wk ( $P = 1.00$  and  $P = 0.16$ , respectively).



**FIGURE 2.** Mean dietary protein (A) and fiber (B) intakes, according to diet, at 6 wk. A: Dietary protein intake during the first 6 wk of dietary intervention, according to 3738 daily food diaries ( $n = 89$  participants,  $n = 42$  study days). Protein intake was significantly different between groups after both 6 and 18 wk of dietary intervention (ANOVA, treatment  $\times$  time interaction:  $P < 0.0001$  for both). B: Total fiber intake after 6 wk of dietary intervention, according to 3-d food protocols (soluble fiber intake, black bars; insoluble fiber intake, light gray bars). HCF, diet high in cereal fiber; HP, diet high in protein; MIX, diet moderately high in both cereal fiber and protein. Insoluble fiber intake was significantly different between groups after both 6 and 18 wk of dietary intervention (ANOVA, treatment  $\times$  time interaction:  $P < 0.0001$  for both). Soluble fiber intake did not differ between dietary groups throughout the intervention (ANOVA, treatment  $\times$  time interaction: 6 wk,  $P = 0.10$ ; 18 wk,  $P = 0.31$ ). Insoluble fiber intake was significantly lower in the control group than in either the HCF or Mix group ( $P < 0.0001$ ) but did not differ between the control and HP groups ( $P = 1.00$ ). Insoluble fiber intake was significantly higher in the HCF group than in the Mix group ( $P < 0.0001$ ). Soluble fiber consumption was comparable between groups and did not change significantly during the dietary intervention.

Insoluble cereal fiber intake was comparable at week 0 (ANOVA, treatment  $\times$  time interaction:  $P = 0.71$ ), but was significantly different between diets after 6 (Figure 2B) and 18 wk (ANOVA, treatment  $\times$  time interaction:  $P < 0.0001$  for both). When the diets were compared separately, insoluble fiber intake was significantly lower in the control group than in either the HCF or the Mix group, both after 6 wk (control,  $13.8 \pm 0.8$  g/d; HCF,  $42.5 \pm 1.7$  g/d; Mix,  $26.1 \pm 0.7$  g/d;  $P < 0.0001$ ) and 18 wk (control,  $14.7 \pm 0.9$  g/d; HCF,  $40.6 \pm 1.9$  g/d; Mix,  $25.6 \pm 0.8$  g/d;  $P < 0.0001$ ), but did not differ between the control and HP groups ( $P = 1.00$  after both 6 and 18 wk). Insoluble fiber intake was significantly higher in the HCF than in the Mix group,



after both 6 and 18 wk ( $P < 0.0001$  for both). Soluble fiber consumption was comparable between groups and did not change significantly during the dietary intervention (ANOVA, treatment  $\times$  time interaction: 0 wk,  $P = 0.85$ ; 6 wk,  $P = 0.10$ ; and 18 wk,  $P = 0.31$ ).

#### Changes in markers of dietary adherence, according to diet, at 6 and 18 wk

Body weight and BMI (Table 3) remained virtually identical with all diets, which indicated that the provision of isoenergetic diets was largely successful. BMI was also unchanged during the intervention when the ITT analysis was conducted ( $n = 111$  participants; ANOVA, treatment  $\times$  time interaction: 6 wk,  $P = 0.71$ ; 18 wk,  $P = 0.23$ ).

Changes in markers of protein intake are shown in Table 3. The urinary ratio of nitrogen to creatinine as a marker for dietary protein intake and absorption was affected by the dietary intervention, after both 6 and 18 wk (ANOVA, treatment  $\times$  time interaction:  $P = 0.006$  and  $P = 0.023$ , respectively), which was driven by increases in the HP group. In the post hoc subgroup analyses, the urinary ratio of nitrogen to creatinine increased from baseline in the HP group after 6 wk but not after 18 wk and was higher in the HP than in the HCF group after both 6 ( $P = 0.011$ ) and 18 ( $P = 0.036$ ) wk. The observed decrease between 6 and 18 wk in the HP group was statistically significant ( $P = 0.020$ ), which indicated lower dietary adherence.

