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In Vivo *Whole-Body Resting Energy Expenditure and Insulin Action in Human Malignant Hyperthermia*

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Background: **Malignant hyperthermia (MH) is a pharmacogenetic disease triggered by volatile anesthetics or succinylcholine. The disorder is heterogenetic and caused by abnormal calcium regulation within skeletal muscle cells. No clear metabolic differences have been found in MH-susceptible (MHS) persons in vivo while not having MH episodes, but some reported signs suggest that insulin action and energy turnover might be altered in muscle of MHS persons.**

Methods: **In fasting and insulin-stimulated conditions, using the glucose clamp technique and indirect calorimetry, we assessed *in vivo* resting energy expenditure (REE) and nutrient utilization rates in 10 MHS, 5 MH-equivocal (MHE) and 10 MH-negative (MHN) persons from 14 families. With a model using the persons' fat-free mass, fat mass, age, and gender, we calculated their predicted REE and compared it with measured REE in 10 MHS and 10 MHN persons (measured − predicted = residual REE).**

Results: ***In vivo*** **measured REE and glucose disposal rates were similar in 10 MHS and 10 MHN persons. Only during insulin stimulation was residual REE greater in MHS persons (6.4%; $P = 0.013$).**

Conclusions: ***In vivo*** **insulin action is unimpaired in MHS persons. Although the absolute values of whole-body REE are the same in MHS and MHN persons, the part of REE independent of the determinants fat-free mass, fat mass, age, and gender is moderately greater in MHS than in MHN persons during insulin exposure. This suggests that MH susceptibility might influence insulin-stimulated energy turnover in muscle. (Key words: Body composition; family, nutrient oxidation rate.)**

MALIGNANT hyperthermia (MH) is a pharmacogenetic disease characterized by hypermetabolic episodes of the skeletal muscle which may occur during or after exposing MH-susceptible (MHS) individuals to trigger agents such as succinylcholine or volatile anesthetics.[1] MH results from the loss of control of muscle cytosolic calcium concentration ($[Ca^{2+}]_c$), caused by the trigger agents in MHS persons. MH susceptibility is diagnosed by the *in vitro* muscle contracture test (IVCT)[2,3] performed on living muscle strips obtained by open muscle biopsy. Genetic aspects of MH susceptibility are complex, with six suspected gene loci at present. The most important loci, with 19 known mutations, is the gene coding for the sarcoplasmic reticulum calcium channel RYR1.[4] These different MH mutations affect the function of RYR1.[5,6]

Several metabolic studies have looked for endocrinologic and energy turnover abnormalities associated with MH susceptibility outside the hypermetabolic episodes. Some revealed a slight increase in fasting plasma glucose[7] and insulin[8] concentrations, together with hyperinsulinemia during an intravenous glucose tolerance test[9] in MHS humans. Muscle of MHS persons has also been reported to contain enhanced levels of myophosphorylase activity[10] and $[Ca^{2+}]_c$.[11] These studies suggest that muscle insulin response could be altered in MHS individuals; phosphorylase promotes glycogen breakdown, whereas insulin allows the disposal of exogenous glucose mainly through glycogen deposition in muscle[12] and an increased $[Ca^{2+}]_c$ inhibits insulin-stimulated glucose uptake by muscle.[13]

When assessed by indirect calorimetry,[7,8,14] no differences have been found in resting or exercise energy

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expenditure in MHS persons compared with controls, but studies using 31-phosphorus (^{31}P) magnetic resonance spectroscopy revealed an increased inorganic phosphate–phosphocreatine ratio in the skeletal muscle of MHS persons.[15,16] When HEK-293 cells are transfected by human wild-type and MH-mutated RYR1, they double their expression of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) over that of untransfected cells.[6] This may represent a compensation mechanism by which the transfected cells augment the reuptake into their stores of the calcium that leaks through the RYR1 into the cytoplasm to maintain a normal resting $[Ca^{2+}]_c$.[6] If present *in vivo* in MHS persons, the energy cost of such an extra work of muscle SERCA could be detected by measuring whole-body resting energy expenditure (REE), because skeletal muscle mass represents about 40% of body weight and can account for as much as 30% of whole-body REE.[17] The principal determinants of whole-body REE are fat-free mass (FFM), fat mass (FM), age, and gender and together explain 73[17] to 81%[18] of interindividual REE variance. This model has established the relative impact of these four physical covariates on the mean whole-body REE that is measured in a studied population. Using this approach, a person's FFM, FM, age, and gender are used to calculate a predicted value of REE for each person, which can be compared with the measured value of REE in the same person. The difference between measured and predicted REE is called residual REE. Whereas a low residual REE has been shown to predict human obesity,[18] this parameter has not been addressed in human MH susceptibility, and its analysis could disclose the energy cost of a compensation mechanism present in MHS individuals.

