ORIGINAL ARTICLE

Taurine in adipocytes prevents insulin-mediated H_2O_2 generation and activates Pka and lipolysis

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Abstract Among many actions assigned to taurine (Tau), the most abundant amino acid in numerous mammalian tissues, it prevents high-fat diet-induced obesity with increasing resting energy expenditure. To sustain this Tau action, the goal of the present study was to explore the acute effects of Tau on baseline and on adrenaline, insulin and their second messengers to modulate lipolysis in white adipose tissue (WAT) cells from rat epididymis. The Tau effects in this topic were compared with those recorded with Gly, Cys and Met. Tau on its own did not modify baseline lipolysis. Tau raised isoproterenol- and dibutyryl-cAMP (Bt_2cAMP)-activated glycerol release. Gly diminished Bt_2cAMP-activated glycerol release, and Cys and Met had no effect. Cyclic AMP-dependent activation of protein kinase A (PKA) in cell-free extracts decreased slightly by Gly and was unaltered by Cys, Met, and Tau. PKA catalytic activity in cell-free extracts was stimulated by Tau and unchanged by Cys, Gly and Met. Gly and Tau effects on PKA disappeared when these amino acids were withdrawn by gel filtration. Insulin-mediated NADPH-oxidase (NOX) raises H_2O_2 pool, which promotes PKA subunit oxidation, and precludes its cAMP activation; thus, lipolysis is diminished. Tau, but not Cys, Gly and Met, inhibited, by as much as 70%, insulin-mediated H_2O_2 pool increase. These data suggested that Tau raised lipolysis in adipocytes by two mechanisms: stimulating cAMP-dependent PKA catalytic activity and favoring PKA activation by cAMP as a consequence of lowering the H_2O_2 pool.

Keywords Hydrogen peroxide · Insulin · Lipolysis · Obesity · PKA · Taurine

Abbreviations

Bt_2cAMP	Dibutyryl-cAMP
PKA	cAMP-dependent protein kinase A
T2	Type 2
TAG	Triacylglycerol
NOX	NADPH-oxidase
NSFA	Non-sterified fatty acids
BSA	Bovine serum albumin
HSL	Hormone-sensitive lipase
WAT	White adipose tissue

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Introduction

Taurine (Tau) is one of the most abundant low molecular weight organic constituents in mammals and numerous physiological actions have been ascribed to it (Huxtable 1992; Schuller-Levis and Park 2003). The beneficial effects of Tau on obesity, lipid accumulation and diabetes are a topic of continuous interest (Kim et al. 2007). Dietary supplementation of Tau reduces high-fat diet-induced arterial lipid accumulation (Murakami et al. 2000) and decreases body weight and abdominal fat pads in genetically obese KK mice (Fujihira et al. 1970). Abdominal fat accumulation, hyperglycemia, insulin

resistance, and serum and liver concentration of triacylglycerols (TAG) were significantly lower in a Tau-supplemented group in a type 2 (T2) diabetes rat model (Nakaya et al. 2000). In this T2 diabetes model, Tau decreased postprandial glucose oxidation, increased muscle glycogen content and reduced excessive TAG presence in epididymis, retroperitoneum, mesentery and serum (Harada et al. 2004). Body weight was reduced significantly after Tau administration for 7 weeks to human obese subjects (Zhang et al. 2004). Furthermore, Tau regulates gene expression for glucose-stimulated insulin secretion, stimulates tyrosine phosphorylation of the insulin receptor in liver and skeletal muscle cells (Carneiro et al. 2009), and prevents high-fat diet-induced obesity with increasing resting energy expenditure (Tsuboyama-Kasaoka et al. 2006). In mice fed a 60% fat diet plus a 5% Tau diet for 18 weeks, changes in white adipose tissue (WAT) included higher mRNA levels of lipoprotein lipase, acyl-CoA oxidase, acyl-CoA synthetase and medium-chain acyl-CoA dehydrogenase than in mice fed the same diet without Tau (Tsuboyama-Kasaoka et al. 2006). These data are in favor of an increase in fatty acids oxidation in WAT, but do not support evidence to promote lipolysis in this tissue in order to provide free fatty acids to be oxidized in other tissues, an unavoidable pathway required to ensure a decrease in stored TAG and in body weight for the experimental subjects previously indicated. Therefore, the objective of this study was to investigate the response of added Tau in regulating lipolysis and/or lipogenesis in cells isolated from WAT of rat epididymis. Fat-depot regulation in WAT cells is mainly regulated by an equilibrium between β-adrenoceptor (β-AR) agonists and insulin actions. β-AR agonists activate lipolysis through an amplification cascade including cAMP-dependent protein kinase A (PKA) phosphorylation of dormant hormone-sensitive lipase (HSL) to hydrolyze TAG (González-Yáñez and Sánchez-Margalet 2006). Insulin impairs lipolysis and activates lipogenesis by two complementary mechanisms: (a) phosphorylation and activation of the insulin receptor and several kinases and phosphatases to stimulate lipogenesis, and (b) restriction of PKA activation by cAMP to hinder lipolysis (Zentella de Piña et al. 2008). The results reported here for Tau are compared with those obtained by its substitution with Gly and with two other sulfur-containing amino acids, Met and Cys. Gly, an anti-inflammatory, immunomodulatory and cytoprotective compound (Zhong et al. 2003), activates hepatic fatty acid metabolism and decreases the triglycerides and plasma non-sterified fatty acids (NSFAs) of sucrose-fed rats (El Hafidi et al. 2004). The reported main results indicate that Tau stimulated β-adrenergic-mediated lipolysis and prevented insulin action to hinder lipolysis.

Materials and methods

Animals

Male Wistar rats weighing 200–240 g fed with a commercial diet (Purina, México) were fasted overnight, but had free access to water. All experiments were conducted in accordance with our Federal Regulations for Animal Care and Use (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico) and were approved by the Ethics Committee of the Mexico City-based School of Medicine, National Autonomous University of Mexico (UNAM).