Fecal isovaleric acid concentrations were measured as an additional marker of dietary protein intake. Changes in absolute fecal isovaleric acid concentrations (data not shown) were different between groups after 6 wk (ANOVA, treatment  $\times$  time interaction;  $P < 0.001$ ; post hoc HP compared with HCF;  $P < 0.001$ ; HP compared with control:  $P = 0.006$ , after Bonferroni correction), but not after 18 wk (ANOVA, treatment  $\times$  time interaction:  $P = 0.125$ ). Values expressed as percentages relative to baseline are shown in Table 3. The increased fecal isovaleric acid concentrations in combination with the lack of change in the ratio of urinary nitrogen to creatinine in the Mix group indicated interference of cereal fibers with protein absorption.

Breath-hydrogen concentrations as a marker of the consumption of soluble fibers that are more readily fermentable in the

colon, as compared with insoluble cereal fibers (42), did not change significantly during the dietary intervention (Table 3).

#### Changes in insulin sensitivity, according to diet, at 6 and 18 wk

The isoenergetic dietary intervention significantly affected whole-body insulin sensitivity (mixed-model analysis for repeated measures, treatment  $\times$  time interaction, 0–18 wk:  $P = 0.025$ ). When the data were analyzed separately for effects after 6 wk (intense dietary advice) and 18 wk (no further dietary advice) of dietary intervention, whole-body insulin sensitivity after 6 wk was significantly affected by the dietary interventions (Table 4; ANOVA, treatment  $\times$  time interaction:  $P = 0.005$ ). Insulin sensitivity in the HCF group improved from baseline [subgroup analyses in the finally included participants, absolute change in M value from  $4.09 \pm 0.37$  (week 0) to  $4.61 \pm 0.38$  mg  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  (week 6);  $P = 0.010$ ] and decreased in the HP group [ $4.20 \pm 0.38$  (week 0) compared with  $3.71 \pm 0.36$  (week 6) mg  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ;  $P = 0.013$ ], which resulted in a significant 25% difference in insulin sensitivity between participants in the HCF and HP groups after 6 wk ( $P = 0.008$ , after Bonferroni correction). After 18 wk, differences in insulin sensitivity were not significant (ANOVA, treatment  $\times$  time interaction:  $P = 0.054$ ), which was, at least in part, explained by lower adherence to the HP diet, as indicated by a significant reduction in the ratio of urinary nitrogen to creatinine in the HP group between weeks 6 and 18 of the dietary intervention ( $P = 0.020$ ). However, insulin sensitivity remained increased by 16% compared with baseline values in the HCF group (week 18 compared with week 0:  $P = 0.017$ ). Insulin sensitivity in the Mix group after 6 wk was not significantly different from that at 0 wk ( $P = 0.15$ ), and no change was seen in the control group. The ITT analysis showed no significant effect of the diets on whole-body insulin sensitivity (ANOVA, treatment  $\times$  time interaction:  $P = 0.26$ ).

Fasting EGP was influenced after 6 wk of dietary intervention (ANOVA, treatment  $\times$  time interaction:  $P = 0.037$ ), and a small increase from baseline was observed in the HP group after both 6 and 18 wk (Table 4). During the clamps, EGP was equally suppressed in all groups ( $P < 0.0001$  on all study days).

TABLE 3

Biomarkers of dietary adherence, according to diet, expressed as a percentage of the baseline value after 6 and 18 wk<sup>1</sup>

	6 wk					18 wk				
	Control	HCF	HP	Mix	<i>P</i> value <sup>2</sup>	Control	HCF	HP	Mix	<i>P</i> value <sup>2</sup>
Urinary N:C ratio	108 $\pm$ 7	106 $\pm$ 9	197 $\pm$ 43 <sup>3,5</sup>	102 $\pm$ 9	0.006	91 $\pm$ 7 <sup>6</sup>	94 $\pm$ 7	150 $\pm$ 29 <sup>3,4,6</sup>	93 $\pm$ 7	0.023
Fecal isovalerate	104 $\pm$ 13	97 $\pm$ 8	139 $\pm$ 12 <sup>5</sup>	142 $\pm$ 20 <sup>4</sup>	0.048	132 $\pm$ 20	124 $\pm$ 13 <sup>6</sup>	153 $\pm$ 22 <sup>5</sup>	141 $\pm$ 18 <sup>5</sup>	0.72
Breath hydrogen	114 $\pm$ 19	153 $\pm$ 33	131 $\pm$ 22	111 $\pm$ 21	0.62	160 $\pm$ 31	136 $\pm$ 29	108 $\pm$ 19	102 $\pm$ 25	0.35
BMI	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	99 $\pm$ 0	0.61	99 $\pm$ 1	100 $\pm$ 1	101 $\pm$ 1	100 $\pm$ 0	0.31

<sup>1</sup> All values are means  $\pm$  SEMs. N:C, nitrogen to creatinine; HCF, diet high in cereal fiber; HP, diet high in protein; Mix, diet moderately high in both cereal fiber and protein. Data were analyzed separately for effects of the intervention periods 0–6 wk (intense dietary advice) and 6–18 wk (no further dietary advice) by using one-factor ANOVA after correction of all values for baseline (week 0).

