Distinct long-term regulation of glycerol and non-esterified fatty acid release by insulin and TNF- α in 3T3-L1 adipocytes

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Abstract

Aims/hypothesis. Adipose tissue lipolysis plays a central part in total body fuel metabolism. Our study was to assess the long-term regulation of glycerol and non-esterified fatty acid (NEFA) release by insulin or TNF- α .

Methods. Fully differentiated 3T3-L1 adipocytes were exposed for up to 22 h to insulin or TNF- α .

Results. Long-term insulin treatment resulted in increased basal glycerol release, reaching sixfold at 22 h with 1 nmol/l insulin. Partial inhibition was observed by pharmacologically inhibiting phosphatidylinositol 3-kinase or the mitogen-activated kinase kinase – extracellular signal-regulated kinase cascades. This represented 50–60% of the response induced by 1 nmol/l TNF- α and approximately 40% of the glycerol release maximally stimulated by isoproterenol (1 μ mol/l, 30 min). The cellular mechanism seemed to be distinct from that of TNF- α : First, glycerol release in response to long-term insulin was progressive with time and did not display a lag-time char-

acteristic of the effect of TNF- α . Second, pretreatment and co-treatment of the cells with troglitazone greatly inhibited TNF- α -induced glycerol release (128.5 ± 10.2 to 35.4 ± 2.1 nmol/mg protein per h) but not the effect of insulin, which was exaggerated. Third, hormone-sensitive lipase protein content was decreased (45%) by TNF- α but not following long-term insulin. Finally, TNF- α was associated with NEFA release to the medium, whereas long-term insulin treatment was not. Moreover, glycerol release during isoproterenol-stimulated lipolysis was additive to the effect of long-term insulin, whereas NEFA release was inhibited by nearly 90%.

Conclusions interpretation. Contradictory to its short-term inhibitory effect, long-term insulin stimulates glycerol release with concomitant stimulation of NEFA re-esterification. [Diabetologia (2001) 44: 55–62]

Keywords Lipolysis, troglitazone, long-term insulin, hormone sensitive lipase.

The regulation of adipocyte lipolysis is increasingly recognized to play a part in the pathophysiology of

obesity, insulin resistance and Type II (non-insulin-dependent) diabetes mellitus. Both non-esterified fatty acids (NEFA) and glycerol, the two end products of lipolysis, are potential mediators for metabolic (dys)regulation, which is associated with these common conditions [1, 2]. Prolonged increases in NEFA concentrations could induce or aggravate skeletal muscle insulin resistance [3], impair pancreatic beta-cell function [4] and result in glucose overproduction [5, 6]. Glycerol has recently been suggested to be used by skeletal muscle as a precursor for intramuscular triacylglycerol synthesis [2], which in turn when excessive, could result in skeletal muscle insulin

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Abbreviations: ERK, Extracellular signal-regulated kinase 1/2; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MEK, mitogen-activated kinase kinase; PKA, protein kinase A; PI 3-kinase phosphatidylinositol 3 kinase; TZD, thiazolidinedione; PDE-3B, phosphodiesterase 3B; KRP, Krebs-Ringer phosphate; RIA, radioimmunoassay.

resistance [7]. Consistent with this is the increased basal lipolysis [5, 8] and the altered catecholamine-stimulated lipolysis [8, 9] seen in cohorts of Type II diabetic and obese patients, further supporting the involvement of altered NEFA and glycerol generation in these pathophysiological conditions. The mechanisms for these abnormalities in both short-term and long-term regulation of adipocyte lipolysis have, however, not been fully resolved.

Beta-adrenergic agents and insulin are important short-term regulators of lipolysis, largely through protein kinase A (PKA)-mediated phosphorylation of hormone-sensitive lipase (HSL) [10–12]. This enzyme has been extensively studied and is considered the major regulatory and rate-limiting enzyme in adipocyte triacylglycerol breakdown [13–15]. Although beta-adrenergic agents stimulate PKA, short-term insulin treatment inhibits HSL activity through the stimulation of phosphodiesterase 3B (PDE-3B) which occurs in a phosphatidylinositol 3-kinase dependent manner [16–19]. Complementarily, insulin stimulates the re-esterification of NEFA to triglycerides in the short term [20–22], providing an additional mechanism for inhibiting NEFA release from adipose tissue.

