Regulation of protein synthesis after acute resistance exercise in diabetic rats

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Farrell, Peter A., Mark J. Fedele, Thomas C. Vary, Scot R. Kimball, Charles H. Lang, and Leonard S. Jefferson. Regulation of protein synthesis after acute resistance exercise in diabetic rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E721–E727, 1999.—These studies determined whether insulin-like growth factor-I (IGF-I) involvement in exercise-stimulated anabolic processes becomes more evident during hypoinsulinemia. Male Sprague-Dawley rats (n = 6–12/group) were made diabetic (blood glucose ≈ 300 mg/dl) by partial pancreatectomy (PPX) or remained nondiabetic (blood glucose ≈ 144 mg/dl). Rats performed acute resistance exercise by repetitive standing on the hindlimbs with weighted backpacks (ex), or they remained sedentary (sed). Resistance exercise caused increases in rates of protein synthesis (nmol Phe incorporated·g muscle·h−1), measured for gastrocnemius muscle in vivo 16 h after exercise for both nondiabetic (sed = 154 ± 6 (SE) vs. ex = 189 ± 7) and diabetic rats (PPXsed = 152 ± 11 vs. PPXex = 202 ± 14, P < 0.05). Arterial plasma insulin concentrations in diabetic rats, =180 pM, were less than one-half those found in nondiabetic rats, =444 pM, (P < 0.05). The activity of eukaryotic initiation factor 2B (eIF2B; pmol GDP exchanged/min) was higher (P < 0.05) in ex rats (sed = 0.028 ± 0.006 vs. ex = 0.053 ± 0.015; PPXsed = 0.033 ± 0.013 vs. PPXex = 0.047 ± 0.009) regardless of diabetic status. Plasma IGF-I concentrations were higher in ex compared with sed diabetic rats (P < 0.05). In contrast, plasma IGF-I was not different in nondiabetic ex or sed rats. Muscle IGF-I (ng/g wet wt) was similar in ex and sed nondiabetic rats, but in diabetic rats was 2- to 3-fold higher in ex (P < 0.05) than in sed rats. In conclusion, moderate hypoinsulinemia that is sufficient to alter glucose homeostasis does not inhibit an increase in rates of protein synthesis after acute moderate-intensity resistance exercise. This preserved response may be due to a compensatory increase in muscle IGF-I content and a maintained ability to activate eIF2B.

growth factors; peptide chain initiation

TRANSLATION of mRNA into new protein is a complex and highly regulated process. Complexity exists both external and internal to the cell, with many hormones (24), including insulin (25) and insulin-like growth factor I (IGF-I) (24), exerting partial regulatory control depending on the physiological need to increase, decrease, or maintain rates of protein synthesis. The first step in translation, referred to as peptide-chain initiation, is of particular importance because under certain conditions the overall rate of protein synthesis can depend on the functional status of the initiation step. The role of insulin in regulating peptide-chain initiation has received considerable attention (33). Much of the available information is based on models in which rates of protein synthesis were measured in the presence or total absence of insulin (1, 28). These studies clearly show that rates of protein synthesis in skeletal muscle are lower in severely hypoinsulinemic rats. Plasma glucose concentrations in some of these studies were 600–900 mg/dl, a degree of hyperglycemia that, if sustained, is not compatible with normal growth or survival. More information is needed on the effects of moderate hypoinsulinemia on protein metabolism.

Insulin exerts many of its effects on peptide-chain initiation by regulating the activity and/or state of phosphorylation of eukaryotic initiation factors (eIFs) (41). One of these factors, eIF2B, regulates the exchange of GTP for GDP complexed to eIF2. During resting conditions, reduced eIF2B activity correlates with reduced rates of protein synthesis in skeletal muscle from severely diabetic rats (28, 31), but this reduction is quickly reversed by insulin treatment. One of the goals of the present study was to determine the importance of insulin in regulating the activity of eIF2B under in vivo conditions that required elevated rates of protein synthesis. Acute resistance exercise provided such conditions.