<sup>2</sup> *P* values refer to the respective treatment  $\times$  time interaction in the full model. Baseline values were not significantly different between groups. Absolute values for the entire study population at baseline are given in Table 1. Results are given from 259 experiments, with 18–22 participants per group and study day.

<sup>3</sup> Significantly different from HCF (ANOVA with Bonferroni post hoc test).

<sup>4</sup> Significantly different from control (ANOVA with Bonferroni post hoc test).

<sup>5</sup> Significantly different from baseline (paired Student's *t* test).

<sup>6</sup> Significantly different from respective value at 6 wk (paired Student's *t* test).

**TABLE 4**Insulin sensitivity, according to diet, expressed as a percentage of the baseline value after 6 and 18 wk<sup>1</sup>

	6 wk					18 wk				
	Control	HCF	HP	Mix	<i>P</i> value <sup>2</sup>	Control	HCF	HP	Mix	<i>P</i> value <sup>2</sup>
M value	101 ± 5	116 ± 5 <sup>3,4</sup>	91 ± 5 <sup>4</sup>	111 ± 7 <sup>3</sup>	0.005	99 ± 5	116 ± 6 <sup>4</sup>	100 ± 8	97 ± 7	0.054
EGP	103 ± 2	103 ± 2	106 ± 3 <sup>4</sup>	97 ± 3 <sup>3</sup>	0.037	104 ± 2	103 ± 2	110 ± 3 <sup>4</sup>	104 ± 3	0.26

<sup>1</sup> All values are means ± SEMs. HCF, diet high in cereal fiber; HP, diet high in protein; Mix, diet moderately high in both cereal fiber and protein; EGP, fasting endogenous glucose production of the liver; M value, insulin-mediated glucose uptake as a measurement of whole-body insulin sensitivity. Data were analyzed separately for effects of the intervention periods 0–6 wk (intense dietary advice) and 6–18 wk (no further dietary advice) by using one-factor ANOVA after correction of all values for baseline (week 0).

<sup>2</sup> *P* values refer to the respective treatment × time interaction in the full model. Results are from 259 experiments with 18–22 participants per group and study day. Absolute values for the entire study population at baseline are given in Table 1.

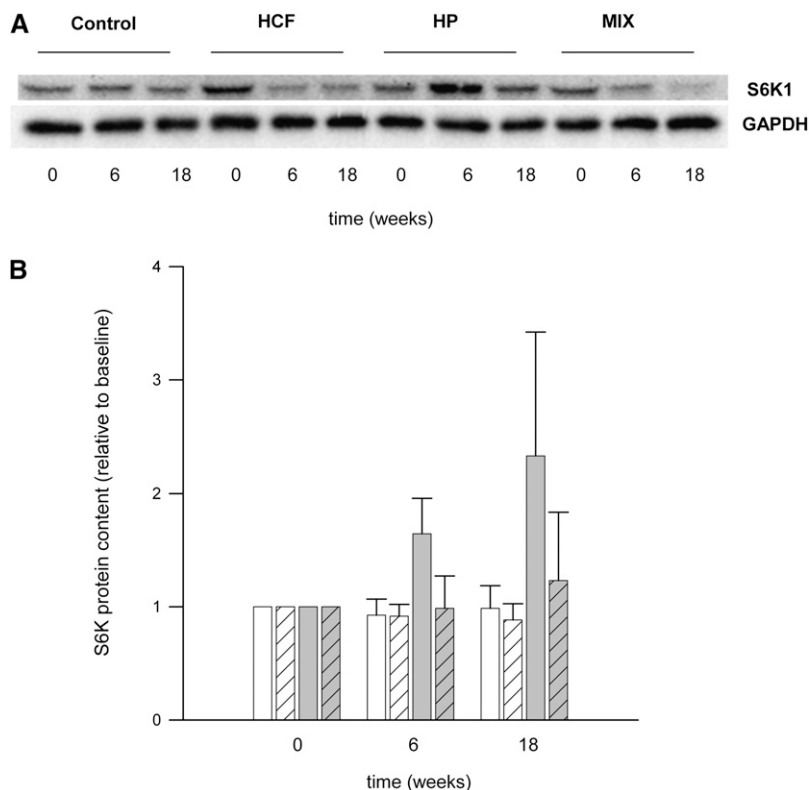
<sup>3</sup> Significantly different from HP (ANOVA with Bonferroni post hoc test).