Adipocyte isolation

Rats were decapitated and the epididymal fat pads from two rats were immediately removed to isolate adipocytes with the purpose of obtaining cells with low basal levels of cAMP (Honnor et al. 1985). In brief: Krebs–Ringer solution was enriched with 25 mM HEPES, 2.5 mM CaCl$_2$, 2 mM glucose, 200 nM adenosine and bovine serum albumin (BSA fraction V, free fatty acid) at various concentrations as detailed later; pH was adjusted at 7.4. One gram of minced fat pads from two rats was digested in 10 ml of collagenase (1 mg/ml) for 30 min, with vigorous shaking in Krebs–Ringer-enriched media supplemented with 1% BSA. Cells were filtered through nylon cloth and washed three times through 1-min centrifugation cycles at 220g. The adipocytes were weighed.

Glycerol release assay

Glycerol release assay as an indicator of lipolysis was performed using 100 µl of packed adipocytes incubated for 30 min at 37°C in a total volume of 1 ml of 4% BSA in Krebs–Ringer-enriched solution to which dibutyryl-cAMP (Bt$_2$cAMP), Cys, Gly, Met and Tau were added. The amino acids were dissolved appropriately to reach the final concentrations indicated in the figures. Adipocytes were dispersed during incubation by shaking at 160 cycles/min. Lipolysis was stopped by transferring tubes from 37°C into an ice bath for 5 min. The tubes were centrifuged at 10,000g and maintained at 4°C for 10 min. A 300-µl aliquot from solution lying below this fat cake was utilized to measure glycerol (Warnich 1986).

Assay of PKA activation and PKA catalytic activity

Once rat adipocytes were prepared as indicated previously, a cell-free preparation suitable to assay cAMP-mediated activation of PKA, as well as PKA catalytic activity was obtained (Gaudiot et al. 2000). In short, 100 µl of packed adipocytes were mixed with 900 µl of Krebs–Ringer buffer

supplemented with 5 mM glucose and 2% BSA and incubated during 1 h at 37°C; the mixture was centrifuged at 2,400g during 5 min. The supernatant was eliminated, and the sediment containing adipocytes was disrupted in 350 µl of lysis buffer (Tris–HCl 50 mM, pH 7.8, 0.33 M sucrose, 1 mM $MgCl_2$, and protease inhibitor). After vigorous shaking, the samples were centrifuged at 2,500g during 4 min to obtain a floating fat cake, an intermediate layer, and sediment. This intermediate layer was the source for measuring PKA activity in adipocytes. To analyze the effect of different amino acid on cAMP-mediated activation of PKA, the amino acids studied were added to the enzyme either before or after the cyclic nucleotide, as indicated in the corresponding figures (Zentella de Piña et al. 2008). Later, the catalytic activity of the activated enzyme was assayed. To measure PKA catalytic activity, a 20-µl aliquot of this cytosolic extract was mixed with 30 µl of 50 mM MOPS buffer of pH 7.0 containing 250 µg/ml BSA, 100 µM ATP, 10 mM $MgCl_2$, and a 100-µM $[\gamma^{32}$-P] (specific activity of 0.5×10^6 cpm/6 µmol) final concentration in the incubation mixture (Zentella de Piña et al.

2007). In all cases, the catalytic reaction was started with 80 µM of kemptide in a total volume of 60 µl. At the end of the incubation period, 25 µl of reaction mixture was placed on a piece (1.5 × 1.5 cm) of Whatman P-81 filter paper (UK), washed twice with 5 ml of 5% phosphoric acid and placed in a vial with scintillation liquid to measure radioactivity.

Gel filtration to separate small molecules from PKA

A 100-µl aliquot of PKA from adipocyte lysates (previously mixed with amino acids and cAMP as indicated in each experiment) was carefully loaded into an insulin syringe containing 700 µl of Sephadex G-50 that had been previously equilibrated with 50 mM Tris–HCl buffer of pH 7.8, supplemented with 1 mM $MgCl_2$, and centrifuged at 3,000 rpm for 3 min at 4°C (Penefsky 1977). The effluent containing macromolecules separated from small molecules was collected in 1.5-ml tubes and used to assay PKA activation and/or PKA enzymatic activity, as described in the previous paragraph.
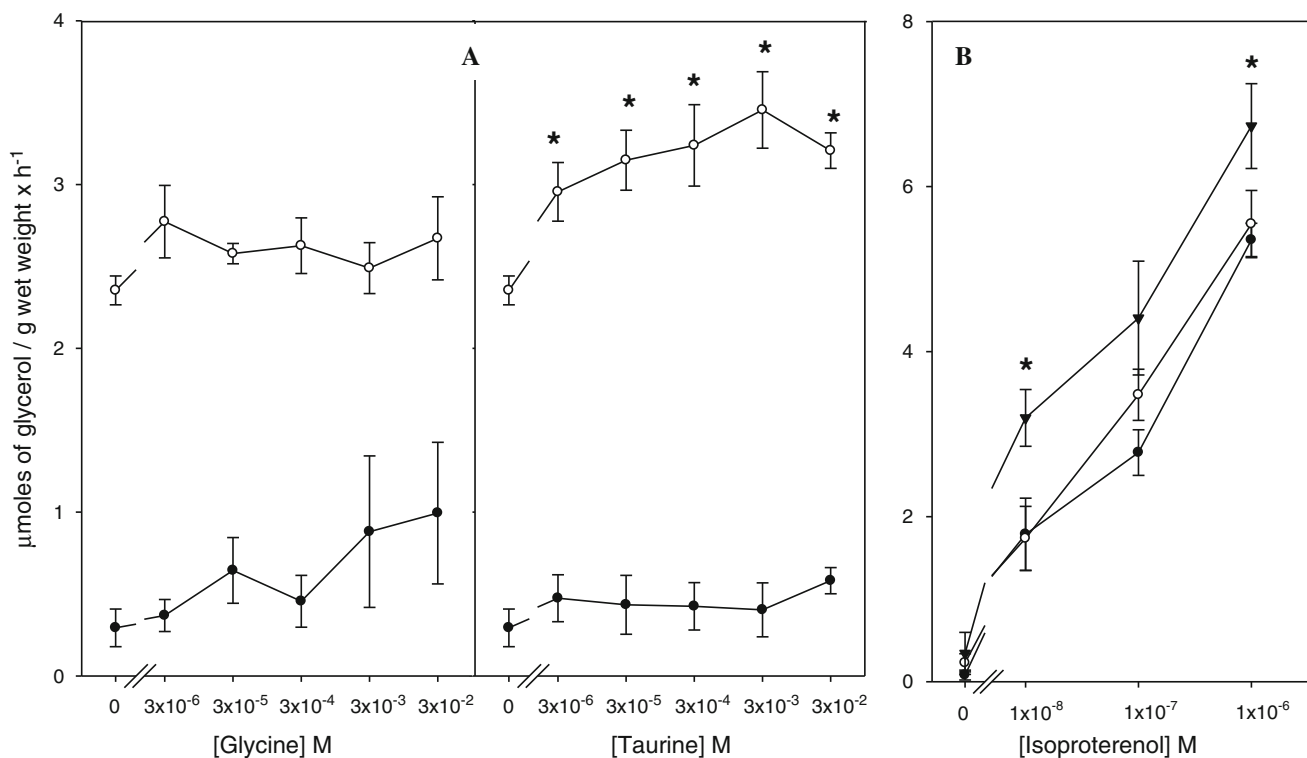

