Impact of hyperinsulinemia on myosin heavy chain gene regulation

JOSEPH A. HOUMARD, D. SEAN O'NEILL, DONGHAI ZHENG, MATTHEW S. HICKEY, AND G. LYNIS DOHM
Human Performance Laboratory, Department of Exercise and Sport Science and School of Medicine, and Department of Biochemistry, East Carolina University, Greenville, North Carolina 27858

Houmard, Joseph A., D. Sean O'Neill, Donghai Zheng, Matthew S. Hickey, and G. Lynis Dohm. Impact of hyperinsulinemia on myosin heavy chain gene regulation. J. Appl. Physiol. 86(6): 1828–1832, 1999.—The purpose of this study was to determine whether hyperinsulinemia alters myosin heavy chain (MHC) gene expression in human skeletal muscle. A biopsy from the vastus lateralis was obtained in young, lean [age 24.6 ± 1.0 (SE) yr, body fat 11.9 ± 1.9%, body mass index 26.1 ± 1.1 kg/m²; n = 10] men before and after 3 h of hyperinsulinemia (hyperinsulinemic-euglycemic clamp). Muscle was analyzed for mRNA of type I, IIA, and IIX MHC isoforms. Hyperinsulinemia (mean of 1,065.7 ± 9.8 pmol/l during minutes 20 to 180) did not change (P > 0.05) the mRNA concentration of either the type I MHC or type IIA MHC isoforms. In contrast, type IIX MHC mRNA increased (P < 0.05) with hyperinsulinemia compared with the fasted condition. These data indicate that hyperinsulinemia rapidly increases type IIX MHC mRNA in human skeletal muscle.

THE IMPACT OF HYPERINSULINEMIA ON MYOSIN HEAVY CHAIN GENE REGULATION

There is some evidence that the morphology of skeletal muscle is altered in the insulin-resistant state. Several research groups have observed a higher percentage of glycolytic, fast-twitch, white (type IIB) muscle fibers in individuals with non-insulin-dependent diabetes mellitus (NIDDM) compared with lean controls (9, 18). An increased percentage of type IIB muscle fibers was also evident in insulin-resistant, first-degree relatives of NIDDM patients (22). Other work indicates an increased expression of type IIB muscle fibers in conditions where insulin resistance is prevalent such as obesity, chronic heart failure (CHF), and NIDDM (2, 13, 14, 16, 17, 19, 31, 33, 35, 36). Together, these data suggest a relationship between the expression of type IIB muscle fibers and the insulin-resistant state.

In support of this relationship, 7 days of hyperinsulinemia increased the concentration of type IIB muscle fibers in the oxidative, slow-twitch, red (type I) soleus in rats (10). This finding, coupled with the observation that fasting and/or postprandial hyperinsulinemia is prevalent with insulin resistance (4), suggests that hyperinsulinemia may regulate the expression of type IIB muscle fibers. To test this hypothesis, we determined whether hyperinsulinemia (hyperinsulinemic-euglycemic clamp) influences myosin heavy chain gene expression (MHC type I, IIA, IIX mRNA) in human skeletal muscle. Hyperinsulinemia was induced with a 3-h hyperinsulinemic-euglycemic clamp, and the mRNA responses of the MHC isoforms were measured as an index of gene expression in muscle samples from healthy volunteers.

METHODS

Experimental design. Subjects were initially screened and tested for anthropometric characteristics to ensure that non-obese, healthy individuals were studied. Subjects refrained from exercise and alcohol consumption and consumed a diet containing a minimum of 250 g of carbohydrate/day in the 48 h preceding testing. Subjects reported to 0630 after a 12-h fast and rested in the supine position for 60 min. A muscle sample was obtained with the needle-biopsy technique from the vastus lateralis. Approximately 20 min after the biopsy, a 3-h euglycemic, hyperinsulinemic glucose clamp was initiated. After 3 h of hyperinsulinemia, muscle tissue was extracted from the contralateral leg. Muscle was subsequently analyzed for MHC mRNA isoform content (I, IIA, IIX).

The rationale for measuring the type IIX isoform in human skeletal muscle was the following. Mature rodent skeletal muscle is characterized by electrophoretically distinct MHCs, the predominant isoforms being the I/IIA or IIX isoforms in humans and type I MHC in rodents. In rats, antimyosin immunocytochemistry indicates that type IIX MHC isoforms correspond with the appropriate histochemically determined fiber type (6, 29, 30, 32). In rats, antimyosin immunocytochemistry indicates that type IIX MHC isoforms correspond with the appropriate histochemically determined fiber type (6, 29, 30, 32). In rats, antimyosin immunocytochemistry indicates that type IIX MHC isoforms correspond with the appropriate histochemically determined fiber type (6, 29, 30). In contrast, in human skeletal muscle, type IIX MHC transcripts are abundant in the histochemically type IIB fibers; there is no type IIB MHC isoform expressed in human tissue (30, 32).

