Potential Role of TNFα and Lipoprotein Lipase as Candidate Genes for Obesity

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ABSTRACT To maintain body weight, metabolic efficiency was promoted during evolution; two candidate genes for body weight regulation are lipoprotein lipase (LPL) and tumor necrosis factor-α (TNFα). Human fat cells do not synthesize lipid, but rely on LPL-mediated plasma triglyceride hydrolysis. Adipose LPL is elevated in obesity. Following weight loss, LPL is elevated further, suggesting attempts to maintain lipid stores during fasting and to replenish lipid stores during refeeding. Muscle LPL is regulated inversely to adipose LPL. Thus, an increased adipose/muscle LPL ratio would partition dietary lipid into adipose tissue and would explain some of the variability in weight gain when humans are exposed to excess calories. Adipose tissue TNFα expression is increased in obese rodents and humans and may be important in obesity. When insulin-resistant rodents were injected with anti-TNF binding protein, insulin action improved, suggesting a link between insulin resistance and TNF. TNF is expressed at higher levels in muscle cells of insulin-resistant subjects, and TNF may inhibit LPL expression. Overall, TNF may function to make the subject less obese by inhibiting LPL and rendering the animal more insulin resistant. Obesity has many components, both metabolic and behavioral. However, the metabolic changes resulting from LPL and TNF likely played a role in regulating body adipose tissue during much of human evolution and continue to affect human obesity today.


KEY WORDS: • tumor necrosis factor • lipoprotein lipase • obesity • insulin resistance

Obesity is defined as an excess of adipose tissue, although a useful classification of obesity is based not on adiposity alone, but as excess adipose tissue leading to a spectrum of health consequences. These health problems range from metabolic disturbances such as hyperlipidemia, insulin resistance, diabetes and hypertension, to sleep apnea, gallstones and an increased risk for several malignancies (Kissebah et al. 1989).

When discussing the etiology of obesity, and the potential causal role of lipoprotein lipase (LPL), tumor necrosis factor-α (TNFα) or any other factor, it is important to bear in mind a number of important observations. First, obesity represents an intake of more energy than is expended. This first point is perhaps trivial, but is often lost in media hype, and in a zealous weight loss industry, which may try to convince some that obese subjects fail to abide by the laws of conservation of mass and energy. In addition, obesity is a complex condition, involving the interaction of both genetic and environmental factors. Finally, obesity is clearly not one disease, but is multifactorial, involving genetic, metabolic and behavioral factors. Thus, any discussion of etiology will eventually prove to “be right” in at least a small percentage of patients, and also to “be wrong” in the rest. With these caveats in mind, this review will focus on arguments in support of LPL and adipose TNFα as etiologic factors in the pathogenesis of obesity.

Adaptation to a hunter-gatherer lifestyle. As reviewed by Eaton et al. (1988), there has been little change in the human gene pool since the appearance of Homo sapiens sapiens ~35,000 years ago. In other words, modern humans are still genetically adapted to a preagricultural hunter-gatherer lifestyle. The study of such a lifestyle has led to the suggestion that a number of modern chronic conditions, prominent among them, obesity, may be due to a maladaptation of our lifestyle to our genome. The hunter-gatherer lifestyle involved a great deal of physical activity, coupled with a diet high in protein and low in fat when food was available. In addition, there were probably frequent periods of famine (Eaton and Konner 1985). It is likely that many metabolic features of modern humans originally evolved as an adaptation to such a lifestyle. Prominent among these metabolic adaptations are the adaptations designed to maintain adipocyte lipid stores.

Adipose tissue lipid accumulation. Because obesity represents excess adipose tissue lipid, it is useful to examine the
origin of adipose tissue lipids. One possible source of fatty acid substrate for adipose tissue triglyceride synthesis is endogenous cell synthesis, which results in the formation of saturated fatty acids. As demonstrated 30 years ago by Hollenberg (1966), the increase in lipid content of rat adipose tissue was due to the accumulation of predominantly unsaturated plasma-derived fatty acids. Human adipose tissue also derives most of its lipid for storage from circulating triglycerides (Shrago et al. 1969, Wilson et al. 1973). Although it is possible that there is some regulation of final triglyceride synthesis (Cianflone et al. 1992), LPL is the most critical step for the provision of lipid substrate and has been described as the “metabolic gatekeeper” (Greenwood 1985).