Changes in rates of protein synthesis after resistance exercise in persons with insulin-dependent diabetes mellitus (type 1) or in rat models of type 1 diabetes have not been reported. We chose a model of mild hypoinsulinemia because data from such a model would be more applicable to insulin-treated type 1 diabetes than would models in which no insulin is available and the life expectancy of the rat is limited to a few days. Given the importance of insulin for regulating peptide-chain initiation, we first wanted to verify a previous finding (14) that moderately hypoinsulinemic rats can elevate rates of protein synthesis after moderate-intensity resistance exercise to the same magnitude as rats with normal pancreatic function. If this ability was maintained, we wanted to determine whether compensatory mechanisms help to explain this finding. In the present study, we determined whether there were associated changes in anabolic factors after resistance exercise in hypoinsulinemic rats. One factor investigated was IGF-I.

Juraskinski and Vary (27) demonstrated that sepsis results in reduced rates of protein synthesis in gastrocnemius of rats because of an inhibition of peptide-chain
initiation, but IGF-I is capable of relieving this block as well as stimulating muscle protein synthesis (27) in both septic and control rats. Severe hypoinsulinemia also results in reduced rates of protein synthesis due to an inhibition of peptide-chain initiation (33). Because we have previously shown that some insulin must be present for normal postresistance exercise elevations in protein synthesis to occur (19), and because Yan et al. (48) demonstrated that IGF-I immunocytoreactivity in tibialis anterior muscle was elevated 4 days after eccentric exercise but not before this time, we reasoned that IGF-I may also help to relieve a block in peptide-chain initiation after resistance exercise in diabetic rats.

The physiological stress of resistance exercise results in transient elevations in rates of protein synthesis (4); however, little is known about the effects of this stress on cytosolic factors that determine the capacity for mRNA translation or the efficiency with which mRNA is translated. As an example, a reduction in the amount of total RNA in muscle is evident in severely diabetic rats (1, 15, 30) and may be present in long-term moderately diabetic rats. Such reductions could limit an ability to increase rates of protein synthesis in response to resistance exercise. Therefore, we designed the present study to assess potential regulators of mRNA translation from the viewpoint of insulin availability.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Pennsylvania State University. Male Sprague-Dawley rats were used in all experiments and were housed in temperature- and humidity-controlled holding facilities with lights on at 0700 and off at 1900. Rats were fed ad libitum a standard rodent diet, PMI Feeds 5001, which contained 24% protein, 12% fat, 50% carbohydrate, 7% ash, 6% fiber, and vitamins.

Partial pancreatectomy. These studies required the use of rats that were diabetic and whose glucose concentrations were not controlled by daily exogenous insulin. Although this can be accomplished (5) by using nonlethal amounts of cytotoxic drugs (streptozotocin or alloxan), such drugs were not used in this study because their effects are not limited exclusively to the pancreatic β-cell (21, 23, 34, 39, 42, 44). The partial pancreatectomy (PPX) procedure was modified to include the use of rats that weighed 110–140 g, as opposed to the weights (90–110 g) suggested by Foglia (20). We find that a larger percentage (=80%) of the animals become diabetic when heavier rats are pancreatectomized. We also used a microcauterizer to eliminate small pancreatic blood vessels and to reduce bleeding during surgery. Sterile conditions were maintained throughout the surgery. Rats were anesthetized using methoxyflurane and were kept on a heated surgical pad. The procedure requires the physical removal of regions while major blood vessels are left intact. This is accomplished by using sterile cotton Q-tips. Pancreatic tissue between the bile duct and the duodenum is not removed, because this approximates 10% of the original total pancreatic tissue. At the conclusion of surgery, rats were given ampicillin (5 mg/100 g body weight) as an antimicrobial agent. Two weeks after PPX, a tail vein blood sample was obtained in the fed state to determine blood glucose concentrations, and rats that were not diabetic (<175 mg/dl) were eliminated from the study. In previous studies (13, 14), we had observed reduced rates of somatic growth after partial pancreatectomy. The timing of the exercise component of these studies was such that the diabetic rats were slightly (<1 mo) older than non diabetic rats. This allowed us to study groups that were not markedly different in total body weight.