Long-term regulatory mechanisms of adipocyte lipolysis are less well characterized. Of the various factors suggested to play a part in impaired adipocyte function in insulin resistance and diabetes, increased TNF- α expression and production and long-term exposure to high circulating insulin concentrations have attracted interest. It has been shown that TNF- α is produced and excreted from adipose tissue in obesity and thus acts in an autocrine fashion to alter adipocyte function [23, 24]. Long-term exposure (> 6 h) to TNF- α has been shown to stimulate lipolysis in adipocytes [25, 26], despite inducing reduced HSL expression [27, 28]. Several cellular mechanisms have been suggested to explain this effect of TNF- α on adipocyte lipolysis. Among them that lower levels of expression of inhibitory G proteins, induced in adipocytes by TNF- α treatment, reduces the tonic basal inhibition of lipolysis rate [29]. Another explanation has been the TNF- α -induced reduction in the expression of perilipin [30], a protein suggested to play a part in regulating HSL access to intracellular triacylglycerol [31, 32]. This effect of TNF- α , as well as the activation of lipolysis, was prevented by thiazolidinediones [28], a class of insulin sensitizing anti-diabetic agents [33]. A recent study suggested that TNF- α reduced the expression of PDE-3B, which resulted in increased PKA activity and thus, enhanced HSL phosphorylation and activity [34].

The possibility that long-term exposure to insulin stimulates glycerol release from adipocytes as opposed to its short-term anti-lipolytic effect is less well established. Two studies in which primary adipocytes were used suggested either enhanced HSL ac-

tivity [35] or alteration in its cellular localization [36] as an underlying mechanism. An earlier study in 3T3-L1 adipocytes indicated a correlation between enhanced glycerol release induced by long-term insulin treatment and activation of lipoprotein lipase (LPL) [37], an enzyme generally considered to function in the vasculature on triacylglycerol stored in lipoproteins, rather than on intracellular triacylglycerol pools [38].

In our study we compared the effect of long-term exposure of 3T3-L1 adipocytes to TNF- α or to insulin, on glycerol and NEFA release, which enabled us to assess their effect on both lipolysis and re-esterification. In addition, we investigated whether the thiazolidinedione (TZD) troglitazone can inhibit long-term insulin-induced lipolysis, as was shown for TNF- α . Our findings indicate that the long-term regulation of lipolysis by TNF- α and insulin occurs through seemingly distinct mechanisms, with TZDs preventing the former but enhancing the latter.

Materials and methods

Cell culture and treatments. The 3T3-L1 adipocytes (American Type Tissue Culture Collection, Manassas, Va., USA) were grown in high glucose (25 mmol/l) Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Beit-Haemek, Israel) and differentiated exactly as described previously [39, 40]. Fully differentiated cells were incubated with serum free DMEM supplemented with 0.5% radioimmunoassay (RIA) grade bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, Mo., USA) and with either recombinant human insulin (Novo-Nordisk, Bagsvaerd, Denmark) or TNF- α (kindly provided by Dr. P.H. Pekala, East Carolina University, Greenville, N.C., USA). For pretreatment and co-treatment with troglitazone or an inactive TZD (10 μ mol/l each, kindly provided by Drs. A. Saltiel and H. Camp, Parke-Davis, Ann-Arbor, Mich., USA), cells were incubated in full DMEM for 6 h before and then in serum free medium during insulin or TNF- α treatments, as described above. Cell viability was monitored by protein recovery, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test and lactate dehydrogenase release to the medium.

Hexose transport measurements. The uptake of 2-deoxyglucose was done exactly as described previously [39, 41], using 50 μ mol/l 2-deoxy-[3 H]-glucose (Nuclear Research Center, Dimona, Israel) ($3.7 \cdot 10^4$ Bq/ml) for 10 min.

Lipolysis measurements. Lipolysis was measured by assessing glycerol and NEFA release at the end of a 22-h incubation without or with insulin or TNF- α . Cells were rinsed three times with PBS and further incubated with Krebs-Ringer phosphate (KRP) buffer (HEPES 50 mmol/l pH 7.4, NaCl 128 mmol/l, KCl 4.7 mmol/l, CaCl $_2$ 1.25 mmol/l, MgSO $_4$ 1.25 mmol/l and sodium phosphate 10 mmol/l) supplemented with 1% RIA grade BSA (results were compared and found to be identical with non-esterified fatty acid-free BSA supplemented buffer). Non-esterified fatty acid concentrations were measured using a commercial kit (Roche, Mannheim, Germany) following the instructions of the manufacturer and calculated according to an oleic acid standard curve done in the same buffer. Glycerol