Fig. 1 a Dose–response curve of two amino acids on baseline and isoproterenol-stimulated lipolysis in isolated rat adipocytes. Baseline conditions (filled circles) or in the presence of 10^{-8} M isoproterenol (open circles), with different concentrations of amino acids as indicated in the figure. b Dose–response curve of isoproterenol on lipolysis in isolated rat adipocytes supplemented with none (filled circles), 3×10^{-5} M Gly (open circles) and 3×10^{-5} Tau (filled circles), 3×10^{-5} M Gly (open circles) and 3×10^{-5} Tau (filled inverted triangles). In each experiment, cells were incubated during 30 min at 37°C in Krebs–Ringer of pH 7.4 and enriched with bovine serum albumin (BSA) at 4%. Glycerol was assayed after incubation in each sample as indicated in "Materials and methods". Values are mean ± standard error (SE) of at least three independent experiments performed in duplicate. Statistical significance as compared with control values in the absence of amino acids, *P < 0.05

Adipocyte H_2O_2 pool value

A total of 100 μl of packed rat adipocytes obtained as described previously was incubated in a total volume of 1 ml of Krebs–Ringer solution containing 4% BSA and enriched with insulin, Cys, Gly, Me or Tau to reach the final concentrations indicated in the figures. Incubation was for 10 min at 37°C and under shaking conditions (160 cycles/min) (Zentella de Piña et al. 2007). Reaction was ended by the addition of 100 μl of 6 M TCA, and the tubes were immediately centrifuged at 10,000g at 4°C for 10 min. The intermediate layer after centrifugation was the source to measure H_2O_2 pool present in adipocytes. A 5-μl aliquot from this layer was employed with 45 μl of 50 mM sodium phosphate buffer, pH 7.4, and 50 μl of Amplex Red solution (commercial kit); samples were maintained during 30 min at room temperature in the dark, and absorbance was measured at 560 nm.

Statistics

Data points shown are means ± standard error (SE) for paired samples, comparisons were carried out with Student's t test and $p < 0.05$ was considered to be significant. Differences in concentration–response curves between nonstimulated, cell-free preparations and stimulated preparations plus modulators were analyzed by rank analysis of variance, followed by Dunn's multiple comparison test, recommended for unpaired samples (Sigma Stat for Windows, Version 3.2; Jandel Corp., San Rafael, CA).

Results

Baseline lipolysis was not modified in the presence of the four amino acids selected (data for Gly and Tau in Fig. 1a); also, Gly (Fig. 1a), Cys and Met (not shown) did not affect isoproterenol-stimulated lipolysis in isolated cells. Stimulated lipolysis with (10^{-8} M) isoproterenol, a selective agonist for β-AR, was enhanced in 50% by 3×10^{-3} M Tau and to a slightly lesser extent by 3×10^{-5}, 3×10^{-4} and 3×10^{-2} M Tau (Fig. 1a). Serum concentrations of Tau in rat within the range of 2.5–4×10^{-3} M have been reported (Harada et al. 2004); therefore, the results obtained in this study with isolated adipocytes fall within these values and probably correspond to the physiological Tau response in the intact animal. These responses of 3×10^{-5} M Gly and Tau were not different when isoproterenol concentration was raised to 10^{-6} M (Fig. 1b).

To define Tau actions on activated lipolysis at the level of the β-receptor in membrane or upon the amplification cascade beginning with cAMP, the experiment presented in Fig. 1 was repeated, substituting isoproterenol with