<sup>4</sup> Significantly different from baseline (paired Student's *t* test).

Apart from small differences in fasting glucose concentrations after 6 wk (changes from baseline: control, −3%; HCF, −4%; HP, +2%; Mix, −1%; ANOVA, treatment × time interaction: *P* = 0.045; HP compared with HCF: *P* = 0.079), commonly used surrogate estimates of insulin sensitivity, such as glycated hemoglobin concentrations, were not affected by the intervention (ANOVA, treatment × time interaction: *P* > 0.74 for all variables).

### Nutrient-dependent signaling cascades in adipose tissue

A representative Western blot for protein immunodetection of S6K1, with the use of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control for protein loading, is shown in **Figure 3A**. Reduced insulin sensitivity (compared with both baseline and HCF) in the HP group after 6 wk was associated with a higher expression of the protein ribosomal subunit serine kinase 6–1 (S6K1) (HP compared with HCF: Mann-



**FIGURE 3.** Ribosomal subunit S6 kinase (S6K1) protein content in subcutaneous adipose tissue, according to diet, at 6 and 18 wk. Protein expression of S6K1 in subcutaneous adipose tissue was measured in a subgroup of matched participants (*n* = 81 experiments, *n* = 6–7 participants per group and study day). **A:** A representative Western blot for protein immunodetection of S6K1 with the use of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control for protein loading. **B:** S6K1 protein content after 6 and 18 wk, according to diet, relative to baseline values (week 0). Differences between groups in the complete model were not significant (Kruskal-Wallis test: 6 wk, *P* = 0.074; 18 wk, *P* = 0.48). HCF, diet high in cereal fiber (white dashed bars); HP, diet high in protein (gray bars); Mix, diet moderately high in both cereal fiber and protein (gray dashed bars); control (white bars).

Whitney *U* test,  $P = 0.025$ ; Figure 3B), although no significant effect was seen in the full model (Kruskal-Wallis test,  $P = 0.074$ ). In the HP group, S6K1 protein expression also tended to be higher than that in the control group after 6 wk (Mann-Whitney *U* test,  $P = 0.063$ ). As observed with insulin sensitivity, differences in S6K1 protein expression were attenuated after 18 wk (Kruskal-Wallis test,  $P = 0.48$ ; HP compared with HCF: Mann-Whitney *U* test,  $P = 0.17$ ).

Mammalian target of rapamycin and downstream factors of the insulin signaling cascade [insulin receptor substrate-1 and protein kinase B (AKT)], including their phosphorylation, showed partly undetectable signals and no differences between groups, which was likely explained by the short-lived effects of protein phosphorylation of the insulin-signaling cascade that cannot be seen in the postabsorptive state.

### Changes in body composition, according to diet, at 6 and 18 wk

Visceral fat mass, subcutaneous abdominal fat, and total body fat tended to decrease comparably in all groups, with no significant differences between groups (Table 5), which was likely explained by the restriction of the dietary fat content to 30% of energy. Changes in liver fat contents were not significantly different between the dietary groups (Table 5). Lean body mass was not significantly different between the groups in the full model (Table 5).

### Other variables

No diet-induced differences were found in inflammatory markers (ANOVA, treatment  $\times$  time interaction for all variables): C-reactive protein (6 wk,  $P = 0.48$ ; 18 wk,  $P = 0.33$ ); plasminogen activator inhibitor-1 (6 wk,  $P = 0.67$ ; 18 wk,  $P = 0.42$ ); the circulating adipokines adiponectin (6 wk,  $P = 0.12$ ; 18 wk,  $P = 0.74$ ) and leptin (6 wk,  $P = 0.82$ ; 18 wk,  $P = 0.83$ ); systolic blood pressure (6 wk,  $P = 0.82$ ; 18 wk,  $P = 0.67$ ); diastolic blood pressure (6 wk,  $P = 0.34$ ; 18 wk,  $P = 0.23$ ); the blood lipids total cholesterol (6 wk,  $P = 0.91$ ; 18 wk,  $P = 0.39$ ), HDL cholesterol (6 wk,  $P = 0.79$ ; 18 wk,  $P = 0.18$ ), and LDL cholesterol (6 wk,  $P = 0.91$ ; 18 wk,  $P = 0.57$ ); triacylglycerols (6 wk,  $P = 0.19$ ;

