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Mechanism for the increased skeletal muscle protein degradation in the obese Zucker rat

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Obese (fa/fa) Zucker rats showed a decreased protein content in skeletal muscle compared with their lean counterparts. This was associated with both a decrease in the fractional rate of protein synthesis and an enhanced fractional rate of protein degradation in skeletal muscle, as studied by pre-loading body proteins with ^{14}C-bicarbonate. The increased protein degradation could be the result of the clearly enhanced expression for several transcripts of the ubiquitin genes in skeletal muscle. The results suggest that the increased protein degradation in the skeletal muscle of the obese animals may be due to increased activity of the ubiquitin-dependent proteolytic system. (J. Nutr. Biochem. 10:244–248, 1999)

Keywords: Zucker rat; protein metabolism; muscle proteolysis; ubiquitin

Introduction

The Zucker fatty (fa/fa) rat appeared as a result of a spontaneous mutation in a cross between the Merck stock M and Sherman rats.[1] The obesity is transmitted as an autosomal mendelian recessive trait and animals that are homozygous suffer the consequences of genetic obesity, accumulating fat progressively throughout their lives (see ref.[2] for review).

Nitrogen balance studies have shown that the obese Zucker rat, when given the same amount of dietary protein as its lean counterpart, tends to deposit amino acid carbon skeletons in the form of fat rather than muscle protein.[3] In fact, it has already been reported that the muscles of obese Zucker rats are smaller and contain less protein, DNA, and RNA than those of their lean counterparts.[4] Lean body mass is also lower in the obese genotype.[5] Several points should be made regarding the metabolic disturbances that may account for the decreased protein accumulation in skeletal muscle. First, in vivo muscle neutral amino acid uptake has been shown not to be affected in animals bearing the fa/fa genotype,[6] in spite of the marked metabolic alterations, including insulin resistance, that are present in the obese animals.[2] However, we have observed that after an intragastric administration of amino acids in the obese animals, these compounds are preferentially channeled into fat rather than taken up by the skeletal muscle for protein synthesis.[7] In relation with muscle protein synthesis, fractional rates have been found to be lower in the obese versus the lean animals.[5] On the other hand, the rates of protein degradation (as estimated by urinary 3-methylhistidine excretion) have been found to be higher in obese rats than in their lean counterparts.[3] As a result of the referred changes in protein metabolism, the obese Zucker rat has a decreased rate of protein deposition in skeletal muscle.[3–5]

Bearing all this in mind, it was the aim of the present study to investigate the mechanisms by which muscle protein turnover is altered and therefore influences muscle protein accumulation in the obese Zucker rat.

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Materials and methods

Animals

Female lean (+/?) and obese (fa/fa) Zucker rats, aged 12 weeks, were fed ad libitum on a chow diet (Panlab, Barcelona, Spain) consisting (by weight) of 54% carbohydrate, 17% protein, and 5% fat (the residue was nondigestible material). Animals had free access to drinking water and were maintained at an ambient temperature of 22 ± 2°C with a 12-hour light/12-hour dark cycle (lights on from 8:00 AM to 8:00 PM).

Biochemicals

All enzymes and coenzymes were obtained from either Boehringer Mannheim, S.A. (Barcelona, Spain) or Sigma (St. Louis, MO USA). ^{14}C-bicarbonate (53 mCi/mmol) was obtained from Amersham (Amersham, Buckinghamshire, UK).