Subjects. Subjects were 10 young [age 24.6 ± 1.0 (SE) yr] men (height 179.2 ± 1.9 cm, weight 82.8 ± 3.8 kg). Inclusion criteria were no medications that could affect metabolism and normative body composition. Body composition was determined from seven skinfold sites (11) (11.9 ± 1.7% body fat) and body mass index (26.1 ± 1.1 kg/m²).

Hyperinsulinemic-euglycemic clamp. The hyperinsulinemic-euglycemic clamp was performed according to a modification of the method of Defronzo et al. (5). An intravenous catheter was placed in an antecubital vein for infusion of insulin and glucose. Another catheter was placed retrograde in a dorsal hand vein for blood sampling. This hand was kept in a warming box at 70°C. Four samples were obtained at 10-min increments to determine fasting glucose and insulin concentrations. A primed continuous infusion of insulin (Humulin, Eli Lilly, Indianapolis, IN) at a dose of 600 pmol · m⁻² · min⁻¹ was used; 4 min after the start of insulin infusion, a variable 20% glucose infusion was initiated. Blood samples were obtained every 5 min, centrifuged, and autoanalyzed for serum glucose (Glucose II Analyzer, Beckman, Fullerton, CA). Samples for insulin were obtained every 10 min and

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
centrifuged, and plasma was stored at -70°C for subsequent analysis. Insulin concentration was determined by microparticle enzyme immunoassay (IMx, Abbott Laboratories, Abbott Park, IL). An M value, which is equal to the rate of whole body glucose disposal at the given insulin concentration, was calculated from the final 60 min of the clamp (5). A higher M value is indicative of enhanced insulin action, mainly in skeletal muscle (5). Because of a computer malfunction in storing the data, an M value was obtainable in 9 of the 10 subjects.

Muscle analysis. After the biopsy, muscle specimens were quick frozen and stored in liquid nitrogen. Total RNA was subsequently isolated by using TRIzol reagent (GIBCO-BRL, Gaithersburg, MD). Briefly, the tissue was homogenized in 1 ml of TRIzol. Chloroform (200 μl) was added, and the sample was vortexed vigorously for 15 s and incubated at room temperature for 5 min. Samples were microfuged for 15 min (12,000 g) at 4°C, and 400 μl of the top aqueous layer were transferred to a fresh microfuge tube. RNA was precipitated by the addition of equal volumes of isopropanol and incubated at room temperature for 10 min. Samples were microfuged for 10 min (12,000 g) at 4°C. RNA pellets were washed with 1 ml of 70% ethanol. After a brief air dry, pellets were resuspended in 50 μl of diethyl pyrocarbonate-treated water.

Muscle was analyzed for MHC isoform (I, IIa, IIx) mRNA by using the RNase protection assay. The cDNA probes for the DNA complementary to type IIa and IIx MHC were obtained from Leslie Leinwand (University of Colorado, Boulder, CO; Ref. 32). The cDNA for type I MHC was obtained from Kirti Bhatt (University of Rochester, Rochester, NY; Ref. 37). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plasmid template was purchased from Ambion (Austin, TX). Plasmids containing type IIa and IIx MHC were linearized with XbaI and XhoI, respectively. Plasmids containing type I MHC were linearized with XhoI. Labeled antisense RNA probes for type I, IIa, and IIx MHC and GAPDH were synthesized by using [α-32P]UTP and a RNA polymerase T3/T7 MAXscript in vitro transcription kit (Ambion, Austin, TX). The RNase protection assay was done with a commercially available kit (RPA II, Ambion). The protected RNA sample was electrophoretically separated on a 6% polyacrylamide gel containing 7 M urea, visualized by autoradiography, and quantitated by using phosphorimagery analysis (Molecular Dynamics, Sunnyvale, CA). Although we hybridized total sample RNA with both GAPDH and MHC probes under conditions of probe excess, the procedure was tested and verified by comparison to single probe hybridization in a previous experiment (unpublished observations). The linear range of the assay was verified by comparing 20 μg yeast RNA and 1, 2, and 5 μg total RNA from human muscle. The phosphorimagery data used for the quantitative analysis were obtained in this linear range. A total of 2 μg RNA per sample were used in the assay. The molecular sizes of protected fragments and full-length probes for types IIx, I, and IIa MHC and GAPDH were verified by comparison with a RNA molecular ladder (Century Size Markers, Ambion, TX). Values were normalized to GAPDH. Data were also normalized by assuming uniform loading and assigning the fasting mRNA reading within a subject a value of 1.0, and relative change was calculated. Values are expressed as dimensionless ratios, and interpretation was similar with use of either normalization technique. Muscle (n = 8) was also mounted in a tragacanth gum-OCT (Miles Laboratories, Elkhart, IN) mixture and frozen in isopentane cooled over liquid nitrogen. Mounted muscle was subsequently sectioned (10 μm) at -20°C and stained by using the ATPase technique at pH 4.54 for the determination of fiber type (3). The muscle samples were magnified and manually classified and counted according to their staining intensity (3).