The aim of the current study was twofold: to compare the insulin sensitivity of MHS with that of MH-negative (MHN) persons and to assess whether MHS persons show an increased residual REE both in the fasting and the insulin-stimulated conditions. Insulin sensitivity was measured using the well-validated euglycemic hyperinsulinemic clamp technique[19] in 10 MHN, 10 MHS, and 5 MH-equivocal (MHE) persons. Indirect calorimetry[20] and body composition analysis were used to determine residual REE in these persons.

Materials and Methods

Subjects

The study participants were 25 Swiss individuals on whom a previous MH diagnostic muscle biopsy had been performed between 1986 and 1996 because of a clinical episode compatible with MH or the existence of an MHS family member. The persons were contacted by mail and asked to enroll in the study on a voluntary basis. The participants were studied between 1995 and 1997 in random order. From the results of their IVCT (table 1), 10 patients had been diagnosed as MHN, 10 as MHS, and 5 as MHEh (IVCT results abnormal for halothane but normal with caffeine). As shown in table 1, the 25 persons were from 14 different families with no known MH genotype. Each person was otherwise healthy, took no medication, and had a normal blood pressure and fasting plasma glucose level. Only two and three persons in the MHN and MHS groups, respectively, had a first-degree hypertensive relative. No participant had a first-degree relative with diabetes other than one MHN person whose father had shown a transient hyperglycemic state while on temporary corticosteroid therapy.

No significant differences in physical parameters among MHN, MHS, and MHE persons was observed (table 1). The study protocol was approved by the Ethical Committee of the Faculty of Medicine of the University of Lausanne. All participants gave informed written consent before taking part in the study.

In Vitro *Contracture Tests*

The muscle biopsies were carried out at the Basel University Hospital. All biopsy samples were taken from the vastus medialis muscle. IVCT was performed according to the protocol of the European Malignant Hyperthermia Group,[2,21] and IVCT results of the first test for both halothane and caffeine were used. The time interval between the muscle biopsy and the muscle contracture test was about 60 min for all patients.

Experimental Plan

The participants were asked to avoid heavy physical exercise and tobacco and caffeine consumption for the 2 days preceding the study. The evening before the glucose clamp procedure (see Euglycemic Hyperinsulinemic Clamp), body composition was measured by the skinfold[22] and bioimpedance[23] methods. Briefly, the bicipial, tricipital, subscapular, and suprailiac skinfold thickness was measured in triplicate by the same investigator using a Harpenden caliper (CMS Weighing Equipment, Ltd., London, UK). Whole-body bioelectrical impedance was determined with a portable device built by the Electronic Department of the Lausanne University

