Journal of Thrombosis and Haemostasis, **2**: 507–513

ORIGINAL ARTICLE

Influence of membrane-bound tumor necrosis factor (TNF)-α on obesity and glucose metabolism

G. VOROS, E. MAQUOI, D. COLLEN and H. R. LIJNEN
Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

To cite this article: Voros G, Maquoi E, Collen D, Lijnen HR. Influence of membrane-bound tumor necrosis factor (TNF)-α on obesity and glucose metabolism. *J Thromb Haemost* 2004; **2**: 507–13.

Summary. *Objective*: To investigate the influence of transmembrane tumor necrosis factor (TNF)-α on adipose tissue development and insulin-mediated glucose metabolism. *Methods and results*: TNF-α and lymphotoxin-α-deficient mice expressing non-cleavable transmembrane TNF-α (Tg-tmTNF-α) and TNF-α/lymphotoxin-α double knockout (control) mice were kept on high-fat diet for 15 weeks. The food intake and feeding efficiency of Tg-tmTNF-α mice were significantly higher compared with control mice. At the end of the study, Tg-tmTNF-α mice had a significantly higher total body weight, as well as subcutaneous and gonadal adipose tissue mass. Histological analysis revealed that the expression of Tg-tmTNF-α resulted in a significantly increased adipocyte area and blood vessel density. Plasma leptin levels correlated positively with adipose tissue mass. The plasma levels of total cholesterol and HDL-cholesterol were significantly increased and LDL-cholesterol levels significantly decreased in Tg-tmTNF-α mice. Fasting blood glucose and plasma insulin levels were not different between the two genotypes and intraperitoneal glucose and insulin tolerance tests did not show significant differences. *Conclusions*: Transmembrane TNF-α enhances adipose tissue formation without altering insulin-mediated glucose metabolism in mice with nutritionally induced obesity.

Keywords: glucose metabolism, insulin, obesity, transmembrane tumor necrosis factor-α.

Obesity is a common disorder in Western type societies; by current estimates about 30% of US adults are obese and another 35% are overweight [1]. Obesity is a major risk factor for dyslipidemia, Type 2 diabetes mellitus and hypertension and it can directly or indirectly cause thrombotic complications and heart disease. Its strong association with insulin resistance has been firmly established in animal models and in humans [2].

Tumor necrosis factor (TNF)-α is a candidate mediator of insulin resistance in obesity. Its adipose tissue mRNA and protein levels are increased in obese rodents and humans [3], and decrease following weight reduction [4]. Mice lacking TNF-α show lower plasma insulin levels and decreased insulin resistance in high-fat diet (HFD) or gold thioglucose-induced obesity models [5,6]. Ob/ob mice with targeted mutations in both p55 and p75 TNF receptors are as obese as control ob/ob mice, but plasma insulin levels are lower and insulin sensitivity is improved [5]. Plasminogen activator inhibitor-1 (PAI-1) is produced by the adipose tissue and its expression is enhanced by TNF-α; PAI-1-overexpressing mice show attenuated nutritionally induced obesity [7]. TNF-α also induces expression of leptin, which is a satiety factor regulating food intake; its plasma levels correlate with degree of obesity [8].

TNF-α exists as a 26-kDa transmembrane form that is cleaved by the TNF-α converting enzyme (TACE or ADAM-17) to yield a 17-kDa soluble molecule; TACE expression was observed in murine adipose tissue [9]. Recently, it was shown that in adipose tissue of obese mice and humans TNF-α occurs predominantly in the transmembrane form [10], possibly explaining the lack of association of circulating TNF-α levels with obesity in humans [4]. Furthermore, inhibition of TNF-α shedding with a matrix metalloproteinase inhibitor (KB-R7785) was shown to improve insulin-mediated glucose metabolism [11,12]. In this study, we have investigated the impact of transmembrane TNF-α on adipose tissue formation and glucose metabolism in mice with nutritionally induced obesity. As a model we have used transgenic mice expressing a mutated non-cleavable transmembrane form of TNF-α, therefore lacking circulating TNF-α [13].

Materials and methods

Diet model

Transgenic mice deficient in both TNF-α and lymphotoxin-α (LT-α), but expressing non-cleavable transmembrane murine

Correspondence: H. R. Lijnen, Center for Molecular and Vascular Biology, University of Leuven, Campus Gasthuisberg, O & N, Herestraat 49, B-3000 Leuven, Belgium.
Tel.: +32 1634 5771; fax: +32 1634 5990; e-mail: roger.lijnen@med.kuleuven.ac.be

Received 15 August 2003, accepted 23 November 2003

TNF-α (Tg-tmTNF-α), and TNF-α/LT-α double knockout (control) mice (mixed C57Bl/6j × 129SvEv genetic background) were a kind gift of C. Mueller (University of Bern, Switzerland) [13]. These mice expressed a transmembrane TNF-α with mutations at the three known cleavage sites. Five-week-old male mice ($n = 9$ for controls and $n = 10$ for Tg-tmTNF-α mice) were kept in microisolation cages on a 12-h day–night cycle and fed water and a HFD *ad libitum* as previously described [14]. Weight of the mice and food intake were measured every 3 days during 15 weeks. Feeding efficiency was calculated as the increase in body weight per kcal food intake. Physical activity was measured during the last week of the diet study, as described [15]; data are represented as number of cycles per 12 h at night ($n = 4$ for each genotype). Blood was collected from the retroorbital sinus with addition of trisodium citrate (final concentration 0.01 mol L^{-1}). At the end of the diet period, following overnight fasting the mice were anesthetized by interperitoneal (i.p.) injection of 60 mg kg^{-1} Nembutal (Abbott Laboratories, North Chicago, IL, USA). Intra-abdominal (gonadal, GON) and inguinal subcutaneous (SC) fat pads were removed and the wet weight determined. Liver, kidney, lungs and quadriceps muscles were also removed and frozen at − 80 °C.

