Metabolic switching of human skeletal muscle cells *in vitro*

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Summary

In this review we will focus on external factors that may modify energy metabolism in human skeletal muscle cells (myotubes) and the ability of the myotubes to switch between lipid and glucose oxidation. We describe the metabolic parameters suppressibility, adaptability and substrate-regulated flexibility and show the influence of nutrients such as fatty acids and glucose (chronic hyperglycemia), and some pharmacological agents modifying nuclear receptors (PPAR and LXR), on these parameters in human myotubes. Possible cellular mechanisms for changes in these parameters will also be highlighted.

Metabolic flexibility

Skeletal muscle is the main tissue involved in lipid and glucose oxidation in the body. In the postprandial state glucose oxidation dominates, however fat oxidation increases both during fasting and sustained exercise [1-3]. The ability to switch from predominantly lipid oxidation during fasting conditions to increased glucose oxidation and suppression of lipid oxidation in response to insulin [2, 3] is called metabolic flexibility, and is a characteristic of healthy skeletal muscle [4, 5]. Kelley and Mandarino termed loss of this ability to switch easily between glucose and lipid oxidation metabolic inflexibility [5]. Obesity, insulin resistance and type 2 diabetes (T2D) are linked to reduced lipid oxidation during fasting, impaired postprandial switch from lipid to glucose oxidation and reduced capacity to increase lipid oxidation during exercise [3, 6-8]. Furthermore, a reduced postprandial switch from lipid to glucose oxidation has been observed in individuals with impaired glucose tolerance [7], suggesting that inflexibility plays a role in the early development of T2D. The usual way to measure metabolic flexibility to carbohydrate *in vivo* is to calculate the difference between steady-state respiratory quotient (RQ) at the end of hyperinsulinemic euglycemic clamp and fasting RQ (ΔRQ) [9]. A low RQ indicates high lipid oxidation, whereas a high RQ indicates high glucose oxidation. An impaired ability to increase muscle and whole body glucose oxidation during clamp has been observed in insulin resistant subjects [5, 10-12]. Additionally, insulin-stimulated glucose disposal rate has been found to correlate with metabolic flexibility and to explain most of the variance in ΔRQ [11, 13], indicating that metabolic flexibility to carbohydrate is primarily reflecting glucose uptake. However, other

studies suggest that impairments in substrate uptake and oxidation do not always occur in parallel [1, 14]. Adaptability to lipid, i.e. the capacity to increase lipid oxidation upon increased fatty acid availability, is less thoroughly investigated. However, Kelley and Simoneau observed a higher postprandial leg RQ in response to a high-fat meal in T2D subjects compared to weight-matched non-diabetic controls [15].

Metabolic switching of skeletal muscle cells

Metabolic switching of human skeletal muscle cells (myotubes) *in vitro* has been described by Ukropcova et al. [16]. Suppressibility was defined as the ability of the cells to suppress fatty acid (FA) oxidation by acute addition of glucose, and adaptability was defined as the capacity of the cells to increase FA oxidation upon increased fatty acid availability [16]. *In vitro* suppressibility was inversely correlated with insulin sensitivity and metabolic flexibility *in vivo*, whereas adaptability was positively correlated with these parameters [16]. This study indicated that metabolic switching is an intrinsic characteristic of skeletal muscle cells. Stull et al. recently showed that race was a contributor of metabolic flexibility *in vivo* [13], signifying that metabolic flexibility is at least partly a product of genetics. Nevertheless, metabolic inflexibility could be due to both intrinsic and extrinsic (induced) factors. Human *in vivo* studies have shown that postprandial impairments in metabolic flexibility can be improved by weight loss [7, 14, 17], and that exercise improves the ability of skeletal muscle to oxidize fatty acids during exercise and fasting [14, 18, 19]. A recent intervention study on men with metabolic syndrome indicated that isoenergetic modulation of dietary fat quality and quantity did not affect respiratory quotient or carbohydrate and lipid oxidation during fasting or after a meal [20]. However, the sample size in this study was relatively small for detecting such changes. The investigation of substrate oxidation started decades ago, and according to Randle cycle [21], fatty acids reduce glucose oxidation, whereas glucose reduces fatty acid oxidation through “reverse Randle cycle” [22, 23]. While the mechanistic basis for Randle cycle is relatively well understood, the molecular mechanism underlying metabolic inflexibility remains to be revealed.