Table 1. Subjects' Characteristics

Subject	Diagnosis	Family	Gender	Age (yr)	Body height (cm)	Body weight (kg)	Body fat (%)	Body FFM (kg)	Body FM (kg)
1	MHN	1	F	33.3	161	65.5	31	45.4	20.1
2	MHN	2	F	30.1	166	63.2	30	44.1	19.1
3	MHN	3	F	44.1	153	55.7	30	39.1	16.6
4	MHN	4	F	40.7	151	44.0	27	31.9	12.1
5	MHN	5	M	27.1	177	64.6	10	58.1	6.5
6	MHN	6	F	35.0	167	59.5	25	44.6	14.9
7	MHN	7	F	38.3	163	48.8	22	38.0	10.8
8	MHN	8	F	21.6	162	54.8	25	41.2	13.6
9	MHN	9	M	32.1	178	59.1	9	53.7	5.4
10	MHN	10	M	20.1	176	80.4	21	63.3	17.1
Mean			7 F/3 M	32.2	165	59.6	23	46.0	13.6
± SE				2.5	3.0	3.2	2.5	3.1	1.6
P (MHN *vs.* MHE)				0.447	0.550	0.414	0.644	0.382	0.907
11	MHEh	11	F	38.6	173	60.9	24	46.3	14.6
12	MHEh	12	M	34.3	174	71.1	18	58.6	12.5
13	MHEh	2	M	28.6	188	86.7	16	73.3	13.4
14	MHEh	6	F	41.1	159	55.3	31	38.4	16.9
15 ± A49	MHEh	9	F	33.7	164	50.5	18	41.3	9.2
Mean			3 F/2 M	35.3	171.6	64.9	21.2	51.6	13.3
± SE				2.2	5.0	6.4	2.7	6.4	1.3
P (MHE *vs.* MHS)				0.607	0.403	0.748	0.675	0.493	0.688
16	MHS	8	F	48.9	161	71.3	33	47.6	23.7
17	MHS	2	F	35.5	164	42.0	18	34.4	7.6
18	MHS	6	M	39.8	179	64.5	15	54.8	9.7
19	MHS	8	M	25.5	163	59.1	8	54.2	4.9
20	MHS	6	M	38.8	183	78.0	20	62.4	15.6
21	MHS	13	F	51.8	157	57.1	28	41.3	15.8
22	MHS	13	F	23.6	170	54.6	21	43.4	11.2
23	MHS	13	F	47.9	162	57.9	24	43.8	14.1
24	MHS	14	F	49.2	175	87.7	40	52.9	34.8
25	MHS	13	F	20.5	155	52.2	24	39.8	12.4
Mean			7 F/3 M	38.2	166.9	62.4	23.1	47.4	15.0
± SE				3.7	3.0	4.2	2.8	2.7	2.7
P (MHN *vs.* MHS)				0.198	0.838	0.592	0.994	0.715	0.670

FFM = fat-free mass; FM = fat mass; MHN = malignant hyperthermia–negative; MHE = malignant hyperthermia–equivocal; MHEh = malignant hyperthermia–equivocal for halothane; MHS = malignant hyperthermia–susceptible.

Hospital[24] to measure the resistance and the reactance in triplicate at 0.5, 50, and 100 kHz after applying a 500-μA current from the right wrist to the right ankle. The arithmetical mean result of both techniques was used to calculate body FM and FFM. The participants then ate a standardized commercial meal (29% protein, 46% carbohydrate, 25% fat) providing 6.7 ± 1.3 kcal/kg body weight (mean ± SD). After a 10-h overnight fast, the participants underwent a hyperinsulinemic euglycemic clamp procedure as previously described.[25]

Euglycemic Hyperinsulinemic Clamp

With the person lying comfortably, two intravenous lines were inserted under local anesthesia (lidocaine 1%): one in a vein of the antecubital fossa of the left arm (injection line) and one in a vein on the dorsal side of the right hand; the right hand was kept in a heated environment (50°C) throughout the test to arterialize the blood samples drawn from this line.

To determine the fasting endogenous glucose production rate, we began the test at time 0 by a primed (16.5 μM/kg), continuous (0.16 $\mu M \cdot kg^{-1} \cdot min^{-1}$) infusion of [6,6-^{2}H]glucose until the start of the insulin infusion at time 150 min (fig. 1). The sterile [6,6-^{2}H]glucose had been diluted to a 1% concentration in NaCl 0.9% under laminar flow and tested for sterility and apyrogenicity. Because we also mixed [6,6-^{2}H]glucose with the exogenous glucose solution necessary to maintain euglycemia[26] (see no. 3 of clamp description), we reduced the [6,6-^{2}H]glucose infusion rate to 0.05 $\mu M \cdot kg^{-1} \cdot min^{-1}$