Protein turnover

Protein turnover rates were determined by a method that, as previously discussed,[8,9] is highly suitable for the liver,[10] but suffers from considerable label recycling in skeletal muscle.[11] However, despite this limitation, it offers the best compromise for simultaneously monitoring protein synthesis and degradation in the same animal.[12] Briefly, apparent rates of synthesis and degradation for proteins of the slow turnover pool were evaluated by measuring the decay in specific and total protein radioactivity after labeling in vivo with a single intraperitoneal dose of sodium ^{14}C-bicarbonate (250 mCi/kg body weight). Fractional rates of protein degradation (k_d), synthesis (k_s), and accumulation (k_a, k_a') were calculated as follows:

$$k_d = 1n \text{ (total protein radioactivity)}/t$$
$$k_s = 1n \text{ (specific protein radioactivity)}/t$$
$$k_a = 1n \text{ (total protein)}/t$$
$$k_a' = k_s - k_d$$

and expressed as percent per day. Tissue protein was determined by the method of Lowry et al.,[13] using bovine serum albumin (BSA) as working standard.

RNA isolation and Northern blot analysis

Total RNA from gastrocnemius muscle was extracted using the acid guanidinium isothiocyanate/phenol/chloroform method.[14] RNA samples (20 μg) were denaturated, subject to electrophoresis in 1.2% agarose gels containing 6.3% formaldehyde, and transferred to Hybond N membranes (Amersham). RNA was fixed to membrane by illuminating with ultraviolet light for 4 minutes. The RNA in gels and in filters was visualized with ethidium bromide and photographed by ultraviolet transillumination to ensure the integrity of RNA, to check the loading of equivalents amounts of RNA, and to confirm proper transfer. RNA was transferred in 20 × standard saline citrate (SSC; 0.15 M NaCl and 15 mM sodium citrate, pH 7.0). Hybridization was done at 65°C overnight in the hybridization buffer (0.25 M Na_2HPO_4, 7% SDS, 1 mM EDTA, 1% BSA, and 10% dextran sulphate), and denatured labeled probes (10^6–10^7 cpm/mL) were added. Radiolabeled probes were prepared by the random primer method (Boehringer Mannheim). The ubiquitin probe used was a cDNA clone containing 12 pairs of the second ubiquitin coding sequence plus complete third and fourth ubiquitin coding sequences and 120 basepairs of the 3′-untranslated region of the chicken polyubiquitin gene UBI.[15] Filters were exposed to Hyperfilm-MP (Amersham) at −70°C for 2 to 4 days and the films quantified by laser densitometry.

Table 1 Muscle weights and protein content in Zucker rats

	Weight (mg)	Total protein content (mg)
Gastrocnemius		
Lean (n = 5)	618 ± 15	98.9 ± 2.4
Obese (n = 5)	530 ± 12†	80.6 ± 1.8‡
Soleus		
Lean (n = 5)	56.0 ± 0.7	9.35 ± 0.2
Obese (n = 4)	51.9 ± 1.0†	7.99 ± 0.2†
Extensor digitorum longus		
Lean (n = 4)	55.3 ± 1.6	9.29 ± 0.3
Obese (n = 4)	50.2 ± 0.4*	8.08 ± 0.1†

For more details, see materials and methods. Weights are corrected by 100 g of initial body weight. Data are means ± SEM for the number of animals indicated in parentheses. Statistical significance of the results (Student's t-test): lean vs. obese: $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.001$.

Statistical analysis

Statistical analysis of the data was performed by means of the unpaired Student's t-test. Significant differences of fractional rates of protein turnover were calculated by analysis of variance on linear regressions.[16]

Results and discussion

The obese Zucker rat is hyperphagic and has a propensity for a differential partitioning of nutrients into adipose fat and body protein. From this point of view, when compared with the non-obese animals, the obese rat incorporates more carbon into adipose tissue and has a reduced rate and total amount of protein accretion, in addition to many alterations in protein metabolism.[2] Bearing this in mind, we investigated the mechanism(s) accounting for the reduced protein accretion.

Muscle weights and protein content

As can be observed from the results presented in *Table 1*, obese animals had lower muscle weights and total protein content compared with their lean counterparts (14% for gastrocnemius, 7% for soleus, and 9% for extensor digitorum longus).