Fig. 1 A, B. Prolonged exposure of 3T3-L1 adipocytes to insulin induces insulin resistance. **A** Western blots of total membranes using anti-IRS-1 or anti-GLUT4 antibodies, representative of 3 independent experiments. **B** 2-deoxyglucose measurements [(■) Basal, (□) following 20-min stimulation with 100 nmol/l insulin] are presented as means $\pm$ SEM of five independent experiments done at least in duplicates. * $p \leq 0.05$ compared with insulin stimulated control cells. # $p \leq 0.005$ compared with insulin-stimulated control cells

was measured spectrophotometrically using glycerokinase and glycerol phosphate dehydrogenase (both from Sigma), as described [42]. Glycerol and NEFA release were linear for at least 2 h. Thus, for basal lipolysis rates 1-h incubation in KRP was used and maximally stimulated lipolysis using 1 μ mol/l isoproterenol was done for 30 min. Fractional re-esterification was calculated as previously described [22], based on the knowledge that glycerol cannot be used to any great extent by adipocyte [43] and that NEFA are mainly used by re-esterification [22, 44]. Primary NEFA generation occurs at a 1:3, glycerol:NEFA ratio and thus the fractional NEFA re-esterification is calculated as: $[(3 \times \text{glycerol released} - \text{measured NEFA released}) / 3 \cdot \text{glycerol released}] \times 100$. This value can vary from 0 to 100%, i.e. no re-esterification at all or complete re-esterification, respectively.

Cell lysates, total membranes and western blots. Cells (1–2 wells of a 6-well plate for each condition) were rinsed three times with PBS. Cell lysates were prepared by adding 0.25 ml of high SDS buffer per well, as we described [28]. Total membranes were prepared after homogenization of the cells in 250 mmol/l sucrose, 20 mmol/l HEPES pH 7.4, 1 mmol/l

EDTA, 0.2 mmol/l sodium vanadate and protease inhibitors as listed above. Western blot analysis of either 15 μ g cell lysate protein (measured using BCA method, Pierce, Rockford, IL., USA) or 10 μ g total membrane protein (measured using Bio-Rad reagent, Bio-Rad Laboratories, Munchen, Germany) were done as described previously [40, 41]. The IRS-1, GLUT4 and HSL protein content were detected using anti-IRS-1 antibody (Upstate Biotechnology, Lake Placid, N. Y., USA), anti-GLUT4 antibody (Chemicon, Temecula, Calif., USA) and an anti-HSL antibody [28], respectively.

Statistical analysis. The student's *t* test was used for comparing values of two groups. We considered a *p* value of 0.05 to be the limit for statistical significance.

Results

Fully differentiated 3T3-L1 adipocytes were exposed for 22 h to either insulin or TNF- α , at a concentration range of 0.01–1 nmol/l. Cells were incubated in serum free medium with up to 1 nmol/l TNF- α , because TNF- α concentrations above 5 nmol/l were associated with a statistically significant reduction in cell viability (assessed as described in Methods). The TNF- α treatment up to 1 nmol/l and for up to 22 h was not associated with statistically significant alterations in basal or in insulin-stimulated glucose transport activity (data not shown). For long-term insulin treatment, this concentration range was preferred to the higher 100–1000 nmol/l used in most studies [45, 46]. The sub-nmol/l range better represents physiological and supra-physiological circulating insulin concentrations measured in vivo. In addition, in 3T3-L1 adipocytes, this range of insulin concentrations induces insulin but not IGF-1 receptor-mediated signalling [47]. To control for statistically significant degradation of insulin during the 22-h incubation [48], the medium was transferred at the end of the incubation to untreated cells and the short-term insulin effect on glucose transport was measured. Insulin activity in the medium was similar to that of a medium to which freshly prepared insulin has been added (data not shown) at both 0.1 and 1 nmol/l, suggesting that the cells were exposed to stable insulin concentrations during the entire incubation.

Exposure of 3T3-L1 adipocytes to 0.1 and 1 nmol/l insulin for 22 h resulted in significantly decreased insulin-stimulated glucose transport activity (Fig. 1). This was associated with reduced total membrane protein content of IRS-1 and GLUT4. These findings are consistent with previous studies, most [45, 46] but not all [48] of which used higher insulin concentrations.

