

## Insulin-Sensitizing Effects on Muscle and Adipose Tissue after Dietary Fiber Intake in Men and Women with Metabolic Syndrome

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**Context:** Dietary fibers have been associated with a reduced incidence of type 2 diabetes mellitus in epidemiological studies; however, the precise mechanisms are unknown.

**Objective:** The objective of the study was to evaluate the efficacy and site of action of an insoluble dietary fiber derived from maize (HAM-RS2) in improving insulin resistance in subjects at increased risk of type 2 diabetes mellitus.

**Design:** This study was a randomized, controlled crossover, dietary intervention study.

**Setting:** The study was conducted at the Centre for Diabetes, Endocrinology, and Research, Royal Surrey County Hospital, Guildford, United Kingdom.

**Participants:** Fifteen men and women with insulin resistance participated in the study.

**Intervention:** The intervention included 40 g/d HAM-RS2 compared with a matched placebo for 8 wk.

**Main Outcome Measures:** After each supplement, participants underwent a two-step hyperinsulinemic-euglycemic clamp study with the addition of glucose tracers; a meal tolerance test; arteriovenous sampling across forearm muscle tissue; and a sc adipose tissue biopsy for assessment of gene expression.

**Results:** There was enhanced uptake of glucose into the forearm muscle measured by arteriovenous sampling ( $65 \pm 15\%$  increase after resistant starch;  $P < 0.001$ ). Adipose tissue function was also affected, with enhanced fatty acid suppression after HAM-RS2 treatment and an increase in gene expression for hormone sensitive lipase ( $P = 0.005$ ), perilipin ( $P = 0.011$ ), lipoprotein lipase ( $P = 0.014$ ), and adipose triglyceride lipase ( $P = 0.03$ ) in biopsy samples. There was no effect on the insulin sensitivity of hepatic glucose production or plasma lipids after HAM-RS2.

**Conclusion:** HAM-RS2 improved peripheral but not hepatic insulin resistance and requires further study as an intervention in patients with or at risk for type 2 diabetes. (*J Clin Endocrinol Metab* 97: 3326–3332, 2012)

In the United Kingdom, it is estimated that 10% of the National Health Service budget is spent in the treatment of type 2 diabetes mellitus (T2DM), currently estimated at £1.3 billion (source: Diabetes UK, February 2010), whereas in the United States, the estimated amount spent is \$119.2 billion, again 10% of all health care costs (1).

A clear priority for diabetes in terms of both public health and economics therefore involves preventing the development of new cases of T2DM, against a background of increased levels of obesity. Lifestyle intervention should play a major part of diabetes prevention and remain a constant theme throughout management.

Maintenance of an appropriate body weight is important because 80% of all patients with T2DM are classified as obese (2); however, weight loss is notoriously difficult to achieve and maintain. It is not only the quantity of the diet but also the quality that may also provide a solution; high fiber intake and a reduction in trans-fatty acids and saturated fats have all been linked to increases in tissue insulin sensitivity (3), a critical target in diabetes prevention. In Westernized countries, especially the United States and the United Kingdom, the intake of dietary fibers and whole grains are particularly low (4), falling well short of modest government targets. There is evidence that filling the fiber gap may make a difference.

Dietary fibers can be classified as either viscous or non-viscous. Viscous fibers cause a well-documented reduction in the rate of glucose absorption with reduction of the glycemic response (5). Nonviscous fibers such as resistant starch (RS) have no direct effect on glucose absorption from the gut yet have been shown in short-term human studies to enhance whole-body insulin sensitivity (6–8). Epidemiological and mechanistic evidence also suggests insoluble fibers are linked to a reduced incidence of T2DM (9). Many fibers currently available, such as RS, are now being reclassified as functional foods, offering health benefits beyond simple nutrition. Using a supplement approach, we have previously determined the metabolic effects of a granular form of type 2 RS, derived from high-amylose maize (HAM-RS2) in healthy subjects. HAM-RS2 increases whole-body insulin sensitivity and significantly reduces adipose tissue lipolysis, findings that could be clinically important for both diabetes care and prevention (reviewed in Ref. 10). The concept that some dietary fibers change peripheral metabolism as a result of changes in short-chain fatty acids originating from colonic fermentation and thus serve as a signal between adipose tissue and muscle are supported by our original work (6), but it is unknown how far these findings go in terms of diabetes prevention. The lack of appropriate intervention human data has been recently highlighted (11). Using an integrated whole-body ap-