All animal experiments were approved by the university ethics committee and were performed in accordance with the guiding principles of the American Physiological Society and the International Society on Thrombosis and Haemostasis [16].

Metabolic parameters

PAI-1 antigen levels were measured with a specific home-made ELISA [17]. Insulin (Mercodia, Uppsala, Sweden) and leptin (R&D Systems, Abingdon, UK) antigen levels were determined with commercial ELISA kits. Blood glucose concentrations were measured using Glucocard strips (Menarini Diagnostics, Florence, Italy) and triglyceride and cholesterol levels were evaluated using routine clinical assays. Lipid extracts of adipose tissue were prepared according to Folch *et al.* [18].

For i.p. glucose and insulin tolerance tests, after an overnight fast, glucose (3 mg g^{-1} body weight) or insulin (0.5 mU g^{-1}) was injected into the peritoneal cavity. Blood was collected via the tail vein and glucose levels were measured before and 15, 30, 45, 60, 90 and 120 min after injection.

Morphometric and histological analysis

The number of adipocytes and their mean size were determined by computer-assisted image analysis on 10-μm paraffin sections of SC and GON adipose tissue examined by fluorescence microscopy. The extracellular matrix between adipocytes was found to have autofluorescent properties, probably due to its high collagen content. For each animal, three areas in four different sections were analyzed; the data were first averaged per section and then per animal. Blood vessels were visualized by staining paraffin sections with the biotinylated Bandeiraea (Griffonia) Simplicifolia BSI lectin (Sigma-Aldrich, St Louis, MO, USA) followed by signal amplification with the Tyramide Signal Amplification Cyanine System (Perkin Elmer, Boston, MA, USA). For each mouse, at least 12 randomly selected fields in nine to 12 sections were analyzed by computer-assisted image analysis, and data were then averaged. Lectin-positive areas were expressed in percent of total section area.

mRNA determinations

Total DNA-free RNA from GON and SC adipose tissue and from skeletal muscle samples was prepared using the RNeasy mini kit (Qiagen, Valencia, CA, USA) and RNA concentrations were determined using the RiboGreen RNA quantification kit (Molecular Probes, Eugene, OR, USA). RNA samples from nine mice were pooled and the mean expression level of murine TNF-α, p55 and p75 TNF-α receptors was determined by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using the GeneAmp Thermostable RNA PCR kit (Applied Biosystems, Foster City, CA, USA) using the following primer pairs: TNF-α, forward primer GGGCC ACCACGCTCTTC, reverse primer CCTCCACTTGGTG GTTTGCT AC; p55 TNFR, forward primer TGCAG GGTTCTTTCTGAGAGAAAG, reverse primer GGATA GAAGGCAAAGACCTAGCAAG; p75 TNFR, forward primer GACTGTGAGGCAAGCATGTATACC, reverse primer TCGACAGCTGCCAGAATGG. Results were normalized to 28S rRNA levels, as described previously [19].

Statistical analysis

Data are generally expressed as mean ± SEM. For the metabolic parameters data are given as median ± SEM. Statistical significance between groups is evaluated using non-parametric *t*-testing. Correlation analysis is performed using the non-parametric Spearman's rank correlation coefficient. Values of $P < 0.05$ are considered statistically significant.

Results

Diet study

At the start of the diet, transgenic mice expressing non-cleavable Tg-tmTNF-α and double TNF-α/LT-α knockout mice (controls) had a comparable total body weight (Table 1 and Fig. 1). When fed a HFD for 15 weeks Tg-tmTNF-α mice gained more weight, resulting in a significantly higher total body weight at the end of the study. The weight of isolated SC and GON fat pads was also significantly higher (Table 1), whereas the weight of other tissues including liver, kidneys and lungs was not different (not shown), indicating that enhanced adipose tissue growth is not aspecifically caused by increased body growth. The triglyceride content in the liver of Tg-tm TNF-α mice was higher than in control mice (13 ± 0.9 vs. 8.8 ± 0.6 mg g^{-1} tissue; mean ± SEM, $n = 10$; $P < 0.001$). Based on the weight of the GON fat pad [20], the percentage of body fat in Tg-tmTNF-α mice was estimated at 3.5 ± 0.24%

Table 1 Effect of transmembrane tumor necrosis factor (TNF)-α expression on total body weight and adipose tissue mass in mice after 15 weeks of high-fat diet