Bt_2cAMP, a cell membrane-permeable cAMP analog. Bt_2cAMP-activated lipolysis was enhanced by Tau, slightly inhibited by Gly, and unmodified by Cys and Met (Fig. 2). Tau concentrations for producing statistically significant enhancement in glycerol release ranged from 3×10^{-4} to 3×10^{-5} M, and Bt_2cAMP concentrations ranged from 10^{-4} to 10^{-2} M. At 10^{-4} M Bt_2cAMP, the Tau effect was more apparent and glycerol release rose by 100%. In 10^{-2} M Bt_2cAMP-incubated adipocytes, 3×10^{-4} M, Gly decreased glycerol release by 21% ($p < 0.05$) (Fig. 2). Based on the fact that enhanced Tau lipolytic action was observed only if lipolysis was activated by the cAMP-mediated route, the next step was to analyze the effect of these selected amino acids on the cAMP
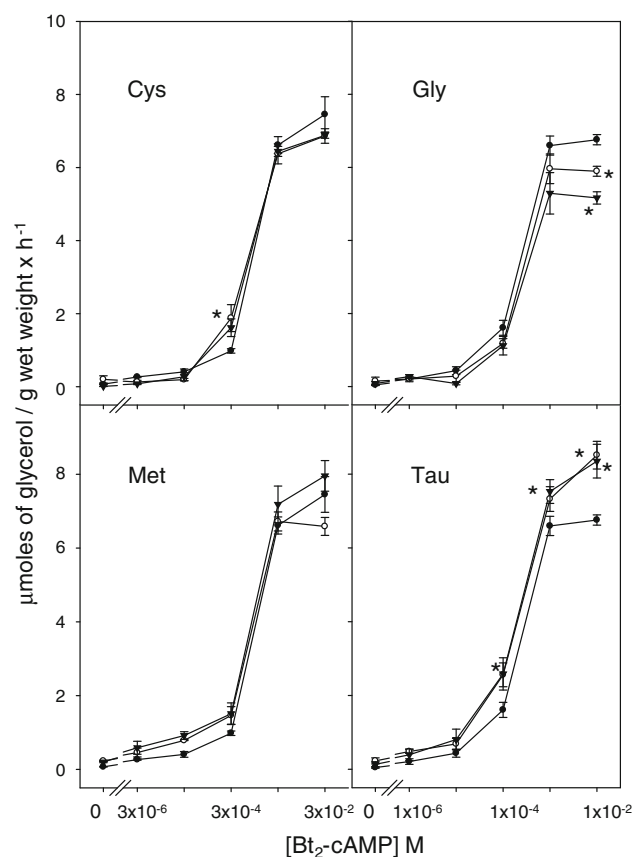

Fig. 2 The effect of four different amino acids on dibutyryl-cAMP (Bt_2cAMP)-stimulated lipolysis in isolated rat adipocytes. Cells were incubated for 30 min at 37°C in a solution containing Krebs–Ringer of pH 7.4 enriched with bovine serum albumin (BSA) at 4%, and different concentrations of Bt_2cAMP alone (*filled circles*) or in the presence of Cys 3×10^{-5} M (*open circles*), Cys 3×10^{-4} M (*filled inverted triangles*), Gly 1×10^{-5} M (*open circles*), Gly 1×10^{-4} M (*filled inverted triangles*), Met 3×10^{-5} M (*open circles*), Met 3×10^{-4} M (*filled inverted triangles*), Tau 1×10^{-5} M (*open circles*), and Tau 1×10^{-4} M (*filled inverted triangles*). Each value represents mean ± standard error (SE) for three individual assays performed in duplicate. Statistical significance as compared with control values in the absence of amino acids, *$p < 0.05$

amplification cascade, i.e., PKA activation by cAMP and PKA catalytic activity. Tau, at the two concentrations assayed, enhanced this enzymatic activity by nearly 30% ($p < 0.05$) (Fig. 3) and this increase was maintained if the cyclic nucleotide, which was required to activate the enzyme, was added to the adipocyte extract 10 min before or after the amino acid. Therefore, this Tau action is not dependent on PKA activation, but rather, on its catalytic activity. Gly at 3×10^{-4} M lowered enzymatic activity by 14% ($p < 0.05$), but such an effect disappeared when cAMP was included 10 min prior to the addition of Gly to the incubation mixture (Fig. 3). In other words, if the enzyme had been previously activated with cAMP, Gly was unable to act on PKA, but if the amino acid was in contact with the enzyme prior to cAMP, nucleotide-mediated PKA activation was lowered by Gly. Cys and Met had no effect on PKA activation or on PKA catalytic activity (Fig. 3).Tau and Gly actions on PKA present in adipocyte lysates supported the effect of both amino acids in releasing glycerol from isolated adipose cells: raised with Tau and inhibited with Gly (Fig. 2). The increase in PKA catalytic activity detected with Tau and the small decrease in PKA activation produced by Gly were not observed when adipocyte extracts in the presence of each of the amino acids were filtered through Sephadex G-50 prior to being assayed for their action on PKA holoenzyme (Fig. 4). These results are indicative that the observed effect of both amino acids is independent of the formation of a covalent bond between PKA and these amino acids.

Cyclic AMP availability in the neighborhood of PKA is a requisite to achieve its activation (Wong and Scott 2004). An approach to lower cAMP availability in the neighborhood of PKA was attempted based on the insulin-mediated H$_2$O$_2$ pool increase in isolated adipocytes (Krieger-Brauer and Kather 1992). Insulin activates an NADPH-oxidase (NOX) system that, in adipocytes, generates a H$_2$O$_2$ pool capable of promoting the formation of a heterodimer between Cys in the catalytic PKA subunit and Cys in its regulatory subunit, which hinders cAMP activation (Zentella de Piña et al. 2008). It can be expected that an amino acid-promoted increase in H$_2$O$_2$ pool might prevent cAMP-mediated PKA activation, but a lower H$_2$O$_2$ pool due to the
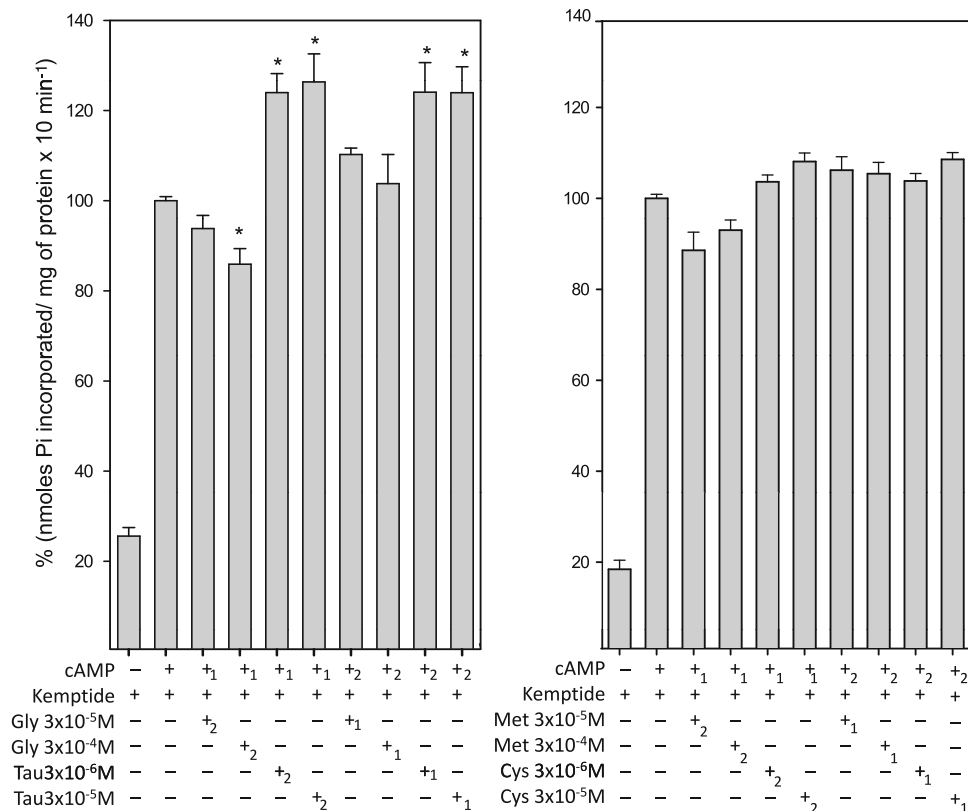