proach, using stable-isotope tracers for the first time to differentiate between changes in hepatic glucose production and peripheral glucose uptake, we have investigated the metabolic potential of HAM-RS2 in obese, insulin-resistant individuals, a key target group in the prevention of overt T2DM.

## Research Design and Methods

All aspects of the study were conducted in accordance with the Declaration of Helsinki and received a favorable ethical opinion from the East Kent Research Ethics Committee and the University of Surrey Ethics Committee. This trial was registered via the International Standard Randomised Controlled Trial Number reference number 56997186. All participants gave written informed consent.

### Research participants

Sixteen (eight female) healthy subjects with insulin resistance as defined by the European Group for the Study of Insulin Resistance criteria but without a diagnosis of T2DM were recruited. Fifteen (seven female) subjects completed the whole protocol. These diagnostic criteria were chosen as the most discriminating for the presence of insulin resistance (12). Their ages ranged from 25 to 70 yr (mean  $48.9 \pm 3.9$  y) with a mean body mass index of  $33.8 \pm 1.9$  kg/m<sup>2</sup> and with a fasting insulin in the range 60–156 pmol/liter. Subjects all exhibited abdominal adiposity (mean waist circumference  $106 \pm 5.1$  cm). Participants were excluded if they had a history of gastrointestinal, endocrine, or cardiovascular disease, and none were currently taking any prescribed medication (with the exception of the contraceptive pill). Female participants were recruited as being either postmenopausal or, if premenopausal, studied while taking oral contraceptives to avoid potential effects of hormonal fluctuation. Habitual diet was assessed by the use of 7-d food records and analyzed using the WinDiets Professional Version program (Robert Gordon University, Aberdeen, UK).

### General protocol

This was a single-blind, crossover dietary intervention comparing HAM-RS2 with placebo. Each starch supplement was consumed daily for 8 wk with an 8-wk washout between treatments. At the end of each supplement, participants underwent the following: 1) a two-step hyperinsulinemic clamp with the addition of stable isotopes to differentiate between peripheral and hepatic insulin sensitivity, 2) a meal tolerance test (MTT) combined with arteriovenous sampling across forearm muscle, and 3) a sc adipose tissue biopsy for gene analysis.

Participants avoided strenuous exercise and alcohol for 48 h preceding each treatment day and were provided with a standardized low-fat/low-fiber evening meal before each study to reduce variability. All participants were recruited as being sedentary (less than three exercise bouts per week) and instructed to maintain normal daily activity levels.

The participants were initially randomized to receive either Hi-maize 260 (Ingredion Inc., Bridgewater, NJ) at 67 g/d ([40 g RS and 27 g rapidly digestible starch (RDS)] or Amioca (Ingredion Inc.) at 27 g/d (0 g RS and 27 g RDS) for 8 wk added to their habitual diet, separated by an 8-wk washout period. For clarity

of the presentation in *Results* and *Discussion*, the Hi-maize supplement will be referred to as HAM-RS2 and the Amioca as placebo. The starches were assayed using a modified version of the Englyst RS method to determine RS and RDS content (13) and were supplied as powder in ready-to-use sachets to mix in with food/drink. Bowel habit and symptom diaries were completed during each intervention to assess tolerance, and diet diaries were completed to assess any changes to background intake. Fasting breath hydrogen (Gastrolyzer; Bedford Scientific, Maidstone, UK) measurements were taken before each clinical intervention as a proxy compliance measure.

