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SHORT COMMUNICATION

Tumor Necrosis Factor α in Various Tissues of Insulin-Resistant Obese Koletsky Rats: Relations to Insulin Receptor Characteristics

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Summary

Tumor necrosis factor α (TNFα) was found to be significantly increased in skeletal muscles and retroperitoneal fat of obese insulin-resistant Koletsky rats as compared to control Wistar rats. This increase was accompanied by a depression of insulin receptor protein tyrosine kinase (PTK) activity. Neither the insulin-binding capacity nor insulin receptor affinity were related to this TNFα increase in these tissues. In the liver, no significant changes of TNFα content and only a lowering of insulin-binding capacity were found. It is concluded that an increased TNFα content in muscles and fat (but not in the liver) contributes to insulin resistance by lowering insulin receptor protein tyrosine kinase activity, while other insulin receptor characteristics (insulin-binding capacity and affinity of insulin receptors to the hormone) do not seem to be influenced by this factor.

Key words

Tumor necrosis factor α • Insulin resistance • Insulin receptor protein tyrosine kinase activity

Recently, tumor necrosis factor α (TNFα) has received much attention for its potential to cause many features of insulin resistance. The TNFα mRNA expression and TNFα protein was greatly increased in adipose tissue from insulin-resistant men and animals as compared with healthy subjects (Hotamisligil *et al.* 1995, Kern *et al.* 1995). TNFα can cause the IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in adipose tissue of obese subjects (Hotamisligil *et al.* 1994, 1996) and thereby a decrease of some postreceptor effects of insulin (Hanner *et al.* 1995). A threefold increase of TNFα was found in muscle cells from insulin-resistant diabetics; this increase was inversely correlated with the glucose disposal rate (Saghizadeh *et al.* 1996).

Different insulin receptor binding characteristics and tyrosine kinase activity were found in

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skeletal muscles, fat and liver of hereditary insulin-resistant obese Koletsky rats (Hřebíček *et al.* 1998). In the present study, we attempted to compare these changes with the content of TNFα and to elucidate some new aspects of insulin resistance.

The experiments were carried out in 8 recessive homozygotes (ff) of Koletsky rats (4 males, 4 females) with a cluster of symptoms of insulin resistance (glucose intolerance, obesity, hyperlipidemia, hyperinsulinemia and hypertension) (Koletsky 1975, Golda and Petr 1988, Golda and Hilgertová 1997) and in 8 normal Wistar rats (4 males, 4 females). After killing the animals by decapitation at the age of 3–4 months, samples of quadriceps femoris muscles, retroperitoneal fat and liver tissue were pulverized in liquid nitrogen, repeatedly homogenized in a solubilizing buffer and centrifuged at 25 000 x *g* and 4 °C for 60 min. TNFα levels in homogenate supernatants were determined using the Biosource International Cytoscreen Rat TNFα ELISA kits (USA). The insulin-binding characteristics and protein tyrosine kinase (PTK) activity of semipurified insulin receptors were described in detail elsewhere (Hřebíček *et al.* 1998). Semipurified insulin receptors were prepared as glycoprotein fraction from affinity chromatography on wheat germ agglutinin (WGA) bounded agarose (Sigma). The binding characteristics – affinity constant K_a and binding capacity R – were calculated from Scatchard's plots (Nyomba *et. al.* 1990). The Boehringer (Mannheim) non-radioactive Tyrosine Kinase kits with biotin- labeled synthetic substrate were used for determination of spontaneous and exogenous insulin-stimulated PTK activity in WGA eluates.

Table 1. TNFα content in homogenates of the quadriceps femoris muscle, retroperitoneal fat and liver of control Wistar rats and Koletsky obese rats

	CONTROL WISTAR RATS			KOLETSKY OBESE RATS		
TISSUE	Both sexes (n=8)	Males (n=4)	Females (n=4)	Both sexes (n=8)	Males (n=4)	Females (n=4)
MUSCLE	7.60 ± 1.5	7.32 ± 1.6	7.89 ± 1.9	12.70 ± 2.8***	14.12 ± 3.2**	11.26 ± 1.5*
FAT	587.4 ± 243.3	738.6 ± 108.3	436.1 ± 243.9	1223.2±308.2***	1206.7±255.8*	1239.6±395.1*
LIVER	465.5 ± 145.2	389.8 ± 156.8	541.3 ± 89.1	542.7 ± 152.2	435.0 ± 58.2	650.5 ± 140.5[a]

*Data (ng/g of protein) are means ± S.D. Significant differences between controls and Koletsky obese rats: *, **, *** $P< 0.05$, $P< 0.01$ and $P< 0.005$, respectively; significant difference between males and females: [a] $P< 0.05$.*

Table 1 illustrates that TNFα content in fat and liver of control animals was nearly 80-60 times higher than in skeletal muscles. Compared to control Wistar rats, TNFα content was substantially higher in fat and muscles of obese Koletsky rats, while the difference in the liver was not significant. Sex differences were not significant in control rats, although a tendency to higher values in the adipose tissue of male rats and in liver of female rats was observed ($P<0.10$). These differences between males and females became significant in the liver (but not in the fat) of Koletsky obese rats.