Methodology used to measure metabolic switching of human myotubes

For a better description of metabolic switching in cells, we have used three parameters, the two parameters suppressibility and adaptability as introduced by Ukropcova [16], and the new parameter substrate-regulated flexibility [24] in functional studies of fuel selection and energy metabolism in cell cultures. Substrate-regulated flexibility was defined as the ability to increase fatty acid (FA) oxidation when changing from a high glucose (5 mM), low FA (5 μM) condition ("fed") to a high FA (100 μM), low glucose (0 mM) ("fasted") condition (Figure 1).

The suppressibility of oleic acid (OA) oxidation, measured as carbondioxide formation (CO_2), when 5 mM glucose was added acutely to the cells, was independent of the fatty acid concentration in the range from 5 to 300 μM (Figure 2A). Moreover, it was observed that maximum suppression by about 50 % was reached at 2-5 mM glucose, and already at 0.5-1 mM glucose acutely we observed a significant reduction in OA oxidation (IC_{50} 0.55 mM). Moreover, we also showed that suppressibility of OA oxidation by glucose was not affected when increasing mitochondrial respiration by addition of the mitochondrial uncoupler 2.4-dinitrophenol (DNP) (Figure 2B). In the opposite situation, suppressibility of glucose oxidation by acute addition of OA was strongly dependent of mitochondrial respiration (Figure 2B) probably because glycolytic metabolism is preferred in human myotubes [25, 26].

The adaptability of OA oxidation in the range from 5 to 300 μM of the fatty acid is shown in Figure 2C, which demonstrate that the adaptability was dependent on FA concentration. For our calculations of this parameter we have mostly used the ratio 100 μM FA/ 5 μM FA (Figure 1).

Addition of insulin (100 nM) to the low FA condition ("fed") condition did not change substrate-regulated flexibility, probably because most of the glucose uptake in human myotubes occurs independent of insulin. Moreover, Ukropcova et al. [16] observed that insulin was not needed for the expression of the metabolic switching phenotype in muscle cells. We also found that this parameter was robust against varying degree of fatty acid uptake by the cells (data not shown). The details about muscle cell cultures used in these studies are given in the legend to Figure 2.

Metabolic switching of human myotubes can be changed by alterations in the extra-cellular milieu

Metabolic switching of myotubes can be altered by changing the extracellular milieu,

and myotubes can be used as a cell model to determine how dietary changes (e.g. fatty acids), altered glycemic control (chronic hyperglycemia) or pharmacological agents acting at nuclear receptors (e.g. PPAR and LXR) may affect overall energy metabolism in cells.

Treatment of myotubes with various fatty acids (100 μM) for 24 h showed that the n-3 fatty acid eicosapentaenoic acid (EPA) increased suppressibility of the acute [^{14}C]oleic acid (OA) metabolism compared to OA pretreatment, as well as adaptability and substrate-regulated flexibility compared to pretreatment with OA and the fatty acid-free control (BSA) (Figure 3 [24]). This suggests a beneficial role for EPA in improving metabolic switching and overall energy metabolism in skeletal muscle. Exposure of myotubes to other long-chain n-3 FAs such as α-linolenic acid (ALA) and docosahexaenoic acid (DHA) also increased substrate-regulated flexibility to the same extent as EPA, indicating that EPA's effect on metabolic switching may be due to a general quality of n-3 FAs [24]. Adaptability of the myotubes was also increased after exposure to palmitic acid (PA) and linoleic acid (LA), suggesting that this is a general fatty acid effect.

For most experiments we have used [^{14}C]OA acutely when measuring the metabolic switching parameters. With OA pretreatment and subsequent incubation with [^{14}C]OA we did not see any change in fatty acid adaptability for OA (Figure 4A). We also observed the same with PA pretreatment and [^{14}C]PA thereafter, thus for determination of the adaptability parameter it seems that another FA acutely is preferred rather than the pretreatment fatty acid. The reason is not clear but it may be an artifact due to isotope dilution. Moreover, adaptability was much higher for labelled OA than for PA (Figure 4A). Also pretreatment with the sulfur-substituted fatty acid analogue tetradecylthioacetic acid (TTA) for 24 h and then using [^{14}C]OA acutely increased the adaptability of the myotubes (Figure 4B). TTA may also work as a PPAR agonist after prolonged incubation of myotubes [27] (see later).