## Metabolic investigations

### Euglycemic-hyperinsulinemic clamp with infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose

After cannulation and after an initial blood sample a primed continuous infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose (170 mg; 1.7 mg/min) was commenced. Once a steady state of enrichment with the stable isotopes was achieved, a further five baseline samples were taken between 100 and 120 min for measurement of the glucose enrichment, glucose, insulin, and nonesterified fatty acid (NEFA) concentration. At 120 min a two-step hyperinsulinemic-euglycemic clamp was initiated. Step 1 consisted of an insulin infusion at 0.3 mU/kg<sup>-1</sup> · min<sup>-1</sup> (Acrapid, low dose; Novo Nordisk, Copenhagen, Denmark) for 120 min to measure the insulin sensitivity of endogenous glucose production (EGP), which is predominantly a measure of hepatic glucose production, and lipolysis. Step 2 consisted of an insulin infusion at 1.5 mU/kg<sup>-1</sup> · min<sup>-1</sup> (high dose) for a further 180 min to measure the insulin sensitivity of glucose uptake. Plasma glucose concentration was maintained at fasting levels using a variable infusion of 20% dextrose spiked with [6,6-<sup>2</sup>H<sub>2</sub>]glucose (8 mg/g for step 1 and 10 mg/g for step 2). Blood samples were taken every 10 min with blood glucose measured immediately by the glucose oxidase method using a Clandon Scientific analyzer (Yellow Springs Instruments, Yellow Springs, OH). Additional blood samples were taken between 210 and 240 min and between 390 and 420 min for glucose and NEFA, representing the two steady-state periods.

### Meal tolerance test with tissue with tissue-specific arteriovenous sampling

To assess the metabolism of skeletal muscle *in vivo*, we measured arteriovenous differences across forearm tissue. Serial blood samples were taken from subjects in the fasting state and for 5 h after a liquid MTT (1804 KJ; 60 g carbohydrate, 12.6 g fat).

Arterialized blood was obtained from a vein draining a hand placed in heated box (55 C). Venous blood from muscle was taken from a vein draining the deep tissues of the contralateral forearm (14). To prevent contamination of the blood from the forearm vein with blood from the hand, venous blood flow from the hand was occluded using a wrist cuff inflated to 20 mm Hg above systolic blood pressure for 2 min before the samples were taken. Oxygen saturation was used to assess the correct positioning/arterialization. A cutoff of oxygen partial pressure less than 65% and oxygen partial pressure greater than 95% were used for the deep-vein and arterialized samples, respectively.

Simultaneous sampling from the two sites began at 0900 h after a 12-h overnight fast. Subjects then ingested the liquid test meal, and further blood samples were taken for 5 h. Forearm

muscle blood flow was assessed by venous occlusion strain-gauge plethysmography following each blood sample.

### Adipose tissue biopsy

An adipose tissue biopsy was performed under local anesthesia (2% lidocaine) 5.5 h after the meal in 10 of 15 subjects. An open biopsy was taken from the upper buttock region, which has previously been shown to exhibit metabolic activity (15). Samples were cleaned and snap frozen in liquid nitrogen and stored at –80 C for later RNA quantification. Total RNA was prepared from the frozen tissue using a commercial kit (Ambion, Austin, TX). RNA integrity (28S to 18S ratio) was determined with the Agilent 2100 bioanalyzer and RNA 6000 labChip kit (Agilent Technologies, Massy, France). RNA preparations were stored at –80 C until real-time quantitative PCR assays.

### Biochemistry

Plasma, triacylglycerol (TG), NEFA, and total cholesterol were measured enzymatically using a Cobas Mira (Roche Laboratories, Antrim, Northern Ireland). Metabolites from the placebo and HAM-RS2 arms of the trial were analyzed together with an intraassay variation less than 2.5% for all metabolites. Concentration of insulin, total ghrelin, leptin, and adiponectin were measured by RIA using commercially available kits (Millipore, Billerica, MA), with an interassay coefficient of variation of less than 5% for all hormones. Blood for total ghrelin analysis was first collected into potassium-EDTA containing 200 Kallikrein-inhibiting units aprotinin per milliliter blood (Sigma Aldrich, Poole UK).