Protein turnover rates

We studied whether the smaller muscle mass and protein content was associated with changes in the fractional constants of protein turnover. Indeed, obese fa/fa rats presented a larger fractional degradation rate and a smaller fractional synthetic rate compared with their lean counterparts (*Table 2*). The result of these changes affected protein accumulation in skeletal muscle, which was clearly lower in the obese animals. Conversely, no significant changes were observed in relation to these constants in the liver (*Table 2*).

These results are in agreement with previous studies that have shown decreased rates of protein synthesis in the muscle of fa/fa rats,[5] and no changes have been found in the liver.[5] Several studies involving urinary excretion of 3-methylhistidine also have indicated that the muscle of the obese animals shows a higher rate of protein degradation.[3]

Table 2 Fractional protein turnover rates in Zucker rats

	Skeletal muscle (gastrocnemius)	Liver
K_d		
Lean	5.65	16.86
Obese	6.64*	13.93
K_s		
Lean	7.92	24.66
Obese	6.94*	20.21
K_a		
Lean	1.93	8.02
Obese	0.65	8.76
K'_a		
Lean	2.27	7.80
Obese	0.30*	6.28

Fractional rates of protein synthesis (k_s), degradation (K_d) and accumulation (k_a) are expressed as percent per day ($n = 5$), For further details see Materials and Methods. Significance of the differences: lean vs. obese: *$P < 0.05$.

This amino acid is derived from the catabolism of myofibrillar proteins and has been used to estimate protein degradation rates.[17] Our study confirms, by a different methodologic approximation (pre-loading of muscle proteins with ^{14}C), the increased protein degradation present in the muscle of obese rats. These changes are related, at least to some extent, to the insulin status of obese animals. Indeed, insulin seems to play a very important role in protein synthesis and possibly degradation (see ref. [18] for review). Chan et al.[19] studied the effects of insulin administration on lean and obese Zucker rats previously made diabetic by alloxan treatment in order to make them comparable to insulin secretion. They found that, at comparable insulin levels, obese rats consistently deposited less protein than their lean counterparts. The hyperinsulinemia that normally is seen in obese rats may be an adaptative response to minimize the impaired balance between protein synthesis and degradation. The higher fractional rate of degradation actually indicates that energy costs per unit of skeletal muscle protein deposition are greater in obese rats than in lean rats.[20] It has been suggested that the increased energy efficiency seen in the obese animals may be explained in part by slower rates of protein turnover.[21] However, the contribution of protein turnover to energy efficiency of the obese Zucker rat depends on the balance between the energy conserved by the slower rate of protein synthesis and increased energy cost due to the larger degradation rate. In addition, Chatzidakis et al.[22] extensively studied substrate cycling in lean and obese Zucker rats (with the goal of accounting for energetic efficiency) and found that neither glucose cycling nor protein synthesis and breakdown rates were significantly different between lean and obese Zucker rats.

Ubiquitin gene expression

We investigated whether the ubiquitin-dependent proteolytic system could be involved in the enhanced protein degradation observed in the muscle of obese Zucker rats. This proteolytic system is postulated to account for the turnover of short-lived proteins[23] or for abnormal proteins formed during stress such as heat shock.[15] Ubiquitin is an 8.6-kDa peptide that is involved in the targeting of proteins undergoing cytosolic adenosine 5′-triphosphate (ATP)-dependent proteolysis. In the cell, ubiquitin can be found free or conjugated in an isopeptide linkage to other cellular proteins. Proteins with multiple ubiquitins are targeted for degradation by an ATP-dependent protease.[24,25] However, it has been suggested that the activity of this system also can be related to the turnover of long-lived proteins, such as those found in skeletal muscle.[26] Recently, this proteolytic system has been involved in the perturbations of protein metabolism consequent to many pathologic states that affect skeletal muscle.[27] During fasting, the glucocorticoids seem to be involved in the activation of the ubiquitin-dependent proteolytic pathway in muscle, because adrenalectomy prevents the rise in ubiquitin conjugates after fasting.[28] However, the mechanism is unclear because the characteristic sequences found on many glucocorticoid-responsive genes are not found on the ubiquitin genes.[29] Wing and Banville[30] cloned the E214k gene responsible for the synthesis of the ubiquitin-conjugated enzyme, and they reported increases in one of the mRNA transcripts with fasting and subsequent decrease with insulin treatment. Thus, the mechanisms involving insulin and glucocorticoids in the modulation of ubiquitin-dependent proteolysis may be completely independent, although insulin can suppress the stimulation by glucocorticoids of muscle proteolysis both in vivo and in vitro.[31] Our group described the fact that administration of tumor necrosis factor-α (TNF) to rats results in an increase in muscle proteolysis both in vivo[32] and in vitro[33] and that it is associated with both an increase in the presence of ubiquitin conjugates and ubiquitin mRNA in skeletal muscle.[34]