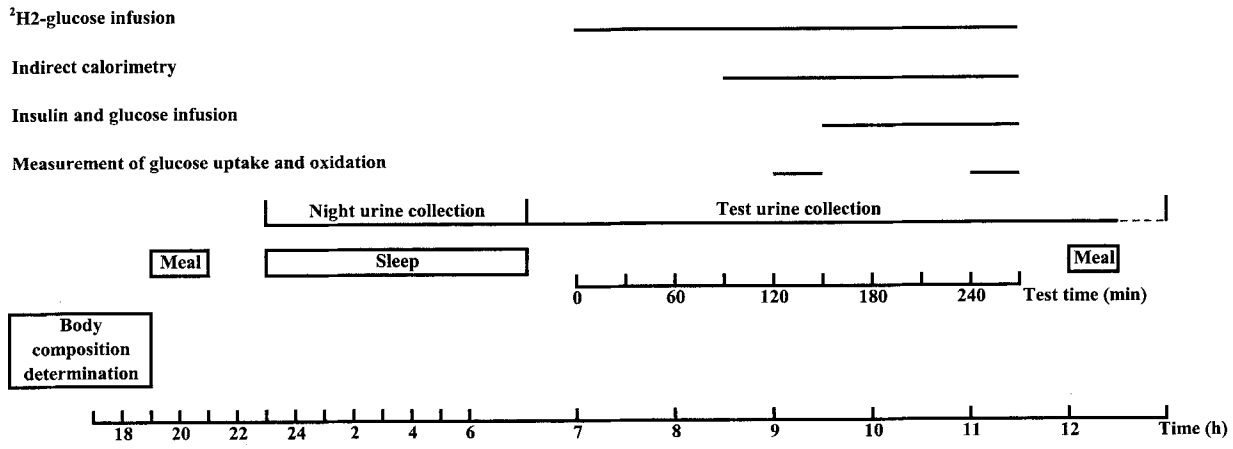

Fig. 1. Diagram of experimental plan.

during insulin infusion between time 150 and 270 min. The plasma deuterated glucose isotopic enrichment was measured in duplicate from samples drawn at 120, 135, 150 min during the fasting period and at 240, 255, and 270 min during the insulin-stimulated state.

The clamp (from time 150–270 min) consisted of: (1) a primed, continuous insulin (Actrapid HM, Novo, Bagsvaerd, Denmark) infusion (1 mU · kg^{-1} · min^{-1}); (2) a 4-mM/h KCl infusion to prevent hypokalemia; and (3) a variable infusion of 17.1% glucose (500 ml 20% unlabeled glucose and 90 ml 1% [6,6-^{2}H]glucose, 0.857 mole % excess) as necessary to maintain the plasma glucose concentration at 5.6 mM for all persons. An amount of NaCl 0.9% replacing the volume of blood drawn (≈ 100 ml) was injected continuously during the clamp. The plasma glucose concentration was measured in duplicate 30, 15, and 1 min before insulin infusion (during fasting) and then every 5 min during insulin infusion. During insulin infusion, the mean glucose and insulin plasma concentrations between 240 and 270 min were (mean ± SE) 5.7 ± 0.1 mM and 76 ± 4 mU/l in the MHN group, 5.6 ± 0.1 mM and 79 ± 5 mU/l in the MHS group, and 5.7 ± 0.1 mM and 83 ± 5 mU/l in the MHE group, with no significant intergroup difference.

Indirect Calorimetry

Respiratory exchange measurements, using a ventilated hood open-circuit indirect calorimeter,[27] started at 90 min and lasted until the end of the test. The airflow through the hood was approximately 40 l/min and was measured at the outlet by a pneumotachograph connected to a differential manometer (model 47303A digital pneumotachograph, Hewlett-Packard, Palo Alto, CA). The rates of carbon dioxide production (VCO_2) and of oxygen consumption (VO_2) were constantly determined by an infrared carbon dioxide analyzer (Uras T2, full-scale 0–1%, Hartmann and Braun, Frankfurt, Germany) and a thermomagnetic oxygen analyzer (Magnos T2, full-scale 19–21%, Hartmann and Braun) in the gas mixture leaving the hood. For fasting and insulin-stimulated calorimetric measurements, we used the four to six consecutive, stable, 5-min periods occurring between 90 and 150 min and 240 and 270 min, respectively (fig. 1).

Urinary collections were carried out the night before (duration [mean ± SD] = 8.3 ± 1.3 h) and during the test (duration = 6.4 ± 0.5 h) for total urinary nitrogen production rate determination (corrected for the change in urea nitrogen pool size,[28] as determined from plasma urea concentration samples drawn at time 120 and 150 min – baseline, and 240 and 270 min – under insulin stimulation). Protein oxidation rate (6.25 g protein/g urinary nitrogen) was calculated at baseline and during the insulin clamp procedure. The nonprotein respiratory quotient (VCO_2/VO_2) was then calculated and allowed the determination of the substrate oxidation rates and REE.[20]

Calculations and Analyses

The baseline endogenous glucose production rate was calculated from the plasma deuterated glucose isotopic enrichment measured between 120 and 150 min (mean ± SE: molar % excess = 1.41 ± 0.05%, 1.44 ± 0.07%, and 1.40 ± 0.03%, and coefficient of variation [CV] = 2.4 ± 1.6%, 2.9 ± 1.3%, and 2.3 ± 2.0% in the MHN, MHS, and MHE groups, respectively).