Long-term insulin and TNF- α induce glycerol release from 3T3-L1 adipocytes. Dose and time effect of TNF- α and insulin on basal (non-stimulated) rates of glycerol release are shown in Figure 2. Long-term ex-

Fig. 2. **A** Dose response of 22-h incubation of 3T3-L1 adipocytes with either insulin (■) or TNF- α (○) on glycerol release. All points are significantly different ($p \leq 0.05$) compared with the control, untreated value. **B** Time course of the stimulation of glycerol release by 1 nmol/l insulin (■) or 1 nmol/l TNF- α (○). * $p \leq 0.05$ compared with basal glycerol release of untreated cells. Each value is the mean $\pm$ SEM of at least three independent experiments done at least in duplicate. **C** The effect of 22 h of treatment with either 1 nmol/l TNF- α (T) or 1 nmol/l insulin (I) on protein content of IRS-1 and HSL as assessed in total cell lysates and compared with untreated cells (C). Shown are blots representative of six independent experiments

posure to insulin resulted in increased glycerol release to the medium, with the maximum effect observed with 1 nmol/l insulin (sixfold over basal value), although significant effects could be observed with concentrations as low as 0.01 nmol/l. The time-course for stimulation of glycerol release shows that the lipolytic effect of TNF- α has a lag period of at least 6 h. In contrast, the stimulation of basal glycerol release by insulin was significant as early as after 2 h of incubation and increased progressively up to 22 h. At this point, glycerol release rate in cells incubated with 1 nmol/l insulin was typically 50–60 % of the value obtained with 1 nmol/l TNF- α and represented 40 % of the maximally stimulated glycerol release achieved after 30-min incubation with 1 μ mol/l isoproterenol (Fig. 3).

Pharmacological inhibitors of several components of the insulin signal were used to find the signalling cascades involved in the induction of glycerol release by long-term insulin. The phosphatidylinositol 3 kinase (PI 3-kinase) inhibitor, Ly 294002 (100 μ mol/l), and the mitogen-activated kinase kinase (MEK) inhibitor, PD 98059 (50 μ mol/l) were added 15 min before and during incubation with insulin for 9 h. Glycerol release was reduced from 66.6 ± 2.5 nmol · mg protein⁻¹ · h⁻¹ to 41.7 ± 3.0 and 47.4 ± 3.2 by the PI 3-kinase and the MEK inhibitors, respectively ($p < 0.01$ for both values compared with the absence of inhibitors). In contrast, the mTOR – p70S6 kinase inhibitor rapamycin was without effect on glycerol release, even at concentrations of 200 ng/ml. Taken together, these results suggest the involvement of both the PI 3-kinase and the MEK-extracellular signal-regulated kinase 1/2 (ERK) cascades in mediating the induction of glycerol release by long-term insulin.

To assess whether the stimulation of glycerol release by TNF- α and long-term insulin is associated with changes in the protein content of HSL, total cell lysates were prepared. As previously shown [28], TNF- α treatment was associated with a decrease in the protein content of HSL as assessed in total cell lysates (Fig. 2). In contrast, long-term insulin was not associated with a statistically significant change in HSL protein content. Densitometry analysis of six independent experiments showed a non-statistically significant increase in HSL protein content in long-term insulin-treated cells, compared with control cells. For comparison, IRS-1 protein content in the same cell lysates is also shown. The distinct time course and effect on HSL expression, suggest that TNF- α and long-term insulin stimulate glycerol release through distinct cellular mechanisms.

To further investigate this notion, the effect of TNF- α and long-term insulin on NEFA release was assessed. Although TNF- α treatment stimulated 13-fold NEFA release to the medium, with a time and dose dependency that paralleled glycerol release (not shown), no significant increase in NEFA release

Fig. 3 A, B. The effect of TNF- α or long-term insulin treatment (1 nmol/l, 22 h) on basal (■) or isoproterenol stimulated (30 min, 1 μ mol/l) (□) glycerol (A) or NEFA release (B) from 3T3-L1 adipocytes. Each value is the mean $\pm$ SEM of three independent experiments done in triplicate. * $p \leq 0.05$. ** $p \leq 0.005$

could be observed after long-term insulin treatment (Fig. 3B, Table 1). This discrepancy between glycerol and NEFA release was observed in all time and concentration ranges tested (not shown). The NEFA re-esterification ratio after 22 h incubation with 1 nmol/l TNF- α was calculated as the difference between the “primary” NEFA production ($3 \times$ glycerol) and the actual NEFA measured, divided by the former, as described in “Materials and methods”. Although overall NEFA re-esterification was typically below 30 % in control cells and those treated with TNF- α , long-term insulin treatment was associated with a large increase in the use of NEFA calculated (Table 1).