The results presented in *Figure 1* clearly show that ubiquitin gene expression was different in the obese animals in relation to their lean counterparts. It is interesting to note that five ubiquitin transcripts were observed in both groups: 6.4, 1.6, 1.1, 0.5, and 0.4 kB. Obese animals had an enhanced expression for three of these transcripts (2.3-fold for the 1.6 kB, 2.5-fold for the 0.5 kB, and 2.3-fold for the 0.4 kB; *Figure 1*). Interestingly, the obese Zucker rat (and other experimental obesity models) shows increased expression of the TNF gene in adipose tissue,[35] which is related to the insulin resistance found in these animals. Furthermore, the circulating levels of TNF also are elevated. Because this cytokine has been shown to activate skeletal muscle ubiquitin-dependent proteolysis in rodents,[36,37] TNF may play a role in the increased muscle protein degradation found in the obese Zucker rat. In addition, in obese patients, an overexpression of TNF has been found in skeletal muscle, and it has been speculated that the cytokine may be responsible for the insulin resistance found in these patients.[38]

Conclusions

This is the first study that involves expression of proteolytic systems in the skeletal muscle during obesity. Our results allow us to suggest that the activity of the ubiquitin-dependent proteolytic system may be responsible for the

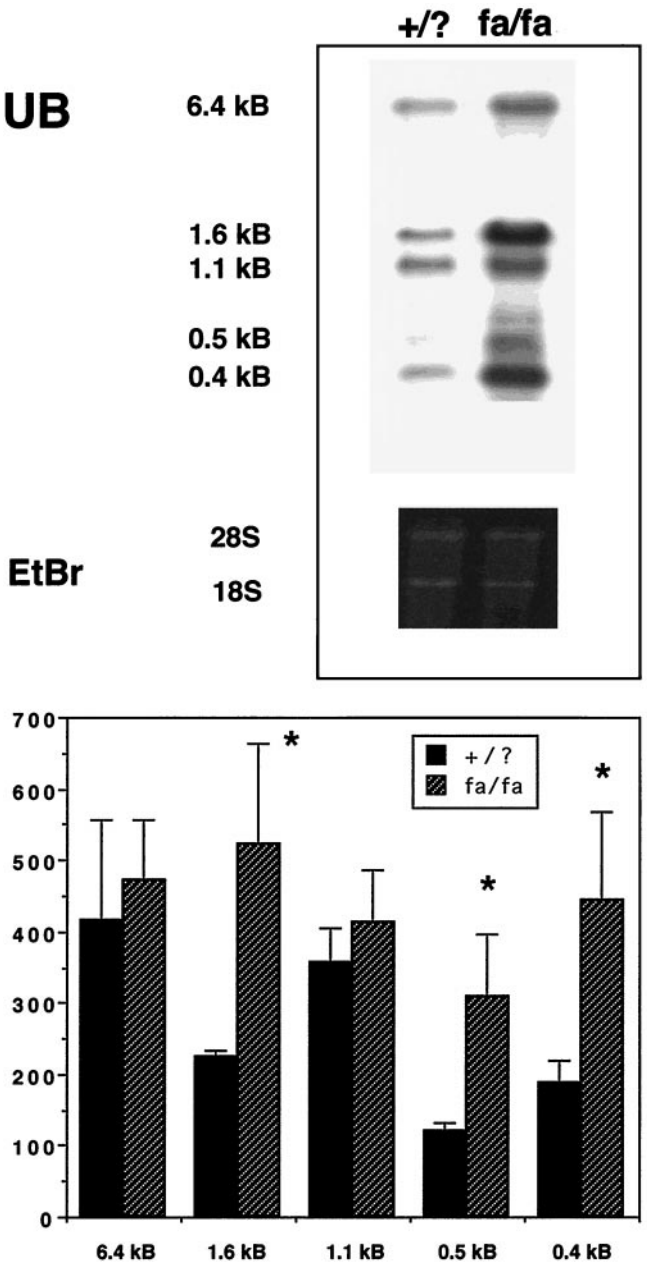

Figure 1 Expression of ubiquitin genes in the skeletal muscle of Zucker rats. Northern blots of gastrocnemius muscle extracts from lean and obese Zucker rats. Expression of ubiquitin genes in skeletal gastrocnemius muscles from lean (+/?) and obese (fa/fa) Zucker rats was determined after hybridization with cDNA probes containing a region of the chicken polyubiquitin gene UBI (see Materials and methods). Ethidium bromide was used as a control of sample loading. Autoradiographs were subjected to scanning densitometry. The results of the densitometric analysis of five different animals are shown in the lower panel and are expressed as arbitrary units. Significance of the differences: lean versus obese, $^{*}P < 0.1$.

increased protein degradation found in the muscle of the obese genotype.

It is interesting to note that the concentration of circulating branched chain amino acids is significantly higher in obese Zucker rats than in their lean counterparts,[6] and these compounds have been associated with an increased rate of protein breakdown during pathologic states that involve muscle wasting.[39] In addition, these compounds seem to modulate the activity of the ubiquitin-dependent system.[40] From this point of view, the higher levels of these amino acids found in the obese Zucker rat may constitute a mechanism for controlling, to some extent, an even greater rate of skeletal muscle protein degradation.

Interestingly, TNF is one of the main activators of muscle proteolysis in pathologic states (and of the ubiquitin-dependent proteolytic system in particular),[32,36,37,41] and it has been found to be overexpressed in adipose tissue in different models of obesity including the Zucker rat.[34,42] This cytokine seems to be responsible for the insulin resistance found in these obesity models (see ref. [43] for review). Therefore, one could speculate that the overexpression of the cytokine during obesity may signal the activation of the proteolytic system in skeletal muscle. This may be a good starting point for designing therapeutic strategies based on the re-channeling of nutrients (amino acids in particular) to skeletal muscle instead of adipose tissue during obesity. However, it must be emphasized that in spite of the genetic defect responsible for this experimental model of obesity, much work remains to be done to define the actual pathogenesis of obesity.