Role of LPL in the hunter-gatherer lifestyle. Many of the regulatory features of LPL are important adaptations to normal eating cycles. For example, LPL is regulated inversely in adipose tissue and muscle in response to feeding and fasting: feeding results in an increase in the adipose enzyme and a decrease in muscle LPL (Eckel 1989). This inverse regulation of LPL would maximize energy storage during times of food availability and maximize energy availability for muscle during periods of food-seeking behavior. An LPL “set point” could have evolved in adipose tissue to divert more plasma triglyceride fatty acids to the adipocyte in response to fat cell shrinkage. Because of the high level of physical activity and low energy density of food, obesity was much less of a danger to the hunter-gatherer than starvation, and therefore an adipocyte set point would have been a survival adaptation. In addition, exercise results in a decrease in adipose tissue LPL, along with an increase in muscle LPL (Simsolo et al. 1993). Therefore, the adipocyte set point may be lower in a well-trained individual and would not have promoted obesity in a hunter-gatherer.

The industrial revolution has brought with it a gradual increase in dietary fat, along with a decrease in physical activity, especially within the last 50 years. These lifestyle factors, combined with a genome programmed for physical activity and a low fat diet, are etiologic in obesity. LPL is likely to be one of many “efficiency genes” that can be particularly detrimental in someone with a sedentary lifestyle consuming a high fat diet.

Regulation of LPL in obesity. Under most circumstances, adipose LPL activity increases with serum insulin levels and the degree of insulin sensitivity. LPL increases in response to feeding (Ong and Kern 1989, Pykalisto et al. 1975), in response to a glucose-insulin infusion (Sadur and Eckel 1982) and after treatment of both insulin-deficient and insulin-resistant diabetics (Ong and Kern 1989, Simsolo et al. 1992, Taskinen and Nikkilä 1979). With progressive obesity, this relationship continues to hold true: adipose tissue LPL is increased in hyperinsulinemic obese subjects (Eckel 1989). However, when obese subjects lost weight and became less hyperinsulinemic, adipose LPL increased further (Kern et al. 1990, Schwartz and Brunzell 1981). When the change in LPL with weight loss was analyzed among patients, who were most obese demonstrated the largest increase in LPL (Kern et al. 1990), suggesting that very obese patients are most likely to have abnormal LPL regulation. Adipose tissue LPL activity from lean, obese and reduced-obese patients from several of our studies are summarized in Figure 1.

There are additional implications for the increased LPL in reduced-obese subjects. If insulin were the only regulatory influence on LPL, one might predict that weight loss would lead to no change in LPL, because reduced-obese patients become more sensitive to insulin in parallel with a fall in plasma insulin. Thus, these studies point to the complexity of LPL regulation in humans. The increase in adipose LPL with weight loss would facilitate the accumulation and storage of lipid. Although the high level of LPL in reduced-obese subjects would not by itself lead to weight regain, it would facilitate adipocyte lipid accumulation from circulating chylomicrons and VLDL in the face of relatively mild energy excess. Therefore, these data suggest that adipose tissue LPL activity may represent an adipocyte set point that is intended to limit adipocyte shrinkage induced by a hypocaloric diet.

Potential role of muscle LPL in lipid partitioning. One possible metabolic etiology for obesity recidivism revolves around increased adipose tissue LPL. However, muscle contains at least half of the total body LPL (Borenstajn 1987), and the free fatty acids (FFA) generated by muscle LPL are catabolized for energy. Therefore, changes in muscle LPL with weight loss could greatly affect the partitioning of circulating lipid between adipose tissue and muscle. For example, if muscle LPL decreased after weight loss at the same time that adipose LPL increased, then the effect of the increased adipose LPL on lipid storage would be considerably greater; circulating lipid would be predominantly directed toward storage in adipose tissue, rather than toward muscle for oxidation. Indeed, one study has already reported decreased muscle LPL activity in reduced-obese subjects (Eckel et al. 1995).

Exercise plays an important role in controlling lipid partitioning. Both short-term and endurance exercise result in increases in human muscle LPL (reviewed in Nikkilä 1987). In a recent study, adipose tissue and muscle LPL were measured in athletes before and after detraining for 2 wk (Simsolo et al. 1993). After detraining, there was not only a large decrease in muscle LPL, but also a twofold increase in heparin-releasable adipose tissue LPL. The combination of fall in muscle LPL and increase in adipose LPL led to a 10-fold increase in the adipose/muscle LPL ratio, suggesting that dietary lipid would be much more likely to be shunted towards adipose tissue for storage.

If changes in muscle LPL are present in obese subjects, these changes may be part of a spectrum of changes that occur in carbohydrate and lipid metabolism. Muscles rich in type I (slow twitch, oxidative) fibers have much higher levels of LPL (Borenstajn 1987) because of an increase in LPL mRNA lev-
Role of LPL in adipose tissue. In 1982, Kawakami et al. (1982) found that endotoxin-stimulated macrophages secreted a substance that inhibited LPL in 3T3-L1 adipocytes. Because LPL was known to play an important role in adipose tissue triglyceride storage, the inhibition of LPL by a macrophage factor was postulated to play a role in cancer cachexia. The 17-kDa protein that was isolated from the macrophage exudate was called cachectin and was subsequently found to be identical with TNF. In addition to the inhibition of LPL, TNF/cachectin also stimulated hormone sensitive lipase (HSL) (Patton et al. 1986, Price et al. 1986). Because of the importance of LPL and HSL in lipolysis and lipogenesis in adipose tissue, cachectin/TNF was suggested to be central to the etiology of cachexia and the hyperlipidemia of cancer and endotoxemia. However, our studies and those of others found that the role of TNF was more complex. TNF did not inhibit LPL in isolated human adipocytes but did inhibit LPL in whole adipose tissue pieces (Fried and Zechnier 1989, Kern 1988, Mackay et al. 1990), suggesting that a paracrine factor was secreted by adipose tissue that affected LPL. This is consistent with our hypothesized role of TNF as a local regulator of adipose metabolism.