	Control	Tg-tmTNF-α
Initial weight (g)	16 ± 0.4	16 ± 0.7
Final weight (g)	28 ± 0.8	33 ± 1.2**
Weight gain (g)	12 ± 1.0	16 ± 0.8**
Food intake (g day^{-1})	2.6 ± 0.07	3.2 ± 0.08**
Feeding efficiency (mg $kcal^{-1}$)	8.6 ± 0.3	10 ± 0.6*
Adipose tissue (mg)		
SC	280 ± 32	660 ± 81**
GON	380 ± 43	1150 ± 110***
Adipocyte number $(mm^2)^{-1}$		
SC	970 ± 120	330 ± 40***
GON	380 ± 47	210 ± 18**
Adipocyte area (μm^2)		
SC	1180 ± 160	3410 ± 380***
GON	2930 ± 370	5070 ± 420**

Data are mean ± SEM of nine or 10 experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. control. SC, subcutaneous; GON, gonadal.

vs. 1.4 ± 0.15% in controls ($n = 9$ or 10; $P = 0.00002$). The average food intake and the feeding efficiency appeared significantly higher for Tg-tmTNF-α mice compared with controls (Table 1 and Fig. 1). The average physical activity of Tg-tmTNF-α mice on HFD was significantly lower than that of the controls (960 ± 560 cycles 12 h^{-1} vs. 4960 ± 840 cycles 12 h^{-1}, $P < 0.05$); these determinations showed a large variability but were on average 5-fold lower in Tg-tm TNF-α mice.

Adipose tissue cellularity

Histological analysis of SC and GON fat pads obtained from mice fed a HFD for 15 weeks suggested that adipocytes were larger in Tg-tmTNF-α mice than in controls. Morphometric analysis confirmed that the number of adipocytes was significantly lower in Tg-tmTNF-α mice, whereas their diameter was larger (Fig. 2). Thus, the mean adipocyte areas of both SC and GON adipose tissue of Tg-tmTNF-α mice were larger (by 290% and 170%, respectively) than those of control mice (Table 1), and the mean volume of adipocytes in both SC and GON fat pads of Tg-tmTNF-α mice was about 3.5-fold enhanced. The lipid (triglyceride) content in SC or GON adipose tissue of Tg-tmTNF-α mice (68 ± 9 or 93 ± 19 mg g^{-1} tissue) was significantly higher compared with control mice (21 ± 6 or 40 ± 8 mg g^{-1} tissue; mean ± SEM, $n = 9$; $P < 0.05$).

Staining of adipose tissues with an endothelial cell-specific lectin revealed smaller stained areas in SC adipose tissue of Tg-tmTNF-α mice vs. controls (Fig. 2). Quantitative analysis confirmed a significantly lower blood vessel density and smaller total stained area in SC, but not in GON tissue of Tg-tmTNF-α mice. However, after normalization to the adipocyte density, blood vessel density was higher in both SC and GON adipose tissue of Tg-tmTNF-α mice (Table 2).

Metabolic parameters

Blood glucose, plasma PAI-1 antigen and triglyceride levels were comparable for both genotypes (Table 3). Plasma insulin levels were somewhat but not significantly higher in the

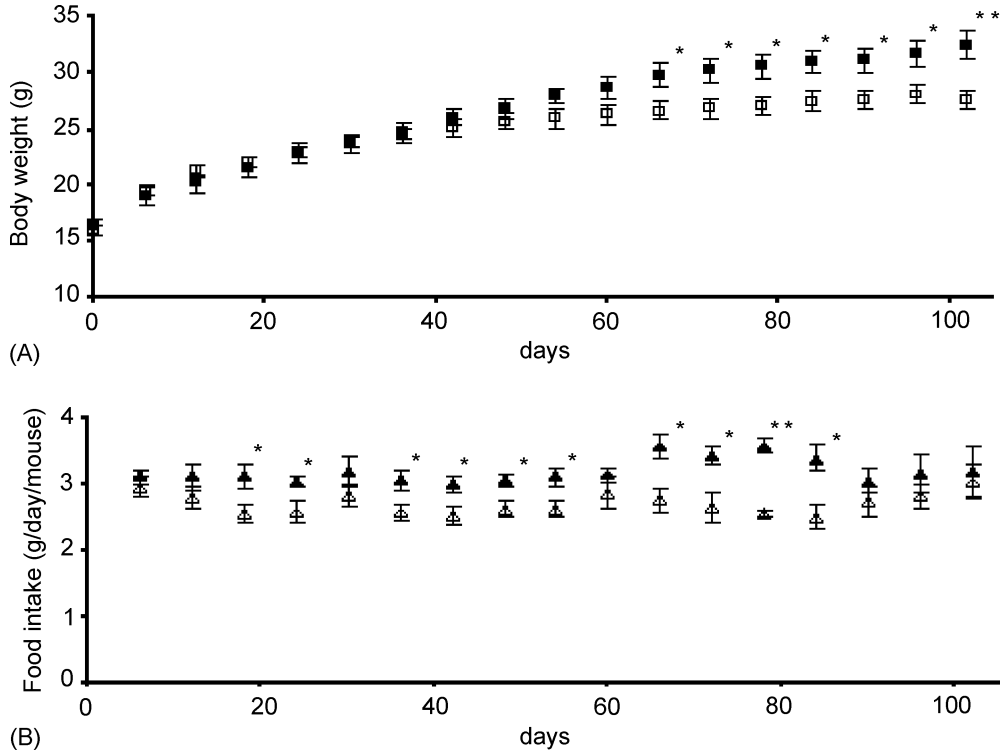

Fig. 1. Growth curve (A) of Tg-tmTNF-α (■) and control (□) mice. Mice were kept on high-fat diet (HFD) for 15 weeks and total body weights are shown ($n = 9$ or 10). Food intake in g day^{-1} per mouse (B) is also shown for Tg-tmTNF-α (▲) and control (Δ) mice. *$P < 0.05$; **$P < 0.01$ vs. controls.