With no change in plasma glucose level, the total

glucose disposal rate during insulin infusion equals the total isotopically determined glucose appearance rate assessed at the same time (molar % excess = 1.07 ± 0.04%, 1.08 ± 0.03%, and 1.03 ± 0.04%, and CV = 1.9 ± 1.3%, 1.7 ± 1.3%, and 1.8 ± 1.0% in the MHN, MHS, and MHE groups, respectively, at 240, 255, and 270 min in the insulin-stimulated state). When the glucose appearance was equal to or smaller than the exogenous glucose infusion rate, the endogenous glucose production rate was assumed to be fully suppressed, and we considered that total glucose disposal rate was equal to the exogenous glucose infusion rate (mean rate administered between time 240 and 270 min). When the glucose appearance was greater than the exogenous glucose infusion rate, endogenous glucose production rate was considered not fully suppressed, and the isotopically-determined glucose appearance rate represented total glucose disposal rate. The total glucose disposal rate was corrected for changes in plasma glucose concentration when necessary by using a volume of glucose distribution equal to the person's extracellular water volume (20% of body weight). The difference between the total glucose disposal rate and the oxidative glucose disposal rate is the nonoxidative glucose disposal rate ("storage rate"). In humans, this rate mainly represents skeletal muscle glycogen deposition rate under insulin stimulation.[12]

We calculated the plasma glucose appearance rate using Steele's steady-state equations,[29] considering the low CVs observed in our isotopic enrichment and calorimetric measurements (in the MHN, MHS, and MHE groups, respectively: fasting VO_2 CV = 2.0 ± 0.3%, 2.0 ± 0.3%, 2.2 ± 0.5%; fasting VCO_2 CV = 2.4 ± 0.4%, 3.3 ± 0.5%, 3.1 ± 0.7%; insulin-stimulated VO_2 CV = 2.0 ± 0.2%, 2.1 ± 0.3%, and 2.5 ± 0.5%; and insulin-stimulated VCO_2 CV = 2.6 ± 0.3%, 3.0 ± 0.4%, 3.2 ± 0.5%).

Plasma glucose concentration was measured using the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, CA), urinary nitrogen was measured using the Kjeldahl method[30] (N Autoanalyzer, Technicon, Tarrytown, NY) and plasma urea concentration was measured enzymatically (Urea Analyzer, Beckman Instruments). Plasma insulin concentrations were assessed in duplicate by radioimmunoassay,[31] and the isotopic enrichment of plasma [6,6-^{2}H]glucose was measured in a gas chromatograph-mass spectrometer (Hewlett Packard, Waldbronn Analytical Division, Waldbronn, Germany) as previously described.[25]

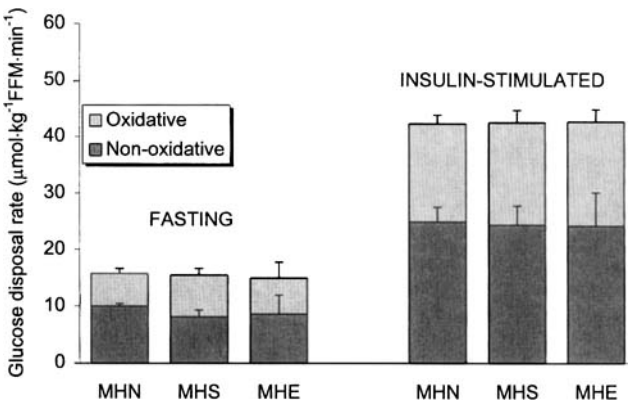

Fig. 2. Total, oxidative, and nonoxidative glucose disposal rates in 10 MHN, 10 MHS, and 5 MHE persons (mean ± SE). Values during both fasting conditions (*left*) and insulin-stimulated conditions (*right*) are shown.

Statistical Analysis

All data are presented as means ± SEM. Intergroup comparisons were performed using the Student unpaired *t* test and intersituational comparisons were performed using the paired *t* test. In the case of an abnormal distribution of one variable, the corresponding nonparametric test was used (Mann-Whitney rank sum test and Wilcoxon signed rank test). The Fisher exact test was used to compare the group gender ratios. Sample correlations are Pearson product-moment correlations. Multiple linear regression analysis was used to calculate predicted REE. A P value < 0.05 was considered to indicate statistical significance.