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References

1 Zucker, L.M. and Zucker, T.F. (1961). Fatty, a new mutation in the rat. *J. Hered.* **52,** 275–278
2 Argilés, J.M. (1989). The obese Zucker rat: A choice for fat metabolism. *Prog. Lipid Res.* **28,** 53–66
3 Dunn, M.A. and Hartsook, E.W. (1980). Comparative amino acid and protein metabolism in obese and non-obese Zucker rats. *J. Nutr.* **110,** 1865–1879
4 Cleary, M.P. and Vasselli, J.R. (1981). Reduced organ growth when hyperphagia is prevented in genetically obese (fa/fa) Zucker rats. *Proc. Soc. Exp. Biol. Med.* **167,** 616–623
5 Reeds, P., Haggarty, P., Wahle, K.W.J., and Fletcher, J.M. (1982). Tissue and whole-body protein synthesis in inmature Zucker rats and their relationship to protein deposition. *Biochem. J.* **204,** 393–398
6 Domènech, M., López-Soriano, F.J., Carbó, N., and Argilés, J.M. (1992). Amino acid metabolism in several tissues of the obese Zucker rat as indicated by the tissue accumulation of α-amino[1-^{14}C]isobutyrate. *Mol. Cell. Biochem.* **110,** 155–159
7 Domènech, M., López-Soriano, F.J., Carbó, N., and Argilés, J.M. (1992). The metabolic fate of an oral ^{14}C-alanine load in the obese Zucker rat. *Int. J. Obesity* **16,** 213–218
8 Tessitore, L., Bonelli, G., and Baccino, F.M. (1987). Early development of protein metabolic perturbations in the liver and skeletal muscle of tumor-bearing rats. A model system for cancer cachexia. *Biochem. J.* **241,** 153–159
9 Tessitore, L., Costelli, P., and Baccino, F.M. (1993). Humoral mediation for cachexia in tumor-bearing rats. *Br. J. Cancer* **67,** 15–23
10 Swick, R.W. and Ip, M.M. (1974). Measurement of protein turnover