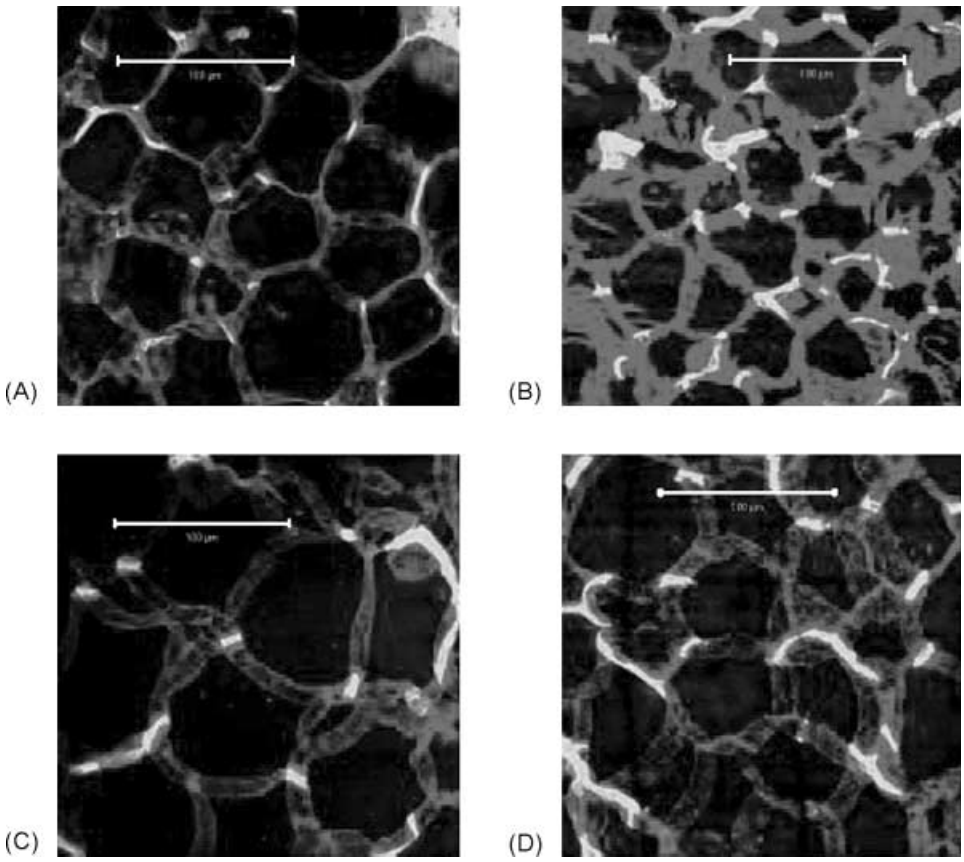

Fig. 2. Staining with the Bandeiraea Simplicifolia lectin of subcutaneous (A,B) or gonadal (C,D) adipose tissue of Tg-tmTNF-α (A,C) and control (B,D) mice. Blood vessels are visualized as white structures.

Tg-tmTNF-α group. Total cholesterol and HDL-cholesterol levels were significantly elevated in the plasma of Tg-tmTNF-α mice, whereas LDL-cholesterol levels were significantly reduced (Table 3). Plasma leptin levels were elevated in Tg-tmTNF-α mice, although due to large interindividual variability statistical significance was not reached. Leptin levels correlated well with total body weight, SC and GON adipose tissue mass in Tg-tmTNF-α mice (total body weight, $r = 0.74$, $P = 0.037$; SC tissue mass, $r = 0.90$, $P = 0.002$; GON tissue mass, $r = 0.79$, $P = 0.021$), as well as in both genotypes pooled (total body weight, $r = 0.67$, $P = 0.003$; SC tissue mass, $r = 0.86$, $P = 0.00001$; GON tissue mass, $r = 0.80$, $P = 0.0001$), but not in the control group (total body weight, $r = 0.41$, $P = 0.27$; SC tissue mass, $r = 0.61$, $P = 0.081$; GON tissue mass, $r = 0.56$, $P = 0.11$).