Results

Fasting Plasma Glucose Concentration

No significant difference was found in plasma glucose and plasma insulin concentrations among the MHN, MHS, and MHE groups (glucose: 5.3 ± 0.1 mM, 5.2 ± 0.1 mM, and 5.2 ± 0.1 mM; insulin: 10 ± 1 mU/l, 8 ± 1 mU/l, and 8 ± 1 mU/l, respectively).

Glucose Disposal Rates

As shown in figure 2, total, oxidative, and nonoxidative glucose disposal rates were not significantly different among the MHN, MHS, and MHE groups, both in the fasting and insulin-stimulated conditions.

Fasting and insulin-stimulated lipid oxidation rates were 1.07 ± 0.08 and 0.44 ± 0.09 mg · kg^{-1} FFM · min^{-1} in the MHN group, 1.13 ± 0.06 and 0.46 ± 0.10 mg · kg^{-1} FFM · min^{-1} in the MHS group, and 0.87 ± 0.25

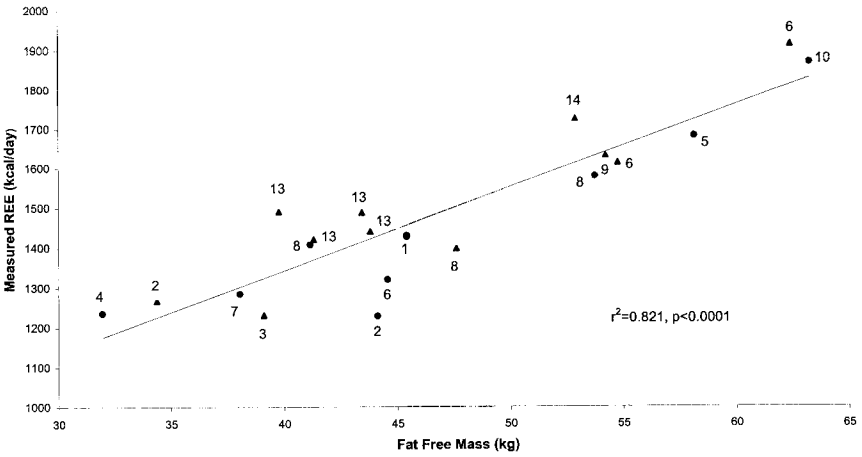

Fig. 3. Relation between insulin-stimulated resting energy expenditure (REE) and fat-free mass (determined by the skinfold thickness and bioimpedance techniques) in 20 volunteers: 10 MHN persons (circles) and 10 MHS persons (triangles). The figures next to the symbols represent the family numbers as they appear in table 1.

and 0.10 ± 0.03 mg · kg^{-1} FFM · min^{-1} in the MHE group. Fasting and insulin-stimulated protein oxidation rates amounted to 1.55 ± 0.15 and 1.29 ± 0.15 mg · kg^{-1} FFM · min^{-1} in the MHN group, 1.26 ± 0.15 and 1.33 ± 0.17 mg · kg^{-1} FFM · min^{-1} in the MHS group, and 1.50 ± 0.47 and 1.51 ± 0.24 mg · kg^{-1} FFM · min^{-1} in the MHE group. Again, there was no significant intergroup difference.

Resting Energy Expenditure

Fasting REE was 1413 ± 55 kcal/day in the MHN group, 1497 ± 61 kcal/day in the MHS group and 1440 ± 85 kcal/day in the MHE group; there was no significant difference among groups. Expressed per unit of FFM, fasting REE was 31.4 ± 1.3, 31.9 ± 1.0, and 28.9 ± 2.4 kcal · kg^{-1} FFM · day^{-1} in the MHN, MHS, and MHE groups, respectively; there was no significant difference among groups.

Under insulin stimulation, REE was 1427 ± 69 kcal/day, or 31.5 ± 1.0 kcal · kg^{-1} FFM · day^{-1}, in the MHN group; 1539 ± 59 kcal/day, or 32.8 ± 1 kcal · kg^{-1} FFM · day^{-1}, in the MHS group; and 1468 ± 100 kcal/day, or 29.4 ± 2 kcal · kg^{-1} FFM · day^{-1}, in the MHE group; there was no significant difference among groups.