In 1993, the production of TNF by rodent adipose tissue was described (Hotamisligil et al. 1993). When compared with lean littersmates, rodents with genetic obesity and insulin resistance expressed 5- to 10-fold more TNF mRNA, and two times more TNF protein in their adipose tissue. In an attempt to reverse the insulin resistance in these animals, a soluble TNF-binding protein was infused into fa/fa rats, resulting in a two- to threefold increase in insulin-stimulated glucose uptake. In a follow-up study (Hotamisligil et al. 1994), the infusion of TNF binding protein into fa/fa rats decreased plasma insulin and FFA levels, and increased autophosphorylation of the insulin receptor (tyrosine kinase) and insulin receptor substrate-1 in both adipose tissue and muscle. This effect was seen only in the obese fa/fa rats, which overexpress TNF in adipose tissue, and not in the lean FaFa rats. A recent study suggested a possible role for the fatty acid binding protein aP2. When aP2 knock-out mice were fed a high fat diet, they became obese but did not become insulin resistant, and also did not overexpress TNF (Hotamisligil et al. 1996). Together, these studies led to several provocative conclusions. TNF overproduction by adipose tissue was involved in the pathogenesis of the insulin resistance of obesity, and could represent a form of “adipostat,” which prevented the animal from becoming obese, and perhaps too slow to evade predators.

We studied TNF expression in the adipose tissue of lean, obese and reduced-obese humans (Kern et al. 1995). The TNF protein was quantitated by Western blotting and ELISA in adipose tissue, and in the medium of cultured adipose tissue; we also developed quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) to better measure TNF mRNA from small samples. TNF mRNA levels were examined in the adipose tissue of 39 nondiabetic subjects, spanning a broad range of body mass index (BMI). As shown in Figure 2, there was a significant increase in adipose TNF mRNA levels with increasing adiposity. However, very obese subjects (BMI > 45 kg/m²) had TNF levels that were lower than moderately obese subjects. Weight loss yielded a significant decrease in adipose TNF protein and mRNA to ~50% of initial levels. Similar observations were made by others, along with a significant relationship between TNF expression and plasma insulin levels (Hotamisligil et al. 1995). Thus, TNF is expressed in human adipocytes and is elevated in most obese subjects, suggesting that the changes in TNF that occur with obesity are regulated by fat cell size, or some other component of the obese state.

As described previously, TNF administration resulted in a decrease in LPL under a variety of conditions (Doerrler et al. 1994, Fried and Zechnier 1989, Grunfeld et al. 1989, Patton et al. 1986). If the elevated adipose TNF expression that occurs with obesity prevents further weight gain, it may accomplish this through a decrease in LPL through an autocrine feedback loop. We measured fasting adipose LPL activity in all of our 39 patients and compared LPL activity levels with adipose TNF expression. We found a significant inverse relationship between TNF expression and LPL activity. Thus, these studies lend supporting evidence for a mechanism by which TNF may control fat cell size.

If endogenous TNF expression in adipose tissue helps limit obesity by increasing insulin resistance and decreasing LPL, then why would very obese subjects (BMI > 45) have relatively low TNF levels? We can identify two possible explanations. Low TNF expression may be permissive for the development of massive obesity. The relatively low levels of TNF in very obese subjects may suggest a failure of TNF to function as an adipostat, leading to efficient fat storage and a lack of inhibition to the development of obesity. However, even in these very obese subjects with low TNF expression, TNF decreased with weight loss, suggesting some level of regulation. Subject selection in these studies may be another explanation. These subjects were all nondiabetic. If adipose TNF were always to increase with progressive obesity, then subjects who became very obese would express very high TNF levels, be highly insulin resistant and would manifest noninsulin-dependent diabetes mellitus (NIDDM). Thus, by excluding diabetics, we may have excluded subjects that had the most vigorous increase in TNF with weight gain.