Expression of TNF-α and TNF-α receptors

Semiquantitative RT-PCR confirmed expression of TNF-α mRNA in SC and GON adipose tissue and in skeletal muscle (quadriceps) of Tg-tmTNF-α mice, whereas it was undetectable in control mice (Fig. 3). Determination of TNF-α mRNA levels in adipose tissue of mice at 5 weeks of age and after 15 weeks on HFD did not reveal a marked effect of the HFD on its

Table 2 Characterization of blood vessels in subcutaneous (SC) and gonadal (GON) adipose tissue of Tg-tmTNF-α and control mice kept on high-fat diet for 15 weeks

	SC		GON	
	Control	Tg-tmTNF-α	Control	Tg-tmTNF-α
Stained area[a]	8.0 ± 1.1	3.8 ± 0.7*	3.2 ± 0.5	2.1 ± 0.2
Vessel density[b]	560 ± 74	270 ± 27*	220 ± 20	180 ± 11
Vessel size[c]	150 ± 19	140 ± 15	150 ± 20	120 ± 9
Vessel density/adipocte density[d]	0.58 ± 0.02	0.83 ± 0.03**	0.60 ± 0.05	0.87 ± 0.05**

[a]Lectin-stained area in percent of the total area. [b]Number of vessels per mm^2 tissue. [c]Vessel size in μm^2. [d]Number of vessels normalized to adipocyte density, which was calculated as number of adipocytes per mm^2 tissue. Data are mean ± SEM of nine or 10 experiments. *$P < 0.01$; **$P < 0.001$ vs. control.

Table 3 Effect of transmembrane tumor necrosis factor (TNF)-α expression on metabolic parameters in mice after 15 weeks on high-fat diet

	Control	Tg-tmTNF-α	*P*-value
PAI-1 (ng mL^{-1})	1.9 ± 0.15	1.9 ± 0.23	0.755
Triglycerides (mg dL^{-1})	32 ± 2.6	42 ± 5.2	0.079
Total cholesterol (mg dL^{-1})	98 ± 5.7	109 ± 4.8*	0.018
LDL-cholesterol (mg dL^{-1})	8.5 ± 1.3	3.0 ± 0.98*	0.015
HDL-cholesterol (mg dL^{-1})	84 ± 4.9	97 ± 4.5**	0.003
Glucose (mg dL^{-1})	77 ± 7.0	106 ± 11	0.10
Insulin (pg mL^{-1})	565 ± 200	1222 ± 260	0.10
Leptin (pg mL^{-1})	2300 ± 350	6200 ± 3100	0.055

Data are median ± SEM of nine or 10 experiments. *$P < 0.05$; **$P < 0.01$ vs. control.

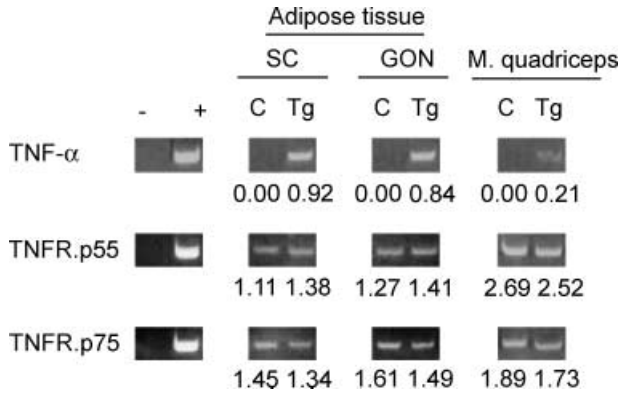

Fig. 3. Expression of TNF-α, p55 and p75 TNF-α receptors in subcutaneous (SC) and gonadal (GON) adipose tissue and in the quadriceps muscle of Tg-tmTNF-α (Tg) and control (C) mice kept on high-fat diet (HFD) for 15 weeks. Lane (−) indicates a blank control without sample, and (+) indicates a positive control (spleen). Expression levels are normalized to 28S rRNA, and mean values are indicated below the panels.

expression (mRNA level, normalized to 28S rRNA, was decreased by 40% in SC tissue and increased by 57% in GON tissue). TNF-α receptors p55 and p75 were expressed in both SC and GON adipose tissue and in skeletal muscle samples, and the mean expression levels were similar for the two genotypes.

Systemic glucose and insulin tolerance

Intraperitoneal glucose tolerance tests were performed 2 weeks before the end of the diet study. Maximal blood glucose levels were achieved 30 min after the injection in both genotypes, whereafter glucose concentrations decreased gradually with time (Fig. 4A). Glucose levels did not differ significantly between the two genotypes, although there was a trend towards higher glucose levels in the Tg-tmTNF-α group after 2 h. Glucose levels at 2 h correlated with SC and GON adipose tissue mass ($P = 0.031$ and $P = 0.032$), but this correlation disappeared after adjustment of glucose levels for body weight. Insulin tolerance tests also yielded similar results for both genotypes (Fig. 4B).

Discussion

To investigate a potential role of tmTNF-α in adipose tissue formation and glucose metabolism we have used a murine model of nutritionally induced obesity with transgenic mice deficient in both TNF-α and LT-α, but expressing non-cleavable transmembrane murine TNF-α [13]. We thus have a model lacking circulating TNF-α (Tg-tmTNF-α mice) and controls of the same genetic background. tmTNF-α can activate the p55 and p75 TNF receptors via cell–cell interactions [13,21]. TNF-α and LT-α are two homologous proteins, which can form homotrimers and activate both TNF receptors [22]. However, LT-α is also able to bind two lymphotoxin-β molecules and this membrane-bound heterotrimer has a distinct receptor, LTβR [23,24]. Studies with knockout mice revealed that the activities of TNF and LT *in vivo* are indeed overlapping, although there are considerable differences in several tissues, including peripheral lymphoid organs, such as spleen, lymph nodes and gut-associated lymphoid tissues [25]. The phenotypic defects in LT knockout mice are generally much more severe than in TNF-deficient mice. In order to study the exclusive effects of tmTNF-α on adipose tissue formation and to avoid compensation by LT-α, we selected mice expressing tmTNF-α in an LT-α-deficient background.