in rat liver with [^{14}C]carbonate. Protein turnover during liver regeneration. *J. Biol. Chem.* **249,** 6836–6841
11 MacDonald, M.L., Augustine, S.L., Burck, T.L., and Swick, R.W. (1979). A comparison of methods for the measurement of protein turnover in vitro. *Biochem. J.* **184,** 473–476
12 Garlick, P.J., Millward, D.J., James, W.P.T., and Waterlow, J.C. (1975). The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. *Biochim. Biophys. Acta* **414,** 71–84
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
14 Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. *Anal. Biochem.* **162,** 156–159
15 Bond, U., Agell, N., Haas, A.L., Redman, K., and Schlesinger, M.J. (1988). Ubiquitin in stressed chicken embryo fibroblasts. *J. Biol. Chem.* **263,** 2384–2388
16 Lee, J.D. and Lee, T.D. (1982). *Statistic and Numerical Methods for Biologists*, Vol. 1. Van Nostrand Reinhold Co., New York, NY, USA
17 Young, V.R. and Munro, H.N. (1978). N^+-methylhistidine (3-methylhistidine) and muscle protein turnover: An overview. *Fed. Proc.* **37,** 2291–2300
18 Sudgen, P.H. and Fuller, S.J. (1991). Regulation of protein turnover in skeletal and cardiac muscle. *Biochem. J.* **273,** 21–37
19 Chan, C.P., Hansen, R.J., and Stern, J.S. (1985). Protein turnover in insulin-treated, alloxan diabetic lean and obese Zucker rats. *J. Nutr.* **115,** 959–969
20 Romsos, D.R. (1981). Efficiency of energy retention in genetically obese animals and in dietary-induced thermogenesis. *Fed. Proc.* **40,** 2524–2529
21 Deb, S., Martin, R.J., and Hershberger, T.V. (1976). Maintenance requirement and energetic efficiency of lean and obese Zucker rats. *J. Nutr.* **106,** 191–197
22 Chatzidakis, C., Lazarus, D.D., and Stein, T.P. (1994). Substrate cycling in lean and obese Zucker rats. *Int. J. Obesity* **18,** 287–293
23 Ciechanover, A., Finley, D., and Varshavsky, A. (1984). Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. *Cell* **37,** 57–66
24 Finley, D. and Chan, V. (1991). Ubiquitination. *Annu. Rev. Cell Biol.* **7,** 25–69
25 Rechsteiner, M. (1991). Natural substrates of the ubiquitin proteolytic pathway. *Cell* **66,** 615–618
26 Hilenski, L.L., Terracio, L., Haas, A.L., and Borg, T.K. (1992). Immunolocalization of ubiquitin conjugates at Z-bands and intercalated discs of rat cardiomyocytes in vitro and in vivo. *J. Histochem. Cytochem.* **40,** 1037–1042
27 Argilés, J.M. and López-Soriano, F.J. (1996). The ubiquitin-dependent proteolytic pathway in skeletal muscle: Its role in pathological states. *Trends Pharmacol. Sci.* **17,** 223–226
28 Wing, S.S. and Goldberg, A.L. (1993). Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. *Am. J. Physiol.* **264,** E668–E676
29 Wiborg, O., Pederson, M.S., Wind, A., Berglund, L.E., Marcker, K.A., and Vuust, J. (1985). The human ubiquitin multigene family: Some genes contain multiple directly repeated ubiquitin coding sequences. *EMBO J.* **4,** 755–759