In addition to various FAs, other components in the extracellular milieu can affect metabolic switching of myotubes. We have previously shown that treatment of human myotubes with chronic hyperglycemia (HG) reduced acute glucose uptake and glycogen synthesis. This reduction accompanied increased accumulation of TAG in the cells and an increased *de novo* lipogenesis [28]. Moreover, recent data show that HG reduced glucose and oleic acid (OA) oxidation, as well as the suppressibility of the cells [26]. However, the adaptability of OA oxidation was not affected after

chronic HG treatment [26]. We also measured substrate-regulated flexibility, which was significantly reduced after HG treatment of the cells (Figure 5).

Metabolic switching of human myotubes can be modified by agonist regulating the nuclear receptors PPARs and LXRs

In addition to various FAs and chronic hyperglycemia, we also examined the possible involvement of peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs) in metabolic switching. The myotubes were incubated for 4 days with compounds known to act agonistic at these receptors.

PPARs are activated by fatty acids and therefore act as lipid sensors in the body [29-31]. Three subtypes of PPARs have been identified, differing in tissue distribution and function. PPARα is predominantly expressed in the liver, but also found in heart and skeletal muscle, where it increases fatty acid oxidation [29, 32-34]. PPARγ is mainly expressed in adipose tissue, and plays an important role in adipocyte differentiation and lipid storage [31, 35-37]. In contrast to the other subtypes, PPARδ (also called PPARβ) is ubiquitously expressed and is relatively abundant in skeletal muscle [38]. The human myotube model used in our experiments express more PPARδ than PPARα (unpublished observations).

The role of PPARδ is currently under investigation, and is so far found to increase fatty acid oxidation in skeletal muscle [39], and might thus play an important role in energy turnover [40]. Treatment of the myotubes with the PPARδ agonists GW501516 and the pan-agonist TTA for 4 days during differentiation increased CO_2 formation from the cells when incubated acutely with labelled OA (Figure 6). The two metabolic parameters suppressibility and adaptability were not changed by PPAR activation, but substrate-regulated flexibility was 2-fold increased by TTA (Figure 7), but not by GW501516. TTA is a pan-PPAR activator that reduces plasma lipids and enhances hepatic fatty acid oxidation in rodents [41]. Dual and pan-PPAR agonists are currently being developed for treatment of type 2 diabetes (T2D) [42], and TTA has been shown to improve glucose metabolism in insulin-resistant rats [43] and to stimulate mitochondrial proliferation in rat skeletal muscle [44]. Recently, we have shown that preincubation of myotubes with TTA enhanced mitochondrial fatty acid and glucose oxidation, indicating increased mitochondrial biogenesis and an improved glucose metabolism [27]. At the same time TTA opposed increased lipid accumulation by the cells. TTA seems more potent in activating rat PPARα than

PPARδ [43, 45], whereas the opposite has been demonstrated for human PPARs [46]. This may partly explain why TTA has particularly strong effects on hepatic metabolism in rodents where PPARα is a major regulator, but relatively modest effects in skeletal muscle where PPARδ may play a more prominent role [38, 47]. In human myotubes, PPARδ seems to play a dominant role over PPARα in controlling lipid oxidation [48], and we have demonstrated that preincubation of myotubes with the PPARδ selective agonist GW501516 and TTA, but not the PPARα-selective agonists fenofibrate and clofibrate (at PPARα-selective concentrations), could induce increased mitochondrial fatty acid oxidation in myotubes [49]. Our data on fatty acid oxidation (Figure 6) also demonstrate that an increased capacity for oxidation of fatty acids (increased CO_2 formation) alone is not sufficient to change the metabolic parameters described in this paper.

LXRs play a crucial role in regulation of cholesterol, lipid and carbohydrate metabolism [50-54]. We have shown that chronic activation of LXRs may affect glucose uptake and oxidation as well as promote a strong effect on lipid metabolism by increasing fatty acid uptake and accumulation as complex lipids and to stimulate lipogenesis in human myotubes [55, 56]. Thus, LXRs could play a role in the regulation of metabolic switching. We have shown that treatment of myotubes with the LXR agonist T0901317 for 2 days did not affect suppressibility or adaptability of the cells *per se*, but counteracted the effect of EPA on these two parameters (Figure 8A and B, [24]). Treatment with T0901317 did not change substrate-regulated flexibility, nor cancel out the effect of EPA on this parameter. Therefore, LXRs do not seem to play a major role, but show some impact on metabolic switching of myotubes.