Resistance exercise. Details of the exercise protocol have been previously described (17). Briefly, rats were operantly conditioned to touch an illuminated bar low on a Plexiglas exercise cage and then were taught to stand and touch an illuminated bar located high on the opposite wall of the cage. Electrical foot shock (<1 mA, 60 Hz) was used to reinforce these movements. Once the learning process was completed (3–4 sessions), weighted vests were strapped over the scapulae, and the rats were required to touch the high bar 50 times during one acute exercise session. We defined "acute" resistance exercise as four separate sessions with one day of rest between sessions. The rats performed 50 repetitions each day with 0.2 (day 1), 0.4 (days 2 and 3), and 0.6 (day 4) g weighted vest/g body weight. Previous work has shown that a rat that was naive to the lifting procedure would not lift the 0.6 g/g body weight on the first day weights were applied to the vest. This protocol can be considered as acute because it does not result in changes in muscle weight (18). Exercise sessions occurred in the dark (red light) in the late afternoon. Rats that did not perform exercise (sedentary) were placed in the lifting cages at least three times during the week of acute exercise and were given five electric shocks to simulate some of the stress experienced by the exercised groups. One of these shock control sessions occurred 16 h before the determination of rates of protein synthesis.

Rates of protein synthesis. All measurements of protein synthesis occurred 16 h after the last bout of acute resistance exercise. Food was withdrawn from the rats during the last 5 h of this 16-h period. Rats were anesthetized with methoxyflurane, and the left carotid artery and right jugular vein were cannulated. Rats remained unconscious after the placement of catheters and during the measurement of rates of protein synthesis. Total time between the onset of anesthesia and completion of surgery was 12–17 min. One milliliter of arterial blood was taken for determination of insulin, IGF-I, corticosterone, and glucose. A flooding dose (22) of L- [2,3,4,5,6-3H]phenylalanine (1 mCi/rat; Amersham Life Science, Arlington Heights, IL) in unlabeled phenylalanine (150 mM; 1 ml/100 g body wt, total volume) was injected immediately after cannulation into the venous catheter over a 15-s period. Arterial blood (1 ml) was taken at 6 and 10 min, and then the gastrocnemius muscle was excised. Muscles were immediately dropped into liquid nitrogen. Frozen muscles were stored at −70°C until phenylalanine incorporated into trichloroacetic acid-precipitable protein was analyzed using dabsylation of the amino acid and measurement on a high-pressure liquid chromatograph (8). Radioactivity in the phenylalanine peak was measured by liquid scintillation counting with appropriate correction for quench. Protein determinations were made using the biuret method. Rates of muscle protein synthesis were calculated using the method of Garlick et al. (22). eF2B activity and RNA analysis. We used different groups of rats to determine eF2B activity, because the latter measures the disappearance of [3H] GDP and measurements of protein synthesis also require the use of tritium (31). The exercise protocol and timing of all experiments for these rats were identical to those described in Resistance exercise and Partial pancreatectomy. Rats were anesthetized, and the gastrocnemius was excised, placed on ice, freed of connective
tissue, and immediately homogenized in 4 volumes of a buffer specifically designed for measuring muscle eIF2B activity (buffer A). Buffer A (31) was made the day before use and contained (in mM) 20 triethanolamine, 2 magnesium acetate, 150 KCl, 0.5 dithiothreitol (DTT), 0.1 EDTA (Na2), 0.1 EDTA (Na3), 250 sucrose, 5 EGTA, and 50 β-glycerophosphate. The homogenate was centrifuged for 20 min at 10,000 g, 4°C, and the postmitochondrial supernatant (PMS) was used immediately for the assay. eIF2 was complexed to [3H]GDP in buffer B, which contained 62.5 mM MOPS, 125 mM KCl, 1.25 mM DTT, and 0.25 mg/ml BSA. Buffer B (104 µl), 42.8 µl H2O, 10.5 µl eIF2, and 2.3 µl [3H]GDP were mixed by tube inversion at 30°C for 10 min. A nonradioactive GDP mix was made by combining 1.2 mg GDP, 10 ml buffer C (buffer B with the addition of 2.5 mM magnesium acetate), and 2 ml H2O. The assay was started with the addition of 40 µl PMS to 161 µl of assay buffer C; 140 µl H2O2, and 40 µl eIF2-[3H]GDP. Sixty-microliter aliquots were taken at 8, 30, 60, 180, 360, and 540 s. The eIF2-[3H]GDP complex was vacuum-captured on nitrocellulose filters, which were then dissolved by vortexing in Filtron-X scintillation fluid. Beta radiation was quantified using liquid scintillation counting, with appropriate correction for quench due to the dissolved filters. Samples from individual muscles were assayed in duplicate.