As is shown in Figure 1, which summarizes the data from both sexes, the significant increase of TNFα in skeletal muscles was accompanied by a significant decrease of insulin receptor binding capacity and by a reduction in both basal and stimulated PTK activity of these receptors (after incubation with exogenous insulin). The affinity of insulin receptors was not substantially changed. On the contrary, an even more enhanced TNFα content in fat corresponded with a significant increase of insulin-binding capacity, a decrease of the affinity index and a significant decrease of stimulated insulin receptor PTK activity. In the liver, neither the TNFα content nor the basal or stimulated insulin receptor PTK activity changed significantly; the only significant change was a decrease of insulin-binding capacity.

TNFα inhibits the proximal steps in insulin receptor signaling by decreasing the insulin receptor

autophosphorylation and subsequent tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1). This inhibitory effect depends on the presence of IRS-1 in intact cells of various tissues, because IRS-1 is converted into an inhibitor of PTK activity (Feinstein *et al.* 1993, Hotamisligil *et al.* 1994,1996). TNFα also stimulates the down-regulation of insulin-sensitive transporter GLUT-4, reduces the transcription of GLUT-4 gene, stimulates the p55 TNFα receptor and activates the sphingomyelinase (Skolnik and Marcusohn 1996, Stephens *et al.* 1997, Peraldi *et al.* 1996). These changes, which were found in the adipose tissue and skeletal muscles of various insulin-resistant animals, are in accordance with our results from Koletsky obese rats, demonstrating a depression of insulin receptor tyrosine kinase parallel to the significant increase of TNFα content in these tissues. Other insulin receptor characteristics – insulin-binding capacity and insulin receptor affinity – changed divergently irrespective of increased TNFα content.

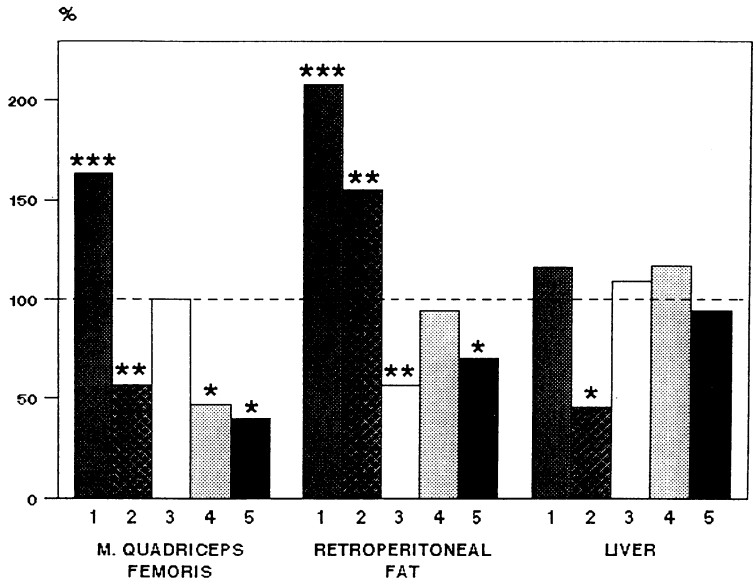

Fig. 1. *TNFα content and insulin receptor characteristics in tissues of obese Koletsky rats as compared with controls (the control values are taken as 100 % and are presented by a dashed line). The columns represent: 1. TNFα content, 2. insulin-binding capacity index R, 3. insulin receptor affinity index K_a, 4. basal PTK activity and 5. stimulated PTK activity of semipurified insulin receptors. Significant differences between controls and Koletsky obese rats: *, **, *** P<0.05, P<0.01 and P<0.005, respectively.*

The TNFα content and insulin receptor PTK activity in the liver was not significantly changed. This corresponds to some studies describing differences between insulin receptor characteristics in the liver and other insulin-sensitive tissues of insulin-resistant animals (Bisbis *et al.* 1993, Yamaguchi *et al.* 1996).

Thus, the patterns of insulin receptor characteristics differed in various tissues of insulin-resistant Koletsky rats. The elevated content of TNFα in muscles and fat was associated with a decrease of insulin receptor protein tyrosine kinase activity (both basal and stimulated), whereas no significant changes of these parameters were found in the liver.

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