Plasma levels of IL-6 and TNF $\alpha$  were measured using commercially available ELISA kits (Gen-Probe Diaclone, Besançon, France), with intra- and interassay coefficients of variation of less than 4.2% and less than 9%, respectively.

The isotopic enrichment of plasma glucose was measured by gas chromatography-mass spectrometry on an HP 5971A mass selective detector (Agilent, Santa Clara, CA). The enrichment was determined using a penta-*O*-trimethylsilyl-*D*-glucose-*O*-methoxime derivative analyzed by selected ion monitoring of the ions at a charge to mass ratio of 319 and 321 (16). Glucose concentration of the same plasma sample was determined by a glucose oxidase method using a Clandon Scientific glucose analyzer (Yellow Springs Instruments).

### Quantification of mRNA

Concentration of the mRNA corresponding to the genes of interest were measured by RT-PCR with a Rotorgene Q (QIAGEN, Courtabouef, France). Primer sequences are available upon request (hubert.vidal@univ-lyon1.fr). First-strand cDNA were first synthesized from 250 ng of total RNA in the presence of 100 U of Superscript II (Invitrogen, Eragny, France) using both random hexamers and oligo (deoxythymidine) primers. The real-time PCR was performed in a final volume of 20  $\mu$ l containing 5  $\mu$ l of a 60-fold dilution of the reverse transcription reaction medium and 15  $\mu$ l of reaction buffer from the Absolute QPCR SYBR Green Mix (Thermo Scientific, Swedesboro, NJ). For quantification, a standard curve was systematically generated with six different amounts (150–30,000 molecules/tube) of purified target cDNA cloned in the pGEM plasma (Promega, Charbonnières, France). Each assay was performed in duplicate. Data were normalized using hypoxanthine phosphoribosyl

transferase 1 mRNA levels measured in each sample by real-time PCR as an internal standard (17).

### Calculations

Fasting insulin sensitivity and  $\beta$ -cell function were assessed by homeostatic model assessment (HOMA) (18).

During the MTT, arteriovenous differences in metabolite concentrations were calculated. Absolute flux was calculated as the product of the arteriovenous concentration difference and tissue blood/plasma flow as appropriate. Total fatty acid uptake into muscle was calculated from the rate of TG and NEFA removal across the tissue as previously described (6). Glucose clearance was calculated as the total glucose flux/insulin area under the curve. During the two-step clamp, the EGP and glucose disposal rate ( $R_d$ ) were calculated, using the model proposed by Steele (19) modified for the inclusion of stable isotopes. The volume of distribution was assumed to be 22% of body weight. The calculation was also modified for inclusion of [6,6- $^2\text{H}_2$ ]glucose in the dextrose infusion (20). Before calculation of glucose turnover, plasma glucose concentration and glucose enrichment time courses were smoothed, using optimal segments technique analysis (21). For each time point, the mean EGP and  $R_d$  were calculated. Data are expressed as the mean EGP and  $R_d$  from five sample values taken during a steady state.

### Statistical analysis

Results are presented as mean  $\pm$  SEM. All statistical analyses were carried out using SPSS version 16 (SPSS Inc. Chicago, IL). End-of-treatment effects were compared between HAM-RS2 and placebo in a crossover design. Time-course data were analyzed by repeated-measures ANOVA. Summary data were analyzed using paired Student's *t* tests when normally distributed or the nonparametric equivalent, the Wilcoxon signed rank test. Values of  $P < 0.05$  were taken as significant.