Mice expressing only tmTNF-α gained weight faster than controls when fed a HFD during 15 weeks. Their adipose tissue

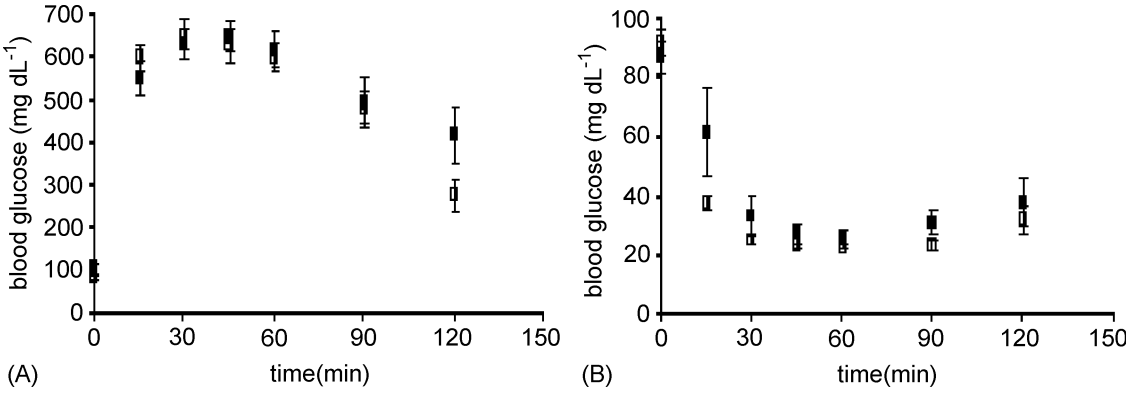

Fig. 4. Intraperitoneal glucose (A) and insulin (B) tolerance tests in Tg-tmTNF-α (■) and control (□) mice after high-fat diet (HFD) for 15 weeks. Blood glucose levels (in mg dL^{-1}) are plotted against time. Data are mean ± SEM of five and six experiments.

area was larger mainly as a result of adipocyte hypertrophy. This observation differs from the results of Xu *et al*. [26], who found that mice producing tmTNF-α were leaner than controls. This discrepancy may be explained by several differences in experimental settings: (i) in the study of Xu *et al*. the mice were kept on a standard chow, whereas we used a HFD to induce obesity; (ii) Xu *et al*. used the aP2 promoter to obtain adipose tissue-specific expression of tmTNF-α, whereas in our mice the expression of tmTNF-α is under the control of its physiological promoter, allowing production by different organs and more physiological regulation of expression; and (iii) the mice used in this study were also deficient in LT-α, which could thus not influence effects of tmTNF-α on adipose tissue formation.

When produced at high concentrations *in vivo* TNF-α contributes to cachexia, a condition characterized by extensive weight loss and reduction of adipose tissue mass [27]. Furthermore, many *in vitro* experiments with different preadipocyte cell lines revealed that administration of TNF-α causes a dose-dependent inhibition of preadipocyte differentiation [28,29]. On the other hand, different murine diet models did not show a significant increase in the development of adipose tissue in TNF-α-deficient and TNF-α receptor-deficient mice compared with wild types [5,6]. In one study p75 TNF-α receptor-deficient mice were even significantly leaner after 16 weeks of HFD [30]. TNF-α may thus have different effects *in vivo* compared with *in vitro* situations, which may depend on its local concentration, the availability of its receptors, and the presence of other modifying factors. We confirmed expression of TNF-α mRNA in adipose tissue of the Tg-tmTNF-α mice, and observed comparable expression of p55 and p75 receptors in adipose tissue of both genotypes. In our experiments, the stimulatory effect of tmTNF-α on the development of adipose tissue during HFD was associated with increased food intake and feeding efficiency and decreased physical activity. TNF-α among other cytokines has a considerable impact on regulation of feeding. Its mRNA, protein and its receptors are present in several brain regions, including the hypothalamus, which contains numerous orexigen and anorexigen centers [31]. Many studies showed that enhanced expression of TNF-α contributes to anorexia in disease [32]. As a member of the cytokine network its production is increased in response to immune stimulation. Among other effects it inhibits feeding after peripheral or central administration [33]. However, it is unclear which regions of the central nervous system are responsible for the anorectic effects of TNF-α, which mechanisms controlling food intake are involved, and whether TNF-α must be in its soluble form to cause anorexia. The influence of LT-α on feeding was less intensively studied. In one report intracerebrovascular administration of LT-α resulted in anorexia in rats [34]. Our study suggests that TNF-α, if confined to the cell membrane, causes increased food intake in LT-α-deficient mice on HFD. The mediators in the hypothalamus involved in this effect remain to be determined.