Statistics. Variables were compared with repeated measures analysis of variance at the P < 0.05 level. Post hoc comparisons were performed by using a Fisher’s protected least significant differences test. Single-order Pearson product-moment correlations (P < 0.05) were used to examine relationships between given variables.

RESULTS

Euglycemic clamp. Fasting glucose and insulin concentrations were 5.1 ± 0.1 mmol/l and 39.0 ± 8.4 pmol/l, respectively. Mean glucose and insulin concentrations for minutes 20–180 of the clamp were 4.9 ± 0.1 mmol/l and 1,065.7 ± 9.8 pmol/l, respectively. Mean M value calculated from the final 60 min of the clamp was 52.2 ± 5.0 μmol·min⁻¹·l⁻¹.

Skeletal muscle. As presented in Figs. 1 and 2, hyperinsulinemia produced a significant (P < 0.05) increase in type IIx MHC mRNA concentration in the vastus lateralis. When fasting MHC mRNA was assigned a value of 1.0 (Fig. 2), the relative change in type IIx MHC mRNA (1.44 ± 0.23) with hyperinsulinemia was significantly (P < 0.05) greater than for the other mRNA (GAPDH, 0.96 ± 0.09; MHC I, 0.92 ± 0.14; type IIa MHC, 1.08 ± 0.09). This difference could not be attributed to sampling from contralateral legs, because we have observed that type IIx MHC mRNA varied by only 6% between legs in similar subjects (n = 10) with no consistent pattern between dominant and nondominant limbs (data not shown). In contrast to studies in adipocyte and hepatoma cell lines (1, 20, 23), we observed no increase in GAPDH mRNA (0.28 ± 0.03 vs. 0.27 ± 0.01 arbitrary units, test-retest correlation of r = 0.76, P < 0.05) in human skeletal muscle with

![Fig. 1. Detection and measurement of type IIx myosin heavy chain (MHC) mRNA in human skeletal muscle. Presented is a representative ribonuclease protection assay with 32P-labeled riboprobes, yielding a 122-bp protected fragment for human type IIx MHC mRNA and a 315-bp fragment for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Two micrograms of total RNA from each individual sample were incubated with GAPDH and type IIx MHC riboprobes, digested with ribonuclease, resolved on polyacrylamide gels, and identified by phosphorimagery. Presented is a typical ribonuclease protection assay of type IIx MHC mRNA in the fasted, control state (lane C) and after 3 h of hyperinsulinemia (lane I) in 3 subjects. Type IIx MHC mRNA increased in 7 of the 10 subjects.](http://jap.physiology.org/Downloadedfrom)
insulin. When normalized to GAPDH, hyperinsulinemia increased MHC IIx mRNA by 25% from a fasting value of 0.17 ± 0.02 to 0.22 ± 0.03 arbitrary units (P < 0.05). There were no changes in type I MHC (0.33 ± 0.02 vs. 0.30 ± 0.05 arbitrary units) and type IIa MHC (0.19 ± 0.01 vs. 0.20 ± 0.01 arbitrary units) with this normalization strategy. Type IIa MHC IIx mRNA increased in 7 of the 10 subjects with either normalization technique.

As presented in Fig. 3, there was a negative relationship between insulin action (M) and the relative percentage of type IIb muscle fibers (r = −0.75, P < 0.05). Histochemically determined muscle fiber type (184 ± 25 fibers counted) was type I, 47.6 ± 3.2%; type IIa, 44.2 ± 3.4%; and type IIb, 8.2 ± 1.7%.