### Results

The inclusion of an additional 40 g/d HAM-RS2 into the habitual diet of participants was well tolerated [mean total fiber intake increased from 20 g/d (range 11.3–29.6 g/d) to 58 g/d]. There was a significant effect of HAM-RS2 intake on fasting breath hydrogen [ $27 \pm 5$  vs.  $8 \pm 2$  ppm ( $P < 0.001$ )]. There was no significant effect of this increase in fiber intake on stool frequency, abdominal pain, flatulence, or bloating as determined by daily bowel habit and symptom diaries. Mean daily macronutrient intake from 7-d diet diaries did not differ between treatment regimens (placebo: 233 g carbohydrate, 76.4 g fat, and 8.5 MJ/d; HAM-RS2: 238 g carbohydrate, 76.9 g fat, and 8.7 MJ/d), and there was no subsequent change in body weight (Table 1).

### Glucose metabolism and insulin sensitivity

There was a significant decrease in both the fasting glucose ( $P = 0.017$ ) and insulin ( $P = 0.041$ ) concentrations after HAM-RS2 treatment (Table 1) and conse-

**TABLE 1.** Anthropometric and fasting plasma measurements taken after 8 wk of supplementation with either 40 g/d HAM-RS2 compared with placebo

|  | Placebo      | HAM-RS2      | P     |
|--|--------------|--------------|-------|
| Body weight (kg)                             | 99.1 (7.7)   | 99.1 (7.3)   | NS    |
| Body mass index (kg/m <sup>2</sup> )         | 33.8 (2.0)   | 33.9 (1.9)   | NS    |
| Fat mass (kg) <sup>a</sup>                   | 37.6 (4.0)   | 37.7 (3.5)   | NS    |
| Fat-free mass (kg) <sup>a</sup>              | 61.5 (5.0)   | 61.5 (4.9)   | NS    |
| Systolic blood pressure (mm Hg) <sup>b</sup> | 126 (5.2)    | 125 (4.2)    | NS    |
| HOMA-%B                                      | 182.5 (12.6) | 175.9 (11.9) | NS    |
| HOMA-IR                                      | 2.9 (0.2)    | 2.5 (0.2)    | 0.029 |
| Fasting glucose (mmol/liter)                 | 5.2 (0.11)   | 5.0 (0.1)    | 0.017 |
| Fasting insulin (pmol/liter)                 | 129 (10.2)   | 108 (8.4)    | 0.041 |
| Fasting leptin (ng/ml)                       | 19.0 (2.2)   | 20.3 (2.2)   | NS    |
| Fasting adiponectin (ng/ml)                  | 8120 (148)   | 7720 (158)   | NS    |
| Fasting total cholesterol (mmol/liter)       | 4.3 (0.3)    | 4.2 (0.4)    | NS    |
| Fasting TG (mmol/liter)                      | 1.2 (0.1)    | 1.4 (0.2)    | NS    |

All values are presented mean (SEM),  $n = 15$ . All measurements were taken in the morning after an overnight fast. Comparisons were made with a Wilcoxon signed-ranks test. HOMA-%B,  $\beta$ -Cell function; HOMA-IR, HOMA for insulin resistance (17); NS, not significant.

<sup>a</sup> Measured using foot-to-hand bioimpedance (Tanita TBF-300, Amsterdam, The Netherlands).

<sup>b</sup> Mean of three readings taken with the subject in the sitting position (Omron MX3 Plus, Omron Healthcare, Milton Keynes, UK).

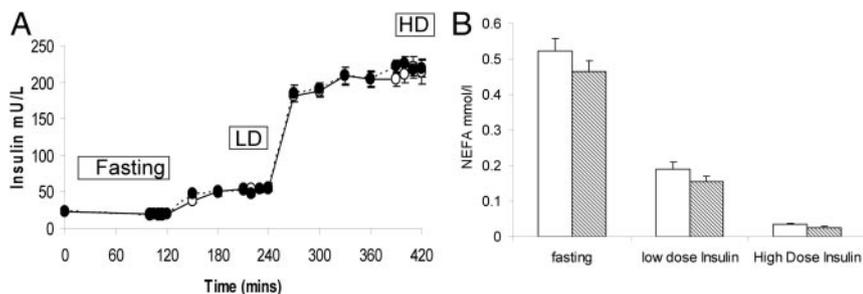
quently a decrease in the calculated HOMA for insulin resistance, with a mean  $10.4 \pm 7.5\%$  improvement in fasting insulin resistance compared with placebo condition ( $P = 0.029$ ).