18 wk,  $P = 0.45$ ); free fatty acids (6 wk,  $P = 0.94$ ; 18 wk,  $P = 0.60$ ); and REE (6 wk,  $P = 0.83$ ; 18 wk,  $P = 0.67$ ).

### DISCUSSION

Epidemiologic observations indicate that HP and HCF diets may affect insulin resistance and diabetes risk differently (6–9, 12–14). Herein we present novel findings from a randomized controlled intervention, which show that implementation over a period of 18 wk of isoenergetic diets varying in cereal fiber and protein contents significantly affected insulin sensitivity in overweight participants at risk of developing type 2 diabetes. Separate analyses of the more strictly controlled first 6 wk of the dietary intervention showed that diet-induced differences in whole-body insulin sensitivity between the HCF and HP diets were striking; however, after 18 wk, the effects of the HP diet were identical to those of the control diet, and differences between the diets were not significant. This might be explained by adaptive processes, with the observed tendency to both reduced abdominal fat and increased lean mass in the HP group as potentially contributing factors. Furthermore, adherence to the HP diet was likely lower in the second phase of the intervention, as indicated by a decrease in the urinary ratio of nitrogen to creatinine. Otherwise, the lack of change in insulin sensitivity in the HCF group from weeks 6 to 18 supported recent findings of others that HCF intake could have prolonged beneficial effects on insulin sensitivity (43).

A rapid onset of insulin resistance in humans exposed to amino acid infusions was reported recently (44, 45), with inhibition of glucose uptake being driven through phosphorylation of downstream factors of the insulin signaling cascade by S6K1 (11, 45), whereas S6K1 knockout mice are protected against diet-induced insulin resistance (46). Therefore, a greater increase in S6K1 protein expression with the HP diet than with the HCF diet after 6 wk of dietary intervention may be linked to the observed significant differences in whole-body insulin sensitivity between groups.

We found that insulin sensitivity was unchanged with the HP challenge when cereal fibers were added to the Mix diet. Normally, ingested dietary proteins are degraded by enzymes originating in the upper gut, which results in the rapid and efficient absorption of amino acids and small peptides by enterocytes in

**TABLE 5**  
Body composition, according to diet, expressed as a percentage of the baseline value after 6 and 18 wk<sup>1</sup>

	6 wk				<i>P</i> value	18 wk				<i>P</i> value
	Control	HCF	HP	Mix		Control	HCF	HP	Mix	
VAT	96 $\pm$ 15 <sup>2</sup>	98 $\pm$ 3	96 $\pm$ 2 <sup>2</sup>	96 $\pm$ 2	0.86	94 $\pm$ 2 <sup>2</sup>	97 $\pm$ 4	96 $\pm$ 2	97 $\pm$ 2	0.80
SCAT <sub>a</sub>	98 $\pm$ 1	99 $\pm$ 2	98 $\pm$ 1	98 $\pm$ 1	0.90	96 $\pm$ 1	98 $\pm$ 1	99 $\pm$ 2	98 $\pm$ 1	0.57
Body fat mass	100 $\pm$ 1	99 $\pm$ 1	99 $\pm$ 1	100 $\pm$ 1	0.76	99 $\pm$ 1	99 $\pm$ 2	98 $\pm$ 2	100 $\pm$ 1	0.94
Body lean mass	100 $\pm$ 0	100 $\pm$ 0	101 $\pm$ 0	100 $\pm$ 0	0.62	100 $\pm$ 0	101 $\pm$ 1	102 $\pm$ 1	101 $\pm$ 1	0.18
Liver fat	101 $\pm$ 6	108 $\pm$ 8	92 $\pm$ 10	98 $\pm$ 10	0.63	97 $\pm$ 6	108 $\pm$ 10	99 $\pm$ 9	109 $\pm$ 10	0.70

<sup>1</sup> All values are means  $\pm$  SEMs. HCF, diet high in cereal fiber; HP, diet high in protein; Mix, diet moderately high in both cereal fiber and protein; VAT, visceral adipose tissue; SCAT<sub>a</sub>, subcutaneous abdominal adipose tissue. Data were analyzed separately for effects of the intervention periods 0–6 wk (intense dietary advice) and 6–18 wk (no further dietary advice) by using one-factor ANOVA after correction of all values for baseline (week 0). *P* values refer to the respective treatment  $\times$  time interaction in the full model. Results are given from 259 experiments with 18–22 participants per group and study day. Absolute values for the entire study population at baseline are given in Table 1.