30 Wing, S.S. and Banville, D. (1994). 14-kDa ubiquitin-conjugating enzyme: Structure of the rat gene and regulation upon fasting and by insulin. *Am. J. Physiol.* **267,** E39–E48
31 Tomas, F.M., Murray, A.J., and Jones, L.M. (1984). Interactive effects of insulin and corticosterone on myofibrillar protein turnover in rats as determined by N-methylhistidine excretion. *Biochem. J.* **220,** 469–479
32 Llovera, M., López-Soriano, F.J., and Argilés, J.M. (1993). Effects of tumor necrosis factor-α on muscle protein turnover in female Wistar rats. *J. Natl. Canc. Inst.* **85,** 1334–1339
33 García-Martínez, C., López-Soriano, F.J., and Argilés, J.M. (1993). Acute treatment with tumour necrosis factor-α induces changes in protein metabolism in rat skeletal muscle. *Molec. Cell. Biochem.* **125,** 11–18
34 García-Martínez, C., Agell, N., Llovera, M., López-Soriano, F.J., and Argilés, J.M. (1993). Tumour necrosis factor-α increases the ubiquitinization of rat skeletal muscle proteins. *FEBS Lett.* **323,** 211–214
35 Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993). Adipose expression of tumor necrosis factor-α: Direct role in obesity-linked insulin resistance. *Science* **259,** 87–91
36 García-Martínez, C., Llovera, M., Agell, N., López-Soriano, F.J., and Argilés, J.M. (1994). Ubiquitin gene expression in skeletal muscle is increased by tumour necrosis factor-α. *Biochem. Biophys. Res. Commun.* **201,** 682–686
37 Llovera, M., García-Martínez, C., Agell, N., López-Soriano, F.J., and Argilés, J.M. (1997). TNF can directly induce the expression of ubiquitin-dependent proteolysis in rat soleus muscles. *Biochem. Biophys. Res. Commun.* **230,** 238–241
38 Saghizadeh, M., Ong, J.M., Garvey, W.T., Henry, R.R., and Kern, P.A. (1996). The expression of TNF-α by human muscle. Relationship to insulin resistance. *J. Clin. Invest.* **97,** 1111–1116
39 Argilés, J.M., Costelli, P., Carbó, N., and López-Soriano, F.J. (1996). Branched-chain amino acid catabolism and wasting. *Oncol. Rep.* **3,** 687–690
40 Busquets, S., Alvarez, B., Llovera, M., Agell, N., López-Soriano, F.J., and Argilés, J.M. (in press). Branched-chain amino acid inhibit ubiquitin-dependent proteolysis in rat skeletal muscle. *Biochem. J.*
41 Argilés, J.M., García-Martínez, C., Llovera, M., and López-Soriano, F.J. (1992). The role of cytokines in muscle wasting: Its relation with cancer cachexia. *Med. Res. Rev.* **12,** 637–652
42 López-Soriano, J., López-Soriano, F.J., Bagby, G., Williamson D.H., and Argilés, J.M. (1997). Anti-TNF treatment does not reverse the abnormalities in lipid metabolism of the obese Zucker rats. *Am. J. Physiol.* **272,** E656–E660
43 Argilés, J.M., López-Soriano, J., Busquets, S., and López-Soriano, F.J. (1997). A journey from cachexia to obesity by TNF. *FASEB J.* **11,** 743–751