Fat-free mass is the most important determinant of whole-body REE,[17] as shown in figure 3, which depicts the relation between FFM and REE in the 10 MHN and 10 MHS persons in the current study.

In Ravussin's model,[17,18] body FM, age, and gender are considered, along with FFM, to explain interindividual REE variance. Using the same model, we determined the respective contribution of these four physical covariates to the REE that we measured in our 20-person population (10 MHN and 10 MHS). By multiple linear regression, we found that FFM, FM, age, and gender together explained 81 and 83% of the observed variance in fasting and insulin-stimulated REE, respectively. For each of the 10 MHN and 10 MHS persons, we calculated a value of *predicted* REE using the following formula:

$$\text{Predicted fasting REE (kcal/day)}: = 700 + 15.0 \cdot \text{FFM} + 3.4 \cdot \text{FM} - 0.5 \cdot \text{age} + 75.5 \cdot \text{gender} \quad (1)$$

$$\text{Predicted insulin-stimulated REE (kcal/day)}: = 671 + 16.8 \cdot \text{FFM} + 4.1 \cdot \text{FM} - 1.6 \cdot \text{age} + 79.0 \cdot \text{gender} \quad (2)$$

in which an arbitrary value of 0 was attributed to female subjects and a value of 1 was attributed to male subjects.

The difference between measured and predicted REE is the residual REE, which represents the part of daily energy expenditure not explained by its four main determinants, FFM, FM, age, and gender.[17] The comparison of fasting and insulin-stimulated residual REE between MHN and MHS persons is shown in figure 4. MHS persons display a 6.4% higher residual REE (Δ kcal/insulin-stimulated REE_{MHN} = 91/1427) than MHN individuals under insulin stimulation. This small difference could not be found in the fasting condition.

Discussion

The main finding of our study was that MHS and MHE persons, compared with MHN persons, do not exhibit any gross alteration of insulin action and energy expenditure. Despite *in vivo* reported signs (higher glucose[7]

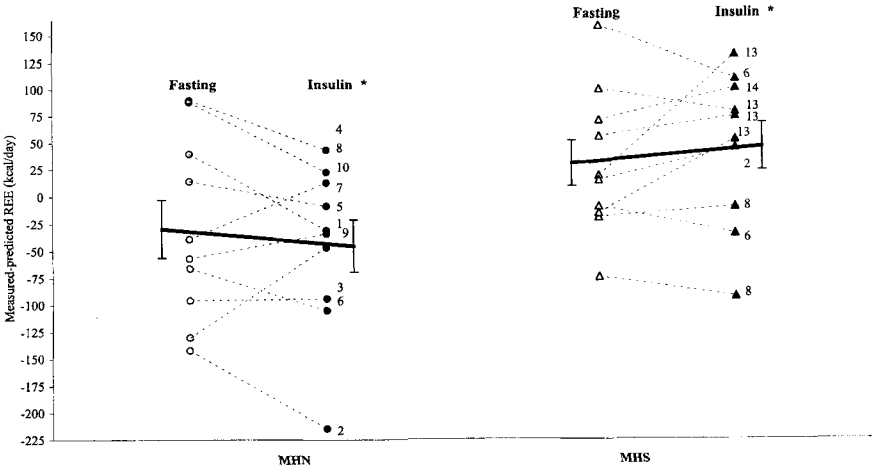

Fig. 4. Comparison of fasting and insulin-stimulated residual REE between 10 MHN and 10 MHS persons. Residual REE was higher in MHS than in MHN persons during insulin stimulation (*P = 0.013), even after exclusion of an MHN person from family number 2 (P = 0.022). Residual REE was not different between MHN and MHS persons in the fasting state (P = 0.1). Circles = MHN; triangles = MHS; open symbols = fasting; filled symbols = under insulin stimulation; continuous line = group mean (± SE). The figures beside the symbols represent the family numbers as they appear in table 1.