Muscle for analysis of total RNA was homogenized in an RNAase-free buffer described by Kelly and Jefferson (29). Total RNA content of muscle homogenate supernatants was determined by spectrophotometry after alkaline hydrolysis (16). Purified RNA standards (Sigma) were processed identically to samples and used to determine linearity of the RNA wavelength absorption over the range of values found in supernatants.

Plasma insulin and corticosterone concentrations were determined by radioimmunoassay (13). The antibody used in the rat insulin assay also recognizes other mammalian insulins but does not cross-react with glucagon, pancreatic polypeptide, somatostatin, or IGFI-1. The useful assay range was 30–900 mU. The corticosterone RIA was specific for corticosterone in that the antibody did not cross-react (<3%) with other steroids. Plasma IGFI-1 was determined by radioimmunoassay using a modified acid-ethanol (0.25 N HCl-87.5% ethanol) procedure with cryoprecipitation, and gastrocnemius muscles were extracted using acid homogenization and Sep-Pak C18 extraction (11, 35). The IGFI-1 antibody does not cross-react with either insulin or IGFI-1, it has a sensitivity of 0.03–0.08 ng/tube, and the intra-assay coefficient of variation is <5%. Hematocrit (Hct) and hemoglobin (Hb) were measured in triplicate using standard procedures. Blood glucose was measured in triplicate using a Yellow Springs Instruments model 23A auto analyzer.

Statistical analysis. Statistical differences between groups were assessed using analysis of variance. The model was a fixed-effects model for two groups (diabetic vs. nondiabetic) and two treatments (exercised vs. sedentary). When significant F ratios were present, a Student-Newman-Kuels post hoc procedure was used to evaluate differences among means.

Table 1. Physical and physiological characteristics of the groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight, g</th>
<th>Blood Glucose, mg/dl</th>
<th>Hct, %</th>
<th>Hb, g/100 ml</th>
<th>Insulin, pm</th>
<th>Corticosterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic sedentary</td>
<td>16</td>
<td>331± 27</td>
<td>144± 20</td>
<td>44.0± 1.9</td>
<td>14.7± 0.7</td>
<td>456± 99</td>
<td>14.1± 6</td>
</tr>
<tr>
<td>Nondiabetic exercised</td>
<td>19</td>
<td>324± 21</td>
<td>145± 25</td>
<td>43.5± 1.8</td>
<td>14.8± 0.6</td>
<td>439± 146</td>
<td>14.7± 3</td>
</tr>
<tr>
<td>Diabetic sedentary</td>
<td>13</td>
<td>276± 42*</td>
<td>294± 69*</td>
<td>44.8± 1.6</td>
<td>15.1± 0.8</td>
<td>205± 103*</td>
<td>13.1± 5</td>
</tr>
<tr>
<td>Diabetic exercised</td>
<td>11</td>
<td>277± 35*</td>
<td>305± 67*</td>
<td>45.9± 1.4</td>
<td>15.2± 0.7</td>
<td>178± 113*</td>
<td>16.3± 6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Hct, hematocrit; Hb, hemoglobin. *Significantly different (P < 0.05) from nondiabetic groups.

Fig. 1. Growth curves for nondiabetic (△, n = 30) and diabetic (●, n = 22) rats during the experimental period. Values are means ± SE.
significantly higher eIF2B activity for both nondiabetic (+89%) and diabetic (+43%) rats. eIF2B activity was not different between diabetic and nondiabetic sedentary rats.

Figure 3 provides plasma concentrations of IGF-I. IGF-I concentrations were significantly higher in exercised diabetic rats compared with sedentary diabetic rats. Sedentary diabetic rats had significantly lower plasma IGF-I compared with sedentary nondiabetic rats. Circulating IGF-I concentrations were not different between exercised and sedentary nondiabetic rats. IGF-I content in gastrocnemius was not changed by exercise in nondiabetic rats; however, exercised diabetic rats had a 158% higher (P < 0.05) amount of IGF-I in the muscle compared with sedentary diabetic rats (Fig. 4). Neither exercise nor insulin status altered total muscle RNA (Fig. 5).