During the two-step euglycemic-hyperinsulinemic clamp, no difference was observed in the basal EGP between treatments (placebo:  $8.5 \pm 0.6 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ ; HAM-RS2:  $8.4 \pm 0.6 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ ) or after low-dose insulin (placebo:  $5.3 \pm 0.6 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ ; HAM-RS2:  $4.7 \pm 0.7 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ ). There was an increase in the peripheral glucose uptake ( $R_d$ ) after high-dose insulin infusion (placebo:  $40.5 \pm 3.5 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ ; HAM-RS2:  $47.1 \pm 4.1 \mu\text{mol/kg}^{-1} \cdot \text{min}^{-1}$ , a mean  $21.1 \pm 9.0\%$  difference between treatments;  $P = 0.031$ ) despite no significant difference in the prevailing insulin concentration during the infusion (Fig. 1).

During the MTT with arteriovenous sampling across forearm tissue, there was a significant increase in glucose uptake after the HAM-RS2 treatment (Fig. 2), with a  $65 \pm 15\%$  increase in total glucose flux ( $P < 0.001$ ).

### Fat metabolism

There was no effect of HAM-RS2 consumption on fasting/postprandial TG or total cholesterol concentrations. Before the clamp, fasting NEFA levels were lower at baseline and suppression of plasma NEFA by insulin was enhanced after HAM-RS2 treatment ( $P = 0.041$ ) compared with placebo (Fig. 1). During the MTT, the net uptake of



**FIG. 1.** Plasma Insulin (A) and NEFA (B) during the two-step hyperinsulinemic-euglycemic clamp. There was no difference in the insulin concentration between treatments, placebo (*open circles*) vs. HAM-RS2 (*closed circles*). After HAM-RS2 treatment, there was significant treatment effect (*open circle*; repeated measures ANOVA,  $P = 0.041$ ), compared with placebo treatment (*closed circle*). Data are means  $\pm$  SEM.

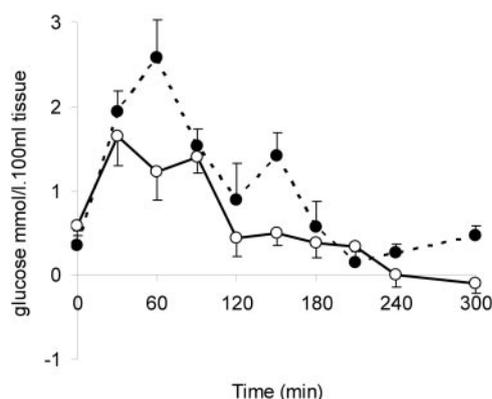
fatty acids into skeletal muscle reflects fatty acids from two sources: those derived from lipoprotein lipase (LPL) lipolysis of TG and those taken up as NEFA from the circulation. The overall uptake of fatty acids into skeletal muscle was increased, approaching significance (+16%,  $P = 0.055$ ) after HAM-RS2. However, the relative contributions of fatty acids did change significantly. HAM-RS2 decreased uptake of fatty acids as NEFA ( $-20\%$ ,  $P = 0.046$ ) and increased TG lipolysis ( $+24\%$ ,  $P = 0.028$ ).

### Hormones and inflammatory markers

There was no significant difference in fasting leptin or adiponectin levels between treatments and no effect on either fasting or postprandial suppression of plasma total ghrelin (data not shown). Despite the participants in this dietary intervention having both insulin resistance and abdominal obesity, the inflammatory markers IL-6 and TNF $\alpha$  were below the limit of detection in 14 of 15 subjects using commercially available assays.