Fig. 3 Effect of four different amino acids on cAMP-activated or non-activated protein kinase A (PKA) from adipocyte extracts. Cell-free extracts were incubated for 15 min with the complete incubation mixture for enzyme assay activity except the kemptide substrate, and supplemented or not with cAMP or with each of the different amino acids as indicted in the figure. Subscript numbers 1 and 2 indicate the order in which either cAMP or the selected amino acid was added. After addition of the first reagent, incubation was maintained during 15 min, and the second reagent and the kemptide substrate were then added to the incubation mixture to start the catalytic reaction during 10 additional min of incubation at 37°C. *$p < 0.05$. Values are means ± standard error (SE) of four independent experiments performed in duplicate. Statistical significance as compared with control values in the absence of amino acids, *$p < 0.05$

Fig. 4 Effect of four different amino acids on protein kinase A (PKA) activity filtered or non-filtered through Sephadex G-50. Two parallel sets of samples were prepared simultaneously. In each of the sets, a 100-μl aliquot of adipocyte lysates was mixed with the indicated amino acid and cAMP. One set was used to measure PKA catalytic activity as described in "Materials and methods" (in *gray*). Each sample of the remaining set was first passed through a small Sephadex G-50 column and subsequently, the eluate was used to measure PKA catalytic activity as described previously (in *white*). Values are mean ± standard error (SE) of at least three independent experiments performed in duplicate. Statistical significance as compared with control values in the absence of amino acids, $*p < 0.05$
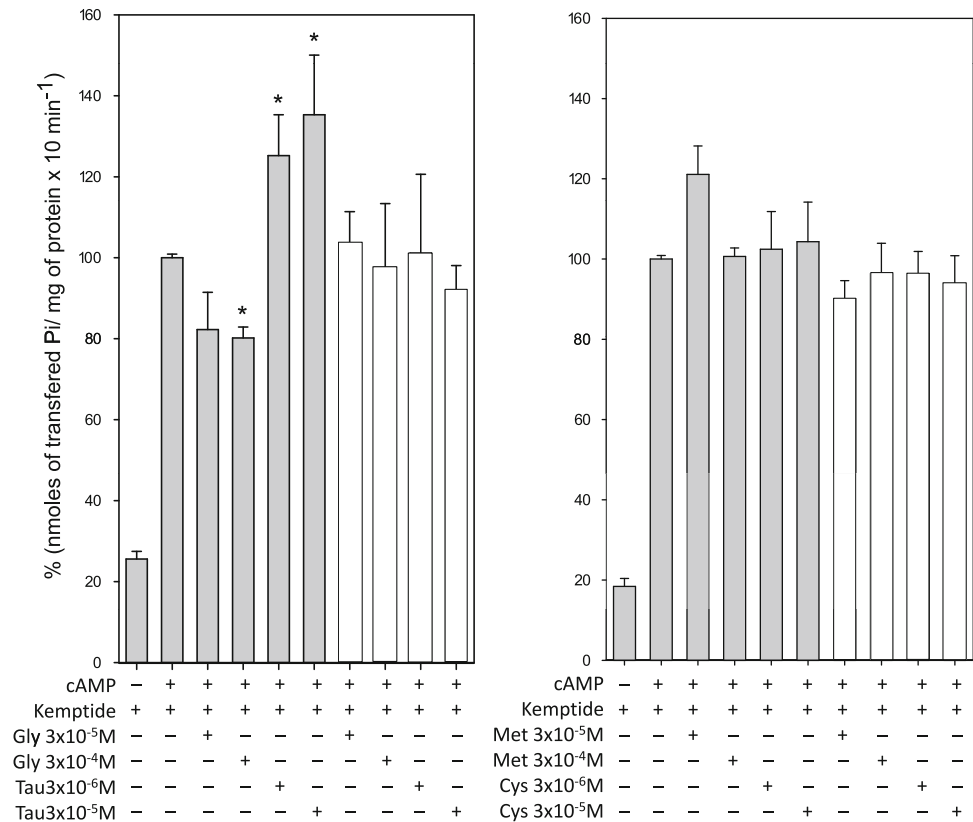
presence of some selected amino acids might facilitate cAMP-mediated PKA activation. Within this context, we explored the ability of Tau and Gly to modify H_2O_2 synthesis by insulin. As presented in Fig. 5, Nox activation by insulin was not modified by Gly used at concentrations from 10^{-6} to 10^{-3} M and by Tau employed at 10^{-6} M. However, Tau 10^{-5} M, produced a 40% inhibition on insulin-mediated Nox activation and a 70% inhibition when Tau concentrations were 10^{-4} and 10^{-3} M.