Long-term insulin alters the lipolytic response to beta-adrenergic stimulation. To further establish that long-term insulin treatment stimulates lipolysis (as measured by glycerol release) while concomitantly stimulating NEFA re-esterification, the isoproterenol stimulated lipolysis was studied after long-term insulin or TNF- α treatments. Cells were incubated for 22 h with 1 nmol/l insulin or TNF- α , rinsed and further incubated for 30 min with 1 μ mol/l isoproterenol. Glycerol release stimulated by isoproterenol was not altered by TNF- α pretreatment and NEFA release was nearly additive (Fig. 3). Notably, isoproterenol-stimulated glycerol release added to the effect of

long-term insulin treatment, whereas the stimulation of NEFA release to the medium was greatly inhibited by insulin pretreatment.

Thiazolidinediones differently modulate the effects of long-term insulin compared with TNF on glycerol and NEFA release. The TZD troglitazone was recently shown to antagonize the lipolytic effect of TNF- α . It prevented stimulation of glycerol and NEFA release, as well as the decreased protein content of HSL, which were induced by TNF- α treatment [28, 30]. To gain further insight into the mechanism underlying the stimulation of glycerol release by long-term insulin compared with the effect of TNF- α , 3T3-L1 adipocytes were incubated with troglitazone and inactive TZD for 6 h before and during treatment with each agent, as described in “Materials and methods”. Troglitazone treatment inhibited 70 and 95 % of the glycerol and NEFA release induced by TNF- α treatment, respectively, whereas the inactive TZD was without effect on TNF- α -induced lipolysis (Fig. 4). Conversely, troglitazone, which had no effect on glycerol or NEFA release in control cells, greatly exaggerated the effect of long-term insulin on glycerol release, without affecting NEFA release.

Discussion

In this study we show that long-term exposure of 3T3-L1 adipocytes to insulin at and near physiological concentrations, results in increased basal glycerol release to the medium without a concomitant rise in NEFA release. These conditions also alter beta-adrenergic-stimulated lipolysis, exaggerating glycerol but nearly preventing NEFA release. Finally, our

Table 1. Effect of TNF- α and long-term insulin on glycerol and NEFA release from 3T3-L1 adipocytes

	Glycerol release (nmol · mg protein ⁻¹ · h ⁻¹)	NEFA release (nmol · mg protein ⁻¹ · h ⁻¹)	% NEFA Re-esterification
control	10.4 $\pm$ 2.9	17.7 $\pm$ 3.3	29.4 $\pm$ 6.5
TNF- α (1 nmol/l, 22 h)	108.3 $\pm$ 11.7 ^b	231.4 $\pm$ 9.5 ^b	25.2 $\pm$ 2.4
insulin (1 nmol/l, 22 h)	64.8 $\pm$ 10.4 ^a	20.8 $\pm$ 1.9	86.8 $\pm$ 2.8 ^a

Each value is the mean $\pm$ SEM of three independent experiments done at least in duplicate. ^a $p < 0.01$ and ^b $p < 0.001$ compared with control

Fig. 4A, B. The effect of pretreatment and co-treatment with 10 $\mu\text{mol/l}$ troglitazone ($\square$) or 10 $\mu\text{mol/l}$ inactive TZD (hatched) on glycerol (**A**) and NEFA release (**B**) from TNF- α and long-term insulin-treated 3T3-L1 adipocytes. Each value is the mean $\pm$ SEM of three independent experiments done in triplicate

data suggest that the cellular mechanisms responsible for the increased glycerol release are distinct from those of TNF- α . Collectively, these data suggest that long-term insulin exposure, while paradoxically increasing basal glycerol release, enhances NEFA re-esterification.