<sup>2</sup> Significantly different from baseline (paired Student's *t* test).

the small intestine (47, 48). Although the protein intake was as high as 20–25% (mean protein intake: 23% after 6 wk) of energy in the Mix group, both S6K1 protein expression and insulin sensitivity were unaffected in these groups and were comparable with values in the control group. It can be hypothesized that a very HP diet, in which the dietary protein content is increased to  $\geq 25$ –30% of energy (mean protein intake: 28% after 6 wk), induces insulin resistance, whereas more moderate increases have no detrimental effects. However, increased fecal isovaleric acid concentrations, which reflect the appearance of dietary protein in the colon and its subsequent metabolism by the gut microbiota (37, 38), combined with the lack of changes in the ratio of urinary nitrogen to creatinine as a marker of protein absorption in the upper gut indicated that cereal fibers in the Mix group may have interfered with the digestion and/or absorption of dietary protein in the small intestine. Various potential mechanisms responsible for the beneficial effects of fiber consumption have been proposed, but there is currently no convincing explanation for the markedly reduced diabetes risk, as particularly shown with diets high in insoluble cereal fibers that are nonviscous and have no major effects on carbohydrate absorption, postprandial increases in plasma glucose concentrations, or blood lipids (42). Under isoenergetic and weight-maintaining conditions, we also observed no differences between groups in body composition, liver fat, inflammatory markers, circulating adipokines, and markers of colonic carbohydrate fermentation, which indicates that these factors did not relevantly contribute to observed changes in insulin sensitivity in the current study. Interference of cereal fibers with protein digestion may, however, provide a novel concept that could contribute to an explanation for the diverse effects of HCF and HP diets on insulin sensitivity and diabetes risk (6–9).

The strengths of our study included the prescription of isoenergetic diets, the provision of supplements to enhance discrimination between diets, the use of several dietary assessment techniques as process measures, the use of biomarkers to assess compliance, and the state-of-the-art measurement of insulin sensitivity. Our study also had several potential limitations. We designed a proof-of-principle study to investigate whether an HP as compared with an HCF intake indeed affects insulin sensitivity, focusing on participants that were likely to adhere to the respective diets. An additionally performed ITT analysis showed no significant effects of the diets on insulin sensitivity. It is likely that the relatively modest effects of a weight-maintaining dietary intervention were obscured by factors known to strongly affect insulin sensitivity, such as changes in body weight and/or physical activity, or intake of certain pharmacologic agents. Furthermore, although a significant effect of the diets was observed in the full model, which was mainly driven by the sustained 16% improvement in insulin sensitivity from baseline in the HCF group, insulin sensitivity in the HCF group was not significantly higher than that in the control group in the Bonferroni-corrected subanalyses. However, this intervention was designed and powered for investigating the effects of an HCF diet compared with those of an HP diet on insulin sensitivity, whereas the effects of control and Mix diets were also studied. Because the control group was provided with a healthy diet that also contained fiber-rich foods, weaker differences between the HCF and control groups could be expected. Finally, because only white subjects participated, our findings cannot be extrapolated to other ethnicities.

In conclusion, under isoenergetic and weight-maintaining conditions we found diverse effects of supplemented HCF and HP diets on insulin sensitivity, at least in the more strictly controlled first 6 wk of the dietary intervention. It needs to be investigated in larger and longer-term randomized controlled trials whether similar differences between HP and HCF diets can be observed under ad libitum conditions and without the use of dietary supplements. However, our data indicate that a very high protein intake, in comparison with an HCF diet, may have unfavorable effects on insulin sensitivity. Interference of cereal fibers with dietary protein absorption provides a novel concept that could contribute to an explanation for the observed diverse effects of these diets on insulin resistance and the risk of type 2 diabetes.

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