Discussion

From the four tested amino acids challenged to modulate lipolysis in cells, WAT, Cys and Met did not affect baseline, isoproterenol-, and Bt2cAMP-stimulated glycerol release, cAMP-mediated PKA activation or PKA catalytic activity. Gly exhibited a moderate, but statistically significant decrease in cAMP-dependent PKA activation (Fig. 3) and a decrease in Bt2cAMP-activated glycerol release (Fig. 2). These data observed with Gly might sustain the reported decrease in NSFAs observed in sucrose-fed rats (El Hafidi et al. 2004). Tau effects were quantitatively higher than those of Gly; these will be discussed in an integrated pattern within isolated adipocytes and are included within the context of systemic lipid regulation in mammals.

As shown in this study, Tau actions on isolated adipocytes augmented those of β-AR agonists. Tau enhances isoproterenol- and Bt2cAMP-stimulated lipolysis (Figs. 1 and 2) by increasing cAMP-dependent PKA catalytic activity (Figs. 3 and 4). These data are suggestive that Tau action does not involve a direct β-AR activation. In this way, Tau action is along the pathway commanded by hormone-sensitive lipase (HSL) (González-Yáñez and Sánchez-Margalet 2006), leaving to one side in this study the pathway ruled by adipose triglyceride lipase (ATGL) (Jenkins et al. 2004, Villena et al. 2004; Zimmermann et al. 2004), which is not a target for PKA-mediated phosphorylation (Villena et al. 2004). Tau actions in isolated cells from WAT support information obtained with the amino acid in experimental models employing whole animals and also resemble data obtained with β-AR agonists in intact animals. In this manner, Tau decreased body weight and abdominal fat pads in genetically obese KK mice (Fujihira et al. 1970), lowered abdominal fat pad accumulation in a T2-diabetes rat model (Nakaya et al. 2000), prevented obesity with increasing resting expenditure and augmented mRNA levels of enzymes involved in fatty acid oxidation in WAT (Tsuboyama-Kasaoka et al. 2006). The body weight in obese young adult males also reduced significantly under Tau supplementation (Zhang et al. 2004). Additionally, rodent models of obesity and diabetes treated with selective β-AR agonists enhanced lipolysis from

WAT, produced marked weight loss and exhibited antidiabetic effects (de Souza and Burkey 2001; van Baak et al. 2002; Wang and Fotsch 2006).

NOX enzymes catalyze NADPH oxidation in the presence of O_2 to generate NADP plus H^+ and superoxide anion. Superoxide reacts spontaneously with H_2O to rapidly generate H_2O_2, which is the product quantified in this work to measure NOX catalytic activity. The enzyme present in WAT cells is the isoform 4 (Mahadev et al. 2004) and it is localized in the cell membrane (Krieger-Brauer and Kather 1992). The dormant enzyme is not activated by cytosolic phosphorylated subunits (Martyn et al. 2006), but rather is turned on by a specific G protein from insulin-activated receptors (Krieger-Brauer et al. 1997). Insulin-generated H_2O_2 modulates the catalytic activity of kinases and phosphatases involved in the physiological action of insulin (Mahadev et al. 2001, 2004; Choi et al. 2006; Zentella de Piña et al. 2008). Of particular relevance for this work, insulin-generated H_2O_2 oxidized highlighted Cys in regulatory and catalytic PKA to form a disulfide bridge between them that impaired its cAMP-dependent activation to prevent lipolysis (Zentella de Piña et al. 2008). Consequently, it might be argued that Tau action lowering insulin-stimulated H_2O_2 synthesis (Fig. 5) will maintain reduced Cys in regulatory and catalytic PKA subunits to allow cAMP-dependent activation and to favor lipolysis.

Tau inhibitory action on NOX activity in WAT might be a more general effect of the amino acid in other tissues to lower H_2O_2 generation. Previous studies of Winiarska et al. (2009) reported Tau-mediated, diminished NOX activity in the kidney cortex of alloxan diabetic rabbits. Tau-mediated NOX activation in other cell types might be underlying the widely recognized antioxidant action of this amino acid as cited in numerous reports (Huxtable 1992; Schuller-Levis and Park 2003; Oprescu et al. 2007).

A controversy emerges from data in Fig. 5: abundant experimental data from the literature support an anti-diabetic effect for Tau and, paradoxically, Tau inhibits insulin action in WAT. Indeed, the anti-diabetic effect of the amino acid was reported first in 1935 (Ackerman and Heinsen 1935) and has been accepted continuously since then (Kim et al. 2007). Additionally, it is known that Tau aids in controlling glucose homeostasis by regulating the expression of genes required for glucose-stimulated insulin secretion, and also by enhancing tyrosine phosphorylation of the insulin receptor in skeletal muscle and liver tissues (Carneiro et al. 2009). Other direct actions of Tau might be added, i.e., overexpressing UCP2 in β-cells (Han et al. 2004) and preventing oxidative stress, which decreases β-cell secretory functions (Xiao et al. 2008). Such a paradox has been described previously for the in vitro actions of β-adrenergic agonists and those of insulin in WAT versus the
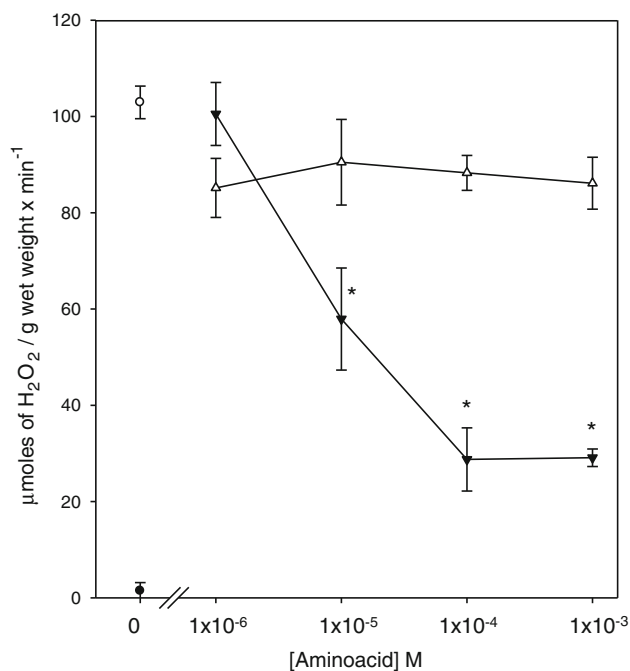