Possible mechanisms for changes in metabolic switching of myotubes

To study whether the effects of FA pretreatment on metabolic switching could be due to altered gene expression, microarray analysis was performed [24]. This revealed that 24 h treatment with 0.1 mM EPA regulated more genes than the other FAs examined, followed by LA and OA, while PA regulated fewest genes. Three of the genes induced by all FAs are involved in fatty acid β-oxidation [24]. Hence, this could reflect the ability of FAs to increase adaptability. Moreover, pathway analysis showed that some pathways involved in carbohydrate metabolism were affected only after

exposure to EPA, supporting the functional data showing specific effects of n-3 FAs on suppressibility and substrate-regulated flexibility compared to the other FAs [24] (Figure 9). *In vivo* studies (both human and animal) have shown that fatty acid oxidation and the activity of β-oxidation enzymes are increased in skeletal muscle of individuals/rats fed a high-fat diet [57-59]. This could be a mechanism to compensate for elevated availability of fatty acids to protect against lipid accumulation, and might be reflected *in vitro* by the adaptability parameter.

Chronic treatment with the PPARδ agonist GW501516 markedly increased OA oxidation and this substance has previously been shown to induce genes involved in fatty acid transport and oxidation in cells [39, 48, 60], and to increase fatty acid oxidation [61, 62]. Moreover, we demonstrate that increasing the capacities for fatty acid oxidation by PPAR activation, or respiration by mitochondrial uncoupling did not change the metabolic parameters in the myotubes. The pan-PPAR agonist TTA also increased fatty acid oxidation, but in contrast to GW501516 it seemed to increase substrate-regulated flexibility. We have previously shown that TTA also positively influences glucose metabolism in myotubes [27], and this fatty acid analogue also increased adaptability after 24 h pretreatment (Figure 4B). Thus, TTA may show PPAR and PPAR-independent effects [41, 45] in myotubes that could explain the difference between the two PPAR agonists on the metabolic parameters. With respect to the different FAs some studies have shown that PUFAs are slightly more potent PPAR agonists than other FAs [29, 30, 63], however no difference in the activation of PPARs has been observed between n-3 and n-6 FAs [29, 63]. Studies have indicated that PPARα interacts almost equally with both saturated and unsaturated FAs and that PPARδ also interacts with saturated and unsaturated FAs, although less effective than PPARα [63, 64]. Based on the gene expression studies we suggest that the effects on metabolic switching by FAs may only partly be explained by changes in gene expression [24].

The effects of distinct FAs on metabolic switching could also be due to differences in accumulation of fatty acids, the level of intracellular lipid species such as acyl-CoA, diacylglycerol (DAG) and triacylglycerol (TAG), and lipid utilization. Ectopic fat deposition is associated with impaired organ function [65], and increased accumulation of intramyocellular lipids (IMCL) correlates with insulin resistance and T2D [66-70]. Intramyocellular lipid intermediates might as well affect metabolic switching. We have shown that EPA *per se* was less incorporated in the cells than the

other FAs examined, probably due to higher oxidation rate [24]. However, treatment with EPA, as well as OA and LA, increased the number of lipid droplets (LDs) per nucleus in myotubes [24]. The content of neutral lipids followed the same pattern as the number of LDs, but lipid distribution was not changed by pretreatment with the various FAs. In accordance with our data, previous studies have shown that treatment with EPA increased acute fatty acid uptake [27, 71]. Furthermore, treatment with EPA has been shown to promote accumulation of TAG and reduce the level of total acyl-CoA in human myotubes [27, 71]. Nevertheless, although various FAs were handled differently in the cells, altered lipid specie distribution could not easily explain the observed effects on metabolic switching. Other possible mechanisms by which metabolic switching can be changed by FAs include altered AMP-activated protein kinase (AMPK) activity, differences in gene regulation through various nuclear receptors, as well as different membrane incorporation of fatty acids and thereby altered membrane fluidity and activity of membrane-associated proteins or dynamics of mitochondrial membranes [72-79].