Histological analysis showed that the mean area of adipocytes was significantly larger in Tg-tmTNF-α mice, whereas the lectin-stained area and the vessel density were significantly smaller in SC adipose tissue of Tg-tmTNF-α mice. The decrease of lectin-stained area and vessel density is likely to be caused by the dilution effect resulting from the increased adipocyte size. When these parameters were normalized to adipocyte density, the lectin-stained area was significantly larger in SC adipose tissue and the vessel density was larger in both SC and GON adipose tissues of Tg-tmTNF-α mice. Thus, individual adipocytes may get a higher blood supply, which may contribute to the faster growth of adipose tissue.

We found slightly increased triglyceride and significantly higher cholesterol levels in the plasma of Tg-tmTNF-α mice. TNF-α increases hepatic synthesis of triglycerides in rats and inhibits synthesis of lipoprotein lipase, both of which may result in hypertriglyceridemia [35]. In rodents and rabbits cholesterol production is induced by infection and other inflammatory conditions, which may be due to an increase in *de novo* hepatic cholesterol synthesis [36]. Interestingly, we found significantly increased HDL- and decreased LDL-cholesterol in the plasma of Tg-tmTNF-α mice. In several studies administration of TNF-α decreased the expression and activity of lecithin:cholesterol acyltransferase (LCAT), which may have accounted for the decreased plasma HDL levels [37]. In our mice the exclusive effect of tmTNF-α may be enough to increase cholesterol levels, but may not be able to inhibit plasma LCAT activity leading to a higher plasma HDL concentration. Inhibition of tmTNF-α cleavage may thus be beneficial for the lipid profile. Plasma leptin levels were increased, although not significantly, in Tg-tmTNF-α mice. Leptin levels correlated well with total body weight and with SC and GON adipose tissue mass in Tg-tmTNF-α mice, but not in the control group. This may be explained by the fact that control mice had very similar total body weights and adipose tissue masses; thus it was difficult to make a correlation with plasma leptin levels. Our data indicate that the relationship between plasma leptin levels and adiposity is preserved in Tg-tmTNF-α mice, suggesting that the expression of transmembrane TNF-α does not impair leptin secretion.

Insulin-mediated glucose metabolism was investigated by measuring blood glucose and plasma insulin levels and performing i.p. glucose and insulin tolerance tests. These experiments showed that insulin-mediated glucose metabolism is only slightly impaired in the Tg-tmTNF-α animals. This is consistent with the results of Xu *et al*. [26], who found only a non-significant improvement of systemic glucose metabolism in tmTNF-α-producing mice despite local insulin resistance. These authors concluded that tmTNF-α, inhibiting adipose tissue development, only indirectly affected systemic glucose metabolism. Our data also suggest that expression of tmTNF-α does not extensively influence glucose metabolism and that the modifications are related to enhanced adipose tissue formation. This is supported by the finding of a weak correlation between glucose levels 2 h after i.p. glucose injection and adipose tissue mass in both genotypes, which disappears after adjustment for body weight.

In conclusion, this study showed that tmTNF-α, in the absence of LT-α, increased weight gain and adipose tissue mass of mice on HFD. The influence of LT-α on these parameters may be evaluated in subsequent diet studies.

Acknowledgements

This study was supported by a grant from the Interuniversity Attraction Poles (IUAP, P5/02), and from the Flemish Fund for Scientific Research (FWO-Vlaanderen, G.0233.03). G.V. was the recipient of a grant from the fund 'KU Leuven–Soros Foundation'.