Fig. 5 Effect of Tau and Gly on insulin-mediated H_2O_2 synthesis in isolated rat adipocytes. H_2O_2 synthesis and measurement was performed as indicated in "Materials and methods". The amount of H_2O_2 generated in the absence of insulin (*filled circles*), in the presence of 10^{-9} M insulin alone (*open circles*) and in the presence of 10^{-9} M insulin supplemented with Tau (*filled inverted triangles*) or with Gly (*open inverted triangles*) is at concentrations indicated in the figure. Values are means ± standard error (SE) of three independent experiments performed in duplicate. Statistical significance as compared with control values in the absence of amino acids, $*p < 0.05$

combined actions of both hormones in the whole organism (de Souza and Burkey 2001; Wang and Fotsch 2006). Whereas in vitro insulin promotes TAG synthesis, β-adrenergic agonists promote TAG degradation; however, β-adrenergic agonists have been used chronically in the whole animal as anti-obesity and anti-diabetic compounds (de Souza and Burkey 2001; van Baak et al. 2002; Wang and Fotsch 2006).

One or more alternatives of those considered previously can help to explain this paradox. (a) A reported bivalent role of NSFAs on β-cells has been described (Newsholme et al. 2007). Acute exposure of pancreatic β-cells to high concentrations of saturated NSFAs results in a dramatic increase in insulin release (Grujic et al. 1997), whereas chronic exposure results in desensitization and suppression of insulin secretion (Oprescu et al. 2007). Thus, the Tau effect reported herein in adipocytes inhibiting an insulin-mediated H_2O_2 rise (Fig. 5), to speed up lipolysis instead of lipogenesis, will contribute to raising glycerol and NSFAs in serum. In turn, acute exposure of β-cells to NSFAs in the whole animal, due to the lipolytic actions of Tau in adipocytes, will promote higher insulin liberation.

(b) PKAs exhibit opposite responses to H_2O_2 tissular pool in WAT and in muscle. In WAT, H_2O_2 favored S–S binding between RII and C subunits in type II PKA subunits, thus preventing its cAMP activation (Zentella de Piña et al. 2008). In muscle, the oxidative disulfide formation of two RI subunits of type I PKA activates the enzyme even in the absence of cAMP (Brennan et al. 2006). (c) If we consider, on a scale, all known pro-insulin Tau actions in numerous tissues including many extra reports not detailed in this study, the Tau anti-insulin data on adipocytes obtained in this study will be placed on the other side of the scale. In the whole organism, available information appears to be in favor of a global pro-insulin action, based on the number of tissues responding to the amino acid and on the higher relative weight (solely muscular tissue represents 50% of the body weight) of all of these tissues.

In conclusion, Tau added to isolated adipocytes modulates two key enzymes from two different pathways previously activated by hormones: it enhances PKA catalytic activity activated by β-AR and inhibits NOX4 catalytic activity inhibited by insulin. Both actions underlie the pro-lipolytic effect obtained here of Tau in adipocytes and support the anti-obesity and anti-diabetic Tau actions when used in vivo.

References

Ackerman D, Heinsen HA (1935) Uber die physiologischs Wikung des Asterubins und anderer, zum Teil neu dargestellter, schwefelhaltiger Guanidinderivate. Z Physiol Chem 235:115–121

Brennan JP, Bardswell SC, Burgoyne JR, Fuller W, Schröder E, Wait R, Begum S, Kentish JC, Eaton P (2006) Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. J Biol Chem 281:21827–21836

Carneiro EM, Latorraca MQ, Araujo E, Beltrá M, Oliveras MJ, Navarro M, Berná G, Bedoya FJ, Velloso LA, Soria B, Marín F (2009) Taurine supplementation modulates glucose homeostasis and islet function. J Nutr Biochem 20:502–511

Choi YH, Park S, Hockman S, Zmuda-Trzebiatowska E, Svennelid F, Haluzik M, Gavrilova O, Ahmad F, Pepin L, Napolitano M, Taira M, Sundler F, Stenson Holst L, Degerman E, Manganiello VC (2006) Alterations in regulation of energy homeostasis in cyclic nucleotide phosphodiesterase 3B-null mice. J Clin Invest 116:3240–3251

de Souza CJ, Burkey BF (2001) Beta₃-adrenoceptor agonists as anti-diabetic and anti-obesity drugs in humans. Curr Pharm Des 7:1433–1449

El Hafidi M, Pérez I, Zamora J, Soto V, Carvajal-Sandoval G, Baños G (2004) Glycine intake decreases plasma free fatty acids, adipose cell size, and blood pressure in sucrose-fed rats. Am J Physiol Regul Integr Comp Physiol 287:R1387–R1393

Fujihira E, Takahashi H, Nakazawa M (1970) Effect of long-term feeding of taurine in hereditary hyperglycemic obese mice. Chem Pharm Bull (Tokyo) 18:1636–1642

Gaudiot N, Ribière C, Jaubert AM, Giudicelli Y (2000) Endogenous nitric oxide is implicated in the regulation of lipolysis through antioxidant-related effect. Am J Physiol 279:C1603–C1610

González-Yáñez C, Sánchez-Margalet V (2006) Signalling mechanisms regulating lipolysis. Cell Signal 18:401–408

Grujic D, Susulic VS, Harper ME, Himms-Hagen J, Cunningham BA, Corkey BE, Lowell BB (1997) Beta₃-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. J Biol Chem 272:17686–17693