Stimulation of lipolysis by long-term insulin, as measured by glycerol release is an unexpected finding because insulin is well known for its short-term anti-lipolytic actions. This finding is, however, in agreement with previous studies, which showed increased glycerol release after prolonged insulin treatment of isolated rat adipocytes [35, 36]. A recent study showed that the effect of long-term insulin depends on the presence of high glucose concentrations in the medium [36]. Yet, the role of the hexoseamine pathway in mediating this effect of long-term insulin was not supported as increased glycerol release occurred irrespective of the presence of glutamine in the medium. In our study, long-term exposure to insulin was done in the culture medium, which contained 25 mmol/l glucose. Yet, at the end of the incubation period, glycerol was collected for 1 h in a buffer without glucose. Thus it seems that extracellular glucose could be necessary as a priming factor, particularly under high glucose conditions but is not required for the actual metabolic flux responsible for glycerol generation. Collectively, these studies show the capacity of prolonged exposure to insulin, even at sub-nanomolar concentrations, to stimulate glycerol release as opposed to its short-term inhibitory effect on lipolysis.

In contrast to the effect on glycerol release, long-term exposure to insulin was not associated with increased NEFA concentrations in the medium, a finding consistent with a previous study [37]. Despite being the two end products of triacylglycerol breakdown, glycerol and NEFA released from adipocytes

rarely reach the expected 1:3 molar ratio. Short-term insulin stimulation rapidly inhibits NEFA release from adipose tissue, through a combined inhibitory action on HSL and a stimulatory effect on NEFA re-esterification [14, 21]. Our study supports the possibility, that although long-term insulin stimulates glycerol release, acting opposite to its short-term anti-lipolytic action, the short-term insulin stimulatory effect on NEFA re-esterification is maintained under prolonged treatment. Because the anti-lipolytic effect of insulin and the stimulation of NEFA re-esterification have been shown to be downstream of PI 3-kinase [17, 21], they seem to diverge downstream of this point. These seemingly independent pathways to affect glycerol and NEFA release from adipocytes have been also shown in response to leptin [49], which was shown to stimulate glycerol but not NEFA release from isolated adipocytes. Whether leptin has a role in mediating the effects of long-term insulin on glycerol and NEFA release from adipose tissue is an intriguing possibility.

The role of long-term regulation of HSL activity, by prolonged exposure to insulin, in mediating the effects on glycerol and NEFA release is questionable. Although increased HSL activity after prolonged exposure of isolated adipocytes to insulin has been reported [35], our study does not support this notion, thus agreeing with a study reporting a lack of effect of insulin at the mRNA expression level of HSL [50]. In addition, it is noteworthy that the expression level of HSL frequently does not correlate with lipolysis rate. For example, our study and others' [27, 28] have shown that TNF- α stimulates lipolysis but decreases HSL expression. Similarly, in adipose tissue of obese patients an increased basal lipolysis rate has been shown but the HSL expression was lower compared with control subjects [8]. These discrepancies suggest several possibilities, including HSL translocation to the lipid droplet [51–53]. Consistent with this mechanism is a study which suggested that long-term insulin results in cellular redistribution of HSL and hence activation of lipolysis [36]. Alternatively, other lipases could be responsible for the effect of long-term insulin. This possibility is in line with the notion that HSL is not the only major regulator of adipocyte lipolysis as shown in HSL knockout mice [54]. Additionally, exposure of 3T3-L1 adipocytes to insulin for

2 days resulted in increased glycerol release attributed to increased LPL activity [37]. Although insulin is known to increase LPL production in adipocytes as well as release to and activation in the vasculature [38], it is, however, not clear whether this enzyme can also function intracellularly on endogenous adipocyte triacylglycerols under the experimental conditions used (no serum as a source of Apo CII).

Tumour necrosis factor and insulin seem to use distinct cellular mechanisms in the activation of lipolysis. This is evident by our findings of distinct time courses, different effects on HSL protein content, unique modulations of the effect of acute lipolysis stimulation by isoproterenol and opposite response to co-incubation with TZD. We found that Troglitazone treatment, although it greatly inhibited TNF- α -mediated lipolysis, seemed to potentiate the effect of long-term insulin which is consistent with an "insulin sensitizer" mode of action. An example for such independent mechanisms would be, that TNF- α modulates HSL activity whereas long-term insulin regulates an alternative lipase. This would be consistent with our result that long-term insulin but not TNF- α has an additive effect on glycerol release induced by isoproterenol. In summary, long-term insulin treatment of 3T3-L1 adipocytes stimulates lipolysis but enhances NEFA re-esterification, acting differently from either its short-term effects or from TNF- α .

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