References

1 Kelner K, Helmuth L. Obesity—what is to be done? *Science* 2003; **299**: 845–9.
2 Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960–1994. *Int J Obes Relat Metab Disord* 1998; **22**: 39–47.
3 Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87–91.
4 Hotamisligil GS, Amer P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409–15.
5 Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 1997; **389**: 610–4.
6 Ventre J, Doebber T, Wu M, MacNaul K, Stevens K, Pasparakis M, Kollias G, Moller DE. Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. *Diabetes* 1997; **46**: 1526–31.
7 Lijnen HR, Maquoi E, Morange P, Voros G, Van Hoef B, Kopp F, Collen D, Juhan-Vague I, Alessi M-C. Nutritionally induced obesity is attenuated in transgenic mice overexpressing plasminogen activator inhibitor-1. *Arterioscler Thromb Vasc Biol* 2003; **23**: 78–84.
8 Kirchgessner TG, Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J Clin Invest* 1997; **100**: 2777–82.
9 Voros G, Maquoi E, Collen D, Lijnen HR. Differential expression of plasminogen activator inhibitor-1, tumor necrosis factor-alpha, TNF-alpha converting enzyme and ADAMTS family members in murine fat territories. *Biochim Biophys Acta* 2003; **1625**: 36–42.
10 Xu H, Uysal KT, Becherer JD, Amer P, Hotamisligil GS. Altered tumor necrosis factor-alpha (TNF-alpha) processing in adipocytes and increased expression of transmembrane TNF-alpha in obesity. *Diabetes* 2002; **51**: 1876–83.
11 Morimoto Y, Nishikawa K, Ohashi M. KB-R7785, a novel matrix metalloproteinase inhibitor, exerts its antidiabetic effect by inhibiting tumor necrosis factor-alpha production. *Life Sci* 1997; **61**: 795–803.
12 Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K. Effect of TNF-alpha-converting enzyme inhibitor on insulin resistance in fructose-fed rats. *Hypertension* 2002; **39**: 578–80.
13 Mueller C, Corazza N, Trachsel-Loseth S, Eugster HP, Buhler-Jungo M, Brunner T, Imboden MA. Non-cleavable transmembrane mouse tumor necrosis factor-alpha (TNFalpha) mediates effects distinct from those of wild-type TNFalpha *in vitro* and *in vivo*. *J Biol Chem* 1999; **274**: 38112–8.
14 Lijnen HR, Maquoi E, Demeulemeester D, Van Hoef B, Collen D. Modulation of fibrinolytic and gelatinolytic activity during adipose tissue development in a mouse model of nutritionally induced obesity. *Thromb Haemost* 2002; **88**: 345–53.
15 Lijnen HR, Demeulemeester D, Van Hoef B, Collen D, Maquoi E. Deficiency of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) impairs nutritionally induced obesity in mice. *Thromb Haemost* 2003; **89**: 249–55.
16 Giles AR. Guidelines for the use of animals in biomedical research. *Thromb Haemost* 1987; **58**: 1078–84.
17 Declerck PJ, Verstreken M, Collen D. Immunoassay of murine t-PA, u-PA and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. *Thromb Haemost* 1995; **74**: 1305–9.
18 Folch J, Lees M, Sloane-Stanely GH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 1957; **226**: 497–509.
19 Maquoi E, Munaut C, Colige A, Collen D, Lijnen HR. Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. *Diabetes* 2002; **51**: 1093–101.
20 Rogers P, Webb GP. Estimation of body fat in normal and obese mice. *Br J Nutr* 1980; **43**: 83–6.
21 Xu H, Sethi JK, Hotamisligil GS. Transmembrane tumor necrosis factor (TNF)-alpha inhibits adipocyte differentiation by selectively activating TNF receptor 1. *J Biol Chem* 1999; **274**: 26287–95.
22 Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, Kohr WJ, Aggarwal BB, Goeddel DV. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984; **312**: 724–9.
23 Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP, Hession C, O'Brine-Greco B, Foley SF, Ware CF. Lymphotoxin beta, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. *Cell* 1993; **72**: 847–56.
24 Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, Browning JL, Din WS, Goodwin RG, Smith CA. A lymphotoxin-beta-specific receptor. *Science* 1994; **264**: 707–10.
25 De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, Smith SC, Carlson R, Shornick LP, Strauss-Schoenberger J. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* 1994; **264**: 703–7.
26 Xu H, Hirosumi J, Uysal KT, Guler AD, Hotamisligil GS. Exclusive action of transmembrane TNF alpha in adipose tissue leads to reduced adipose mass and local but not systemic insulin resistance. *Endocrinology* 2002; **143**: 1502–11.
27 Yoneda T, Alsina MA, Chavez JB, Bonewald L, Nishimura R, Mundy GR. Evidence that tumor necrosis factor plays a pathogenetic role in the paraneoplastic syndromes of cachexia, hypercalcemia, and leukocytosis in a human tumor in nude mice. *J Clin Invest* 1991; **87**: 977–85.
28 Kawakami M, Watanabe N, Ogawa H, Kato A, Sando H, Yamada N, Murase T, Takaku F, Shibata S, Oda T. Cachectin/TNF kills or inhibits the differentiation of 3T3-L1 cells according to developmental stage. *J Cell Physiol* 1989; **138**: 1–7.
29 Ron D, Brasier AR, McGehee RE Jr, Habener JF. Tumor necrosis factor-induced reversal of adipocytic phenotype of 3T3-L1 cells is preceded by a loss of nuclear CCAAT/enhancer binding protein (C/EBP). *J Clin Invest* 1992; **89**: 223–33.
30 Schreyer SA, Chua SC Jr, LeBoeuf RC. Obesity and diabetes in TNF-alpha receptor-deficient mice. *J Clin Invest* 1998; **102**: 402–11.
31 Breder CD, Hazuka C, Ghayur T, Klug C, Huginin M, Yasuda K, Teng M, Saper CB. Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration. *Proc Natl Acad Sci USA* 1994; **91**: 11393–7.
32 Plata-Salaman CR. Immunomodulators and feeding regulation: a humoral link between the immune and nervous systems. *Brain Behav Immun* 1989; **3**: 193–213.
33 Fantino M, Wieteska L. Evidence for a direct central anorectic effect of tumor-necrosis-factor-alpha in the rat. *Physiol Behav* 1993; **53**: 477–83.
34 Kapas L, Krueger JM. Tumor necrosis factor-beta induces sleep, fever, and anorexia. *Am J Physiol* 1992; **263**: R703–7.
35 Feingold KR, Soued M, Staprans I, Gavin LA, Donahue ME, Huang BJ, Moser AH, Gulli R, Grunfeld C. Effect of tumor necrosis factor (TNF) on lipid metabolism in the diabetic rat. Evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for TNF-induced hyperlipidemia. *J Clin Invest* 1989; **83**: 1116–21.
36 Cabana VG, Siegel JN, Sabesin SM. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J Lipid Res* 1989; **30**: 39–49.
37 Ly H, Francone OL, Fielding CJ, Shigenaga JK, Moser AH, Grunfeld C, Feingold KR. Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters. *J Lipid Res* 1995; **36**: 1254–63.