Han J, Bae JH, Kim SY, Lee HY, Jang BC, Lee IK, Cho CH, Lim JG, Suh SI, Kwon TK, Park JW, Ryu SY, Ho WK, Earm YE, Song DK (2004) Taurine increases glucose sensibility of UCP2-overexpressing beta-cells by ameliorating mitochondrial metabolism. Am J Physiol Endocrinol Metab 287:E1008–E1018

Harada N, Ninomiya C, Osako Y, Morishima M, Mawatari K, Takahashi A, Nakaya Y (2004) Taurine alters respiratory gas exchange and nutrient metabolism in type 2 diabetic rats. Obes Res 12:1077–1084

Honnor RC, Dhillon GR, Londos C (1985) cAMP-dependent protein kinase and lipolysis in rat adipocytes. J Biol Chem 260:15122–15129

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72:101–163

Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW (2004) Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 279:48968–48975

Kim SJ, Gupta RC, Lee HW (2007) Taurine–diabetes interaction: from involvement to protection. Curr Diabetes Rev 3:165–175

Krieger-Brauer HI, Kather H (1992) Human fat cells possess a plasma membrane-bound H_2O_2-generating system that is activated by insulin via a mechanism bypassing the receptor kinase. J Clin Invest 89:1006–1013

Krieger-Brauer HI, Meda PK, Kather H (1997) Insulin-induced activation of NADPH-dependent H_2O_2 generation in human adipocyte plasma membranes is mediated by $G\alpha_{i2}$. J Biol Chem 272:10135–10143

Mahadev K, Wu X, Zilbering A, Zhu L, Lawrence TR, Goldstein BJ (2001) Hydrogen peroxide generated during cellular insulin stimulation is integral to activation of the distal signaling cascade in 3T3–L1 adipocytes. J Biol Chem 276:48662–48669

Mahadev K, Wu X, Motoshima H, Goldstein BJ (2004) Integration of multiple downstream signals determines the net effect of insulin on MAP kinase vs PI $3'$-kinase activation: potential role of insulin-stimulated H_2O_2. Cell Signal 16:323–331

Martyn KD, Frederick LM, von Loehneysen K, Dinauer MC, Knaus UG (2006) Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. Cell Signal 18:69–82

Murakami S, Kondo Y, Nagate T (2000) Effects of long-term treatment with taurine in mice fed a high-fat diet: improvement in cholesterol metabolism and vascular lipid accumulation by taurine. Adv Exp Med Biol 483:177–186

Nakaya Y, Minami A, Harada N, Sakamoto S, Niwa Y, Ohnaka M (2000) Taurine improves insulin sensitivity in the Otsuka Long-

Evans Tokushima Fatty rat, a model of spontaneous type 2 diabetes. Am J Clin Nutr 71:54–58

Newsholme P, Keane D, Welters HJ, Morgan NG (2007) Life and death decisions of the pancreatic beta-cell: the role of fatty acids. Clin Sci (Lond) 112:27–42

Oprescu AI, Bikopoulos G, Naassan A, Allister EM, Tang C, Park E, Uchino H, Lewis GF, Fantus IG, Rozakis-Adcock M, Wheeler MB, Giacca A (2007) Free fatty acid-induced reduction in glucose-stimulated insulin secretion: evidence for a role of oxidative stress in vitro and in vivo. Diabetes 56:2927–2937

Penefsky HS (1977) Reversible binding of P$_i$ by beet heart mitochondrial adenosine triphosphatase. J Biol Chem 252:2891–2899

Schuller-Levis GB, Park E (2003) Taurine: new implications for an old amino acid. FEMS Microbiol Lett 226:195–202

Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y, Ezaki O (2006) Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 147:3276–3284

van Baak MA, Hul GB, Toubro S, Astrup A, Gottesdiener KM, DeSmet M, Saris WH (2002) Acute effect of L-796568, a novel beta 3-adrenergic receptor agonist, on energy expenditure in obese men. Clin Pharmacol Ther 71:272–279

Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS (2004) Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem 279:47066–47075

Wang M, Fotsch C (2006) Small-molecule compounds that modulate lipolysis in adipose tissue: targeting strategies and molecular classes. Chem Biol 13:1019–1027

Warnich GR (1986) Enzymatic methods for quantification of lipoprotein lipids. Methods Enzymol 129:101–123

Winiarska K, Szymanski K, Gorniak P, Dudziak M, Bryla J (2009) Hypoglycaemic, antioxidative and nephroprotective effects of taurine in alloxan diabetic rabbits. Biochimie 91:261–270

Wong W, Scott JD (2004) AKAP signaling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5:959–970

Xiao C, Giacca A, Lewis GF (2008) Oral taurine but not N-acetyl-cysteine ameliorates NEFA-induced impairment in insulin sensitivity and beta cell function in obese and overweight, non-diabetic men. Diabetologia 51:139–146

Zentella de Piña M, Vázquez-Meza H, Agundis C, Pereyra MA, Pardo JP, Villalobos-Molina R, Piña E (2007) Inhibition of cAMP-dependent protein kinase A: a novel cyclo-oxigenase-independent effect of non-steroidal anti-inflammatory drugs in adipocytes. Auton Autocoid Pharmacol 17:85–92

Zentella de Piña M, Vázquez-Meza H, Pardo JP, Rendón JL, Villalobos-Molina R, Riveros-Rosas H, Piña E (2008) Signaling the signal, cyclic AMP-dependent protein kinase inhibition by insulin-formed H$_2$O$_2$ and reactivation by thioredoxin. J Biol Chem 252:12373–12386

Zhang M, Bi LF, Fang JH, Su XL, Da GL, Kuwamori T, Kagamimori S (2004) Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. Amino Acids 26:267–271

Zhong Z, Wheeler MD, Li X, Froh M, Schemmer P, Yin M, Bunzendaul H, Bradford B, Lemasters JJ (2003) L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent. Curr Opin Clin Nutr Metab Care 6:229–240

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306:1383–1386