and insulin[8,9] plasma concentrations) and biochemical muscle findings (elevated $[Ca^{2+}]_c$[11] and myophosphorylase activity[10]) compatible with a chronic insulin-resistant state of MHS individuals, we were unable to document evidence of insulin resistance in MHS persons in this study. Our results are in agreement with values of glucose total, oxidative, and nonoxidative disposal rates measured in normal control subjects[32] under physiologic insulin stimulation (1 mU · kg^{-1} · min^{-1}). These parameters were similar in the 10 MHN and 10 MHS persons in the current study, thus showing an unimpaired *in vivo* insulin action[12] in MHS persons. In addition, unlike Rutberg *et al.*[7] who found a 12% higher plasma glucose level in MHS persons, we measured normal fasting plasma glucose concentrations in our MHS persons, as did Campbell *et al.*[8] Although a 133% greater fasting insulinemia was reported in MHS persons,[8] we observed normal fasting plasma insulin concentrations in our MHS participants, which confirms the findings of other studies.[7]

The body composition data show no difference between MHS and MHN persons in terms of body weight, height, FM, and FMM (table 1). This is in agreement with previous results[7] but in contrast to other data; using the skinfold method[22] Campbell *et al.*[33] found a slightly lower (4.6%) body percent fat in male, but not female, MHS humans compared with biopsied control subjects. Similarly, MHS pigs have a larger lean body mass (2–3%) than control pigs.[1] This may be due to the genetic heterogeneity of human MH compared with MH in pigs which is associated with one single genetic mutation.

In agreement with the results of previously published studies,[7,8,14] fasting whole-body REE was the same in our 10 MHS and 10 MHN persons. REE was also the same in both groups under insulin stimulation. Despite an increase in nonoxidative glucose disposal, which corresponds to a stimulation of muscle glycogen synthesis, REE under insulin stimulation was not increased, as reported by Thiébaud *et al.*[32] with the same insulin infusion in control subjects. This might be due to an insulin-induced inhibition of gluconeogenesis in the liver, which is an energy-requiring process.

REE represents 60–70% of daily total energy expenditure in humans and can vary among individuals with the same body size and composition.[17] Skeletal muscle accounts for as much as 40% of body weight, as much as 30% of REE,[17] and explains as much as 50% of the whole-body REE variability in humans.[34] The major energy-requiring process of calcium transport in the muscle cell is the SERCA, which maintains calcium homeostasis by pumping calcium from the cytosol back into the sarcoplasmic reticulum. According to Chinet *et al.*,[35] the active Ca^{2+} pumping by the mouse muscle sarcoplasmic reticulum might account for 25–40% of the total muscle energy expenditure in basal or near-basal conditions (*i.e.*, a possible calculated 7.5–12% of whole-body REE). HEK-293 cells, which do not spontaneously express RYR1, exhibit a twofold increase in their SERCA expression when transfected by RYR1 from human wild-type and MH-mutated-type above untransfected cells,[6] as in an attempt to compensate for the leak of calcium through the RYR1 into the cytoplasm and maintain a normal resting $[Ca^{2+}]_c$. If MH susceptibility implied a permanent leak of calcium out of its intracellular muscle stores, then the energy cost of a compensation mechanism maintaining calcium homeostasis in muscle might be reflected in whole-body REE. Whereas the absolute value of REE was the same in MHN and MHS persons, we

found a slightly higher (6.4%) residual REE in MHS persons during insulin stimulation which was undetectable in the fasting condition.

The significantly greater residual REE observed in MHS persons under insulin stimulation might be related to a role played by calcium, which is incompletely understood in insulin-stimulated target tissues. In adipocytes,[36] insulin exposure has been reported to increase cytosolic calcium concentration. In mouse skeletal muscle fibers,[37] "near-membrane cytosolic" calcium concentration is dose-dependently elevated by insulin stimulation. This calcium increase might be enhanced in MHS muscle, leading to a corresponding extra amount of energy necessary for SERCA to maintain a normal cytosolic calcium concentration, such as directly measured in MHS human muscle cells.[38,39]

This study indicates that 10 MHN, 10 MHS, and 5 MHE persons with the same body composition have similar fasting plasma glucose and insulin concentrations, whole-body energy expenditure, and insulin sensitivity. In groups formed by 10 MHN and 10 MHS persons, we calculated a value of REE predicted by the determinants FFM, FM, age, and gender. The difference between the measured and the predicted REE, (*i.e.*, the residual REE) was slightly higher in MHS persons, but only during insulin stimulation. This finding suggests that insulin might challenge the energy balance of MHS muscle. Further studies are needed to investigate the role of MH mutations in muscle energy expenditure and its calcium homeostasis.

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