DISCUSSION

We expected to find that reduced insulin availability would have negative effects on potential regulators of peptide-chain initiation and that, after resistance exercise, such reductions should inhibit elevations in rates of protein synthesis. Previous studies from this laboratory using in situ bilateral hindlimb perfusions (18) demonstrated that normal elevations in rates of protein synthesis after resistance exercise were ablated when the muscle that performed the exercise was totally deprived of insulin. In the present study, partial PPX resulted in a 50% reduction in plasma insulin that resulted in moderate hyperglycemia. Despite an inability to adequately regulate glucose, diabetic rats were able to increase rates of protein synthesis after exercise. In a previous study, we demonstrated that rates of protein synthesis in muscles of moderately diabetic rats are increased during moderate (but not severe) acute resistance exercise (14). This later finding was verified in the present study; thus moderate hypoinsulinemia per se is not sufficient for reducing an anabolic response to moderate resistance exercise. A preserved ability to increase rates of protein synthesis by diabetic rats could be due to numerous compensatory mechanisms. Our data suggest that one such factor may be IGF-I.

IGF-I is a growth-promoting hormone whose receptor shares structural and functional similarities with the insulin receptor. Relevant to our study, IGF-I stimulates protein synthesis in skeletal muscle (45). Juraskinski and Vary (27) demonstrated that IGF-I has an independent ability to stimulate protein synthesis in nondiabetic rats during a hindlimb perfusion. Stimulation of protein synthesis was even more pronounced in septic animals, which, like severely diabetic animals,
have reduced rates of muscle protein synthesis (27). Thus a higher concentration of IGF-I in muscles of diabetic rats (Fig. 4) may contribute to the maintained ability to increase synthesis after exercise. No such elevation occurred in muscle of exercised nondiabetic rats. This finding is consistent with a report by Yan et al. (48), who found that 1 day after eccentric exercise there was no change in muscle immunocytochemically measured IGF-I; however, muscle IGF-I became elevated, but not until 4 days after the last bout of eccentric exercise. DeVol et al. (7) reported a greater amount of IGF-I mRNA in muscle cells during hypertrophy that was induced by surgical tendon ablation in nondiabetic rats (7). Because the first measure of this increased message was made 2 days after surgical ablation of the gastrocnemius tendon, it is difficult to relate this increase directly to the elevated rates of synthesis we observed 16 h postexercise. DeVol et al. also reported that the increase in IGF-I mRNA after exercise was greater in hypophysectomized rats. This augmented (compensatory?) response is somewhat similar to the increase we found in diabetic but not nondiabetic rats. Our previous report using this model of resistance exercise (18) also shows no change in gastrocnemius RNA concentrations than rats used in previous work (2, 15, 31). Another reason for the conflicting results may be that hypoinsulinemia is not sufficient to inhibit an exercise-induced elevation in this initiation factor.

We expected lower eIF2B activity in diabetic than in nondiabetic rats in the nonstressed state, because severe short-term diabetes results in reduced activity of this factor as well as lower rates of protein synthesis in skeletal muscle (28). The activity of eIF2B in sedentary diabetic rats was not different from that found in nondiabetic rats. This observation could be due to the rats in the current study having higher plasma insulin concentrations than rats used in previous work (2, 15, 31). Another reason for the conflicting results may be that our rats were diabetic for ~5 wk, whereas most other studies used 3- to 7-day diabetic rats. No other data exist on the effects of long-term diabetes on eIF2B activity in skeletal muscle.

The ability to translate mRNA has been separated into translational capacity, which depends on the total RNA available, and translational efficiency, which is how rapidly the existing RNA is translated into new proteins (38). Reduced skeletal muscle total RNA is a consistent finding in severely diabetic rats (15, 30), and this deficit is reversed when rats are provided with exogenous insulin. Thus, the retained capacity (Fig. 5) for translation (total RNA) may explain the similar rates of protein synthesis between groups. Limited information is available on either the effects of long-term diabetes or resistance exercise on muscle RNA.