**DISCUSSION**

The main finding of the present study was that hyperinsulinemia rapidly increased the mRNA of the type IIx MHC isoform in human skeletal muscle. Because type IIx MHC mRNA is the predominant isoform in human type IIb muscle fibers (30, 32), our data support the findings of Holmang et al. (10) that 7 days of hyperinsulinemia increased the relative percentage of type IIb muscle fibers in rat soleus. The present data extend this finding (10) by demonstrating that MHC IIx mRNA increases with only 3 h of hyperinsulinemia in human skeletal muscle.

The MHC isoforms are thought to be primarily transcriptionally controlled (12, 25, 29, 30). Insulin can directly affect the rate of gene transcription and/or mRNA stability through various signaling mechanisms or indirectly control transcription through the suppression of plasma free fatty acid concentration or other factors (15, 20, 23). The cellular mechanism by which insulin could modify MHC mRNA content is not evident from our findings. Regardless, the physiological situation studied closely replicates the cellular milieu that the skeletal muscle cell is exposed to with hyperinsulinemia. Insulin may thus exert a rapid regulation at the transcriptional level for the type IIx MHC isoform, as with other genes (1, 20, 23). On the other hand, no changes in the type I or IIa MHC isoforms were apparent with this relatively acute stimulus (Fig. 2); the impact of more chronic hyperinsulinemia on these isoforms is not evident from the present findings.

Although some of the variance in fiber type between individuals can be genetically explained, the majority has been attributed to environmental factors (25, 29, 30). Data from the present study indicate that hyperinsulinemia provides, at least in part, a potential explanation for the increase of type IIb muscle fibers in conditions where either a fasting or postprandial hyperinsulinemia is present. This relationship has been...
proposed by others (10, 18, 21) but has not been directly tested in human skeletal muscle.

In the present study, MHC protein distribution was not measured because it was doubtful that a change would occur in such an acute period of time, due to the relatively long half-life of the MHC proteins (12, 25). In support of an eventual regulation at the protein level, an increased concentration of type IIb muscle fibers, which predominantly express MHC type IIX mRNA (32), has been reported in human skeletal muscle in the insulin-resistant states of obesity and NIDDM (2, 13, 14, 16, 17, 19, 31, 35, 36). An increase in type IIb fibers has also been reported with hyperinsulinemia in rodents (10). As more direct evidence, an increase in the proportion of type IIX MHC fibers has been reported in skeletal muscle (vastus lateralis) obtained from patients with CHF (33). Pronounced insulin resistance and hyperinsulinemia are evident with CHF (24, 34). Insulin has also been demonstrated to exert a regulatory role in controlling MHC isofrom expression at the mRNA and protein levels in cardiac muscle (8).

Despite such evidence, we cannot definitely conclude from our data that muscle morphology is altered at the MHC protein level with hyperinsulinemia. For example, significant and rapid increases in GLUT-4 mRNA in human skeletal muscle were evident during insulin infusion (15, 28). GLUT-4 protein levels were not, however, altered in insulin-resistant/hyperinsulinemic conditions such as obesity or NIDDM (7). This indicates that protein expression is controlled at multiple levels with insulin exposure (20, 23), which may indeed also be the case with the type IIX MHC isofrom. The present results are still, however, important in that they indicate that hyperinsulinemia exerts a rapid influence at the transcriptional level for the type IIX MHC protein. This effect, in combination with other mechanisms, would ultimately determine MHC IIX protein expression in human skeletal muscle.

The regulation of the MHC IIX isofrom is potentially important because an increased expression could have an impact on functional capacity in insulin-resistant individuals. The contractile characteristics of the type IIb and IIX MHC protein are not energetically efficient and not conducive toward sustaining contractile activity (30). An increased expression of IIb and IIX fibers could thus be at least partially responsible for the reduced exercise tolerance reported in male and female NIDDM patients and their insulin-resistant relatives (21, 27).

In conclusion, these data indicate that hyperinsulinemia rapidly increases type IIX MHC mRNA in human skeletal muscle. Insulin may thus contribute, at least in part, to regulating MHC gene expression.

The authors thank C. Torrence, T. Chaplinksi, R. Israel, D. Snyder, K. Ways, L. Morgan, S. Atkinson, and the Ambulatory Medical Unit at Pitt County Community Hospital. This work was supported by National Institute on Aging Grant AG-10025 (J. A. Houmard).

Address for reprint requests and other correspondence: J. A. Houmard, Human Performance Laboratory, Ward Sports Medicine Bldg., East Carolina Univ., Greenville, NC 27858 (E-mail: HOUMARD)@MAIL.ECU.EDU.

Received 5 October 1998; accepted in final form 28 January 1999.

REFERENCES