### Adipose tissue gene expression

To supplement the physiological findings of the effects of HAM-RS2 on adipose tissue lipolysis, the expression of key



**FIG. 2.** Glucose flux into muscle tissue measured with arteriovenous sampling across the forearm and venous-occlusion strain gauge plethysmography. There was a significant increase in glucose uptake after HAM-RS2 treatment (*closed circles*) compared with placebo treatment (*open circles*) ( $P < 0.001$ ). Data are means  $\pm$  SEM.

genes were measured in sc adipose biopsies (Table 2). There was enhanced expression of several genes involved in fatty acid metabolism including LPL, adipose triglyceride lipase (ATGL), perilipin, adiponectin, and hormone-sensitive lipase (HSL), in all cases representing at least a 2.0-fold increase in tissue expression. There was no effect of HAM-RS2 consumption on the expression of either visfatin or peroxisomal proliferator-activated receptor (PPAR)- $\gamma$ .

## Discussion

In this study we have built on our own published data and that of others on the potent metabolic potential of a dietary fiber derived from cereal, in healthy individuals, and translated this into the potential treatment of insulin resistance (6, 8, 22) in those with an elevated risk of T2DM. In addition, we have demonstrated for the first time changes at the transcriptional level in sc adipose tissue and that the potential mechanism linking HAM-RS2 to increased insulin sensitivity involves peripheral metabolism (adipose and muscle) without any change in hepatic glucose handling. This is an important finding in determining the site of action of dietary fiber in modulating insulin sensitivity, especially for the design of complementary drug/lifestyle treatments.

Adipose tissue expression of LPL, HSL, and ATGL, enzymes involved in lipid uptake and mobilization, are typically reduced in both obesity and T2DM (23) (24), with low HSL expression constituting a possible primary defect in obesity. In this study there was a clear stimulatory effect on the expression of adipose tissue (AT) LPL, ATGL, and HSL after fiber intake. An increase in both HSL and LPL mRNA in AT (25) may indicate increased adipocyte differentiation within the tissue (26). A failure of AT to differentiate may be the critical step that marks the transition from obesity to T2DM.

**TABLE 2.** Gene expression in sc adipose tissue biopsies taken after 8 wk supplementation with 40 g/d HAM-RS2 compared with placebo

|                | Placebo      | HAM-RS2      | P     |
|----------------|--------------|--------------|-------|
| LPL            | 96.2 (19.8)  | 261.0 (52.3) | 0.014 |
| ATGL           | 48.3 (4.9)   | 82.7 (12.7)  | 0.03  |
| Visfatin       | 5.3 (0.5)    | 5.3 (0.7)    | NS    |
| Perilipin      | 294.0 (40.5) | 570.7 (76.6) | 0.011 |
| Adiponectin    | 146.9 (14.9) | 250.2 (45.4) | 0.053 |
| HSL            | 33.2 (4.5)   | 61.3 (6.0)   | 0.005 |
| PPAR- $\gamma$ | 8.1 (0.9)    | 9.2 (2.0)    | NS    |

Data are expressed as mean (SEM),  $n = 10$ . Values are arbitrary units relative to the constitutively expressed housekeeping gene *HPRT*. Comparisons were made using the Wilcoxon signed-ranks test.

HAM-RS2 does not seem to impact on obesity *per se* because the observed changes occur without an effect on body weight but would appear to affect the function of the existing AT. This would appear to demonstrate that tissue insulin sensitivity is increased due to a change in the physiological function of AT with the effects observed similar to those found with pharmacological diabetes treatment.