Our previous report using this model of resistance exercise (18) also shows no change in gastrocnemius RNA concentration during in vivo experiments. In two of three acute resistance exercise protocols used by Wong and Booth (47), no change in total RNA (mg/muscle) was found 12 and 36 h after a single bout of resistance exercise in rats, a time when rates of protein synthesis were elevated. When the same data were expressed as milligrams RNA per milligram protein, all three protocols resulted in higher RNA after exercise. In the present study, we found no statistically signifi-
cant elevation in RNA due to exercise in diabetic or nondiabetic rats when RNA was expressed as milligram RNA per gram wet weight of muscle. Watt et al. (46) also found no change in RNA (µg/muscle) in three of the four muscles studied after 4 days of resistance exercise in rats despite increases in protein synthesis rates in most of these muscles. In humans, Chesley et al. (6) also found no change in total RNA either 4 or 24 h after a single bout of acute resistance exercise. It should be noted, however, that all of the subjects in the latter study engaged in regular resistance exercise training before the study and thus may have adapted to the stress. The literature seems consistent in a finding of stable RNA after acute resistance exercise, and we confirm this finding even in the presence of moderate hypoinsulinemia.

Increases in translational efficiency after resistance exercise have been reported in both human (6) and animal studies (18, 19). Calculations (not shown) based on the data provided in Table 2 and Fig. 5 confirmed this finding. Resistance exercise also increased translational efficiency in diabetic rats. Thus the capacity and efficiency of translation were not altered by moderate hypoinsulinemia.

The model we used to create a diabetic status is not similar to the commonly used model involving the chemical toxins streptozotocin and alloxan. These toxins result in severe hyperglycemia and such rats do not gain weight. The PPX model results in moderate hyperglycemia (although a wide range of fed-state glucose concentrations existed), but the rats gained weight at only a slightly reduced rate compared with nondiabetic rats. This difference in models probably accounts for the findings that total muscle RNA, rates of protein synthesis, and eIF2B activity are not lower in diabetic vs. nondiabetic sedentary rats.

The mean total body weight for the diabetic rats was significantly lower than that observed for nondiabetic rats; however, muscle-specific rates of protein synthesis were not different between groups. Several potential explanations exist for this observation. Diabetic rats have higher rates of proteolysis compared with nondiabetic rats (2, 43), perhaps due to a hypoinsulinemia-induced activation of the ubiquitin-proteasome pathway (40). It is also possible that the 5-h fast had differential effects on rates of protein synthesis between diabetic and nondiabetic rats. This possibility requires further exploration because, to our knowledge, the time course for fasting vs. rates of protein synthesis in diabetic vs. nondiabetic rats has not been completely defined.

Another consideration related to reduced body weight of the diabetic rats is that they may have experienced a greater relative stress compared with nondiabetic rats. The amount of weight lifted was relative to body weight, but this does not ensure that muscles contract identically to lift these loads. If so, however, this did not translate into a failure to increase rates of protein synthesis in response to moderate exercise. Such a failure has been reported when diabetic rats are forced to perform severe intensity (50 repetitions with 1-g weighted backpacks/g body weight) acute resistance exercise (14). It is also possible that surgery itself created a pathophysiological condition that confounded the stress of exercise. In a previous study we found no differences between sham-operated and control rats in the ability to elevate rates of protein synthesis after similar exercise (14).

In summary, we conclude from the data presented that hypoinsulinemia that is sufficient to cause poor glucoregulation is not sufficient to cause a loss in the ability to regulate protein synthesis. A potential cause for this maintained ability to increase muscle protein synthesis after resistance exercise may reside in an increased content of intramuscular IGF-I. Also, moderate hypoinsulinemia did not affect basal or exercise-stimulated increases in the activity of eIF2B. Because moderately diabetic rats can elevate rates of protein synthesis after moderate-intensity resistance exercise, carefully controlled human studies would determine whether persons with type I diabetes mellitus can benefit from the anabolic consequences of resistance exercise could be conducted in the future. This would be an important potential extension of the present studies, because loss of muscle mass is a well-documented consequence of long-term, poorly controlled diabetes. Human studies in addition to those already available (9, 37) should commence to document the safety and feasibility of this type of exercise in a diabetic population.

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