If adipose TNF secretion plays a role in insulin resistance, then one would expect the infusion of an anti-TNF binding protein to improve insulin sensitivity, as occurred in fa/fa rats (Hotamisligil et al. 1993). However, when obese, diabetic humans were injected with a single dose of an anti-TNF binding protein, no improvement in insulin sensitivity was observed (Ofei et al. 1996). There are several possible explanations for these data. Although systemic TNF inhibition reversed insulin resistance in rodents, perhaps TNF acts locally in humans and is less accessible to antibody inhibition. In addition, the single dose of anti-TNF binding protein given to the patients (Ofei et al. 1996) may have been insufficient to reverse insulin resistance. Patients with NIDDM may not be good candidates for anti-TNF therapy because insufficient insulin secretion is part of the etiology of the hyperglycemia. Finally, it is possible that TNF does not play a causative role in the insulin resistance, but represents a protein expressed in response to insulin resistance.
**Muscle TNF.** When a soluble TNF binding protein was infused into fafa rats, there was a two- to threefold increase in insulin-stimulated glucose uptake (Hotamisligil et al. 1993). Because muscle accounts for most in vivo glucose disposal (DeFronzo et al. 1981), these data suggested that muscle responded to the anti-TNF treatment. Further studies involving the infusion of the anti-TNF binding protein demonstrated an improvement in insulin receptor autophosphorylation in both adipose tissue and muscle (Hotamisligil et al. 1994). There are several possible explanations for these changes in muscle insulin responsiveness after anti-TNF treatment:

1) TNF was secreted by adipose tissue, circulated through plasma, and inhibited muscle insulin responsiveness. However, Hotamisligil et al. (1993) were unable to demonstrate elevated plasma TNF levels in obese patients; indeed, it has been difficult to detect TNF in plasma, even in patients with metastatic cancer (Grunfeld and Feingold 1992, Socher et al. 1988).

2) Adipose tissue TNF inhibited muscle glucose transport in a paracrine fashion. Some adipose tissue depots are in close proximity to muscle. The TNF secreted by these adipose cells may have inhibited muscle insulin action.

3) TNF was expressed by muscle itself and acted in an autocrine fashion to inhibit muscle glucose transport. Hence, when TNF binding protein was infused (Hotamisligil et al. 1993 and 1994), it directly inhibited the action of muscle TNF.

We attempted to determine whether muscle expressed TNF, and if so, whether muscle TNF was regulated by diabetes or insulin resistance. Because Hotamisligil et al. (1993) did not detect any TNF mRNA in muscle by Northern blotting, we used RT-PCR to determine whether TNF was expressed in muscle (Saghizadeh et al. 1996). Although the levels of expression were low, TNF mRNA was detected and quantitated by PCR in human skeletal muscle (vastus lateralis) and human heart.

TNF was measured in the muscle tissue of 15 subjects, who covered a spectrum of insulin sensitivity, as defined by euglycemic clamping. TNF mRNA levels in the muscle were significantly higher in both the insulin-resistant subjects and the diabetic subjects (Fig. 3). When the relationship between in vivo insulin action, as represented by the maximal glucose disposal rate, and muscle TNF expression was analyzed, there was a significant linear relationship: increased muscle TNF expression was associated with a decreased in vivo glucose disposal rate ($r = -0.60, P < 0.02$).

In addition to measuring TNF in whole-muscle biopsies, myocytes from muscle biopsies were studied. Using the method of Henry et al. (1995), cells from a muscle biopsy were placed into culture for 4 wk and then differentiated into fused myotubes; TNF mRNA expression was measured. Myocytes cultured from diabetic patients contained significantly more TNF mRNA than myocytes from nondiabetic subjects and also secreted more TNF protein into the medium.

Thus, TNF was expressed by human muscle and by muscle cells in culture. The increased expression of TNF in the muscle cells of subjects with diabetes or insulin resistance suggests that TNF could have functioned in an autocrine fashion to inhibit muscle glucose transport. Because muscle TNF was increased in insulin-resistant nondiabetics as well as in subjects with established NIDDM, the elevated muscle TNF cannot be a result of hyperglycemia and the diabetic milieu.

**FIGURE 2** Adipose tumor necrosis factor (TNF) mRNA expression (copies $\times 10^3$/mg RNA) with increasing body mass index (BMI). *$P < 0.05$ vs. BMI $< 25$ and BMI $> 45$ groups (adapted from Kern et al. 1995).
TNF expression was elevated in muscle cells cultured from diabetics. These cells had been in culture and removed from the diabetic environment for 4 wk, yet they still displayed elevated TNF. There are several possible interpretations for these data. Disordered TNF regulation in muscle may be an important part of insulin resistance. Muscle TNF may be a primary or early pathophysiological marker of the insulin resistant state and could be part of the complex genetic syndrome characterized phenotypically by obesity, insulin resistance and NIDDM. On the other hand, it is possible that elevated muscle TNF expression is in response to the insulin-resistant state and that cells maintain this overexpression when cultured.

**LITERATURE CITED**


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