Colonic fermentation of HAM-RS2 increases plasma levels of the short-chain fatty acids acetate and propionate (6), which are taken up into AT and skeletal muscle tissue. Both acetate and propionate are highly bioactive and could explain most, if not all, of the improvements in insulin sensitivity found with HAM-RS2. This study also demonstrated a decrease in circulating NEFA after HAM-RS2, suggesting an improvement in the suppression of lipolysis with insulin. Interestingly, after HAM-RS2, we also found increased tissue expression of perilipin, which may be the molecular mediator of this observed antilipolytic effect (27). HAM-RS2 may mimic the effects of drug treatment by acting on the same molecular pathways; for example, propionate has been reported to potentially act as ligand for PPAR $\gamma$ . We found no increase in the expression of PPAR $\gamma$  in AT but cannot exclude an effect on actual activity, as supported by increased expression of LPL and adiponectin, two well-known target genes of PPAR $\gamma$  and thiazolidinediones in human adipose tissue (28,29). HAM-RS2 would therefore appear to exert its beneficial effect on insulin sensitivity primarily in the periphery (adipose and muscle) rather than by reducing hepatic glucose production. Importantly, the effects are also independent from any change in either macronutrient/energy intake or body weight.

Consistently, dietary HAM-RS2 shows no effect on plasma TG or cholesterol (30). The most important clinical effect seen with HAM-RS2, namely the increased uptake of glucose into skeletal muscle, we propose as secondary to primary changes in AT function. The increase in postprandial glucose uptake using arteriovenous sampling directly across skeletal muscle showed a dramatic improvement ( $\sim 65\%$ ), in excess of what has been reported with drug treatments (29) using a similar sample size and methodology. Interestingly, there was also enhanced uptake of fatty acids by skeletal muscle that might, in normal circumstances, be expected to lead to increased intramyocellular TG storage and a reduction in insulin sensitivity. The reverse is in fact true: despite increased fatty acid uptake, muscle TG storage when measured by magnetic resonance imaging is reduced by HAM-RS2 (8).

An important aspect of this study is that the metabolic benefit achieved; a mean 65% increase in postprandial glucose disposal and a 10% reduction in fasting insulin resistance (HOMA) was independent from any change in lifestyle or body weight. Recent work has demonstrated the benefi-

cial effects of extreme energy restriction (2.5 MJ/d) on metabolic control in T2DM (31). In this 8-wk nonrandomized intervention, a mean 15 kg loss in body weight resulted in significant decrease in EGP but no effect on peripheral insulin sensitivity, which directly opposes the results found with HAM-RS2 (using identical methodology). Weight loss has been shown to reduce intrahepatic TG (32), so an improvement in EGP is as expected; however, the lack of an effect on muscle insulin sensitivity with weight loss demonstrates that energy restriction alone cannot restore the metabolic milieu in those with insulin resistance/T2DM and nutrient quality may also be critical. Weight loss combined with a high-fiber intake may then be expected to have complementary benefits for glucose metabolism (addressing both hepatic and peripheral insulin resistance simultaneously), although this remains to be tested.

Another proposed mechanism linking fiber intake specifically to insulin sensitivity relates to modifying systemic inflammation via changes in both gut microbiota and intestinal permeability (33). HAM-RS2 has indeed been demonstrated to have mild prebiotic effects (34), although in this study levels of inflammatory markers were below the level of detection for 14 of 15 participants, indicating that any improvement in insulin sensitivity was not secondary to a change in inflammatory status in this group. This is in line with other human fiber feeding studies ((8, 22).

A limitation of this crossover study is the small sample size; however, in contrast, a strength is that we have manipulated only one component of the diet. Larger randomized controlled feeding studies manipulating only fiber intake, with fewer detailed measurements, would be required now to add to the evidence for HAM-RS2 in the prevention of diabetes. In this study we have demonstrated the efficacy of dietary HAM-RS2 in the improvement tissue insulin resistance in a small cohort, by increasing peripheral glucose uptake, through a cascade that may originate in adipose tissue, although the exact molecular mechanism warrants further investigation. The next logical step is to assess the role of HAM-RS2 for the first time as a potential therapeutic treatment for patients with T2DM.

## Acknowledgments

The HAM-RS2 supplements were provided free of charge from Ingredion Inc. (Bridgewater, NJ). There was no industrial involvement in the design of the study or the interpretation of the data. This trial was registered via the International Standard Randomised Controlled Trial Number reference number 56997186.

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