The effects of chronic hyperglycemia (HG) on metabolic switching were not due to changes in gene expression, as microarray analysis revealed that no genes were significantly regulated by HG treatment [26]. This is a potential important finding, and suggests that the effects of HG could be due to post-translational modifications, such as glycosylations or glycations. Moreover, ATP concentration was reduced in HG cells. This indicates that hyperglycemia induces some kind of mitochondrial dysfunction. The amount of mitochondrial DNA was not changed indicating that the mitochondrial content was unaffected. Yet, the mitochondrial function might be impaired. This is in accordance with the findings of Gao et al. showing that HG treatment of 3T3-L1 adipocytes induced insulin resistance, loss of mitochondrial membrane potential, as well as resulted in smaller and more compact mitochondria with no effect on mitochondrial DNA [80]. Another mechanism by which HG theoretically could affect metabolism is through altered AMPK activity, since glucose infusion in rats has been shown to reduce phosphorylation of AMPK and its substrate acetyl-CoA carboxylase (ACC) in red but not white muscle [81]. In our study [26], AMPK activity could not explain the metabolic impairments because phosphorylation of AMPK and ACC was not changed after HG exposure. Taken together, chronic HG impaired metabolic switching of myotubes probably due to an induced mitochondrial dysfunction.

Conclusion

The *in vitro* metabolic parameters suppressibility, adaptability and substrate-regulated flexibility can be modified by fatty acids or by chronic hyperglycemia (HG) in human myotubes. Eicosapentaenoic acid (EPA) increased all three parameters, and the effect of EPA could be counteracted by LXR activation. The fatty acid analogue tetradecylthioacetic acid (TTA) could also modify metabolic switching depending on treatment time. Increased fatty acid oxidation after PPARδ agonist treatment did not change metabolic switching of myotubes. Treatment with HG negatively influenced suppressibility and substrate-regulated flexibility of the cells.

Captions to illustrations

Figure 1. Calculations of metabolic flexibility parameters.

Figure 2. Suppressibility of oleic acid (OA) oxidation by glucose and of glucose oxidation by OA, and adaptability of OA oxidation at different OA concentrations. *Suppressibility (A).* Myotubes underwent CO_2 trapping for 4 h with different concentrations of [^{14}C]oleic acid (OA) in the presence or absence of 5 mM glucose. *Suppressibility with mitochondrial uncoupling (B).* Myotubes were incubated with radiolabeled substrates in the absence or presence of 2.4-dinitrophenol (DNP, 100 μM) for 4 h to collect CO_2. Figure shows suppressibility by acute glucose or by acute oleic acid. Figure left shows suppressibility of [^{14}C]oleic acid (OA) (100 μM) oxidation in presence of acute glucose (5 mM), and right shows suppressbility of [^{14}C(U)]glucose (200 μM) oxidation in presence of acute OA (100 μM). *Adaptability (C).* Myotubes underwent CO_2 trapping for 4 h with different concentrations of [^{14}C]OA in the absence of glucose. Results represent means ± SEM for n=5 (A), n=4 (B), n=4-17 (C). n, number of separate experiments with cells from individual donors. *Human skeletal muscle cell cultures.* Satellite cells were isolated from the M. obliquus internus abdominis of healthy donors. The biopsies were obtained with informed consent and approval by the regional committee for research ethics, Oslo, Norway. The cells were cultured in DMEM (5.5 mM glucose) with 2% foetal calf serum (FCS), 2% Ultroser G, penicillin/streptomycin (P/S) and amphotericin B until 70-80% confluent. Myoblast differentiation to myotubes was then induced by changing medium to DMEM (5.5 mM glucose) with 2% FCS, 25 pM insulin, P/S and amphotericin B. Experiments were performed after 7-8 days of differentiation.

Figure 3. Suppressibility of oleic acid (OA) oxidation by glucose, adaptability of OA oxidation and substrate-regulated flexibility of OA after pretreatment with different fatty acids. *Suppressibility (A).* Myotubes were pretreated for 24 h with 100 μM OA, EPA, LA, PA, 40 μM BSA (control, all FA treatments contain 40 μM bovine serum albumin, BSA), and thereafter underwent CO_2 trapping for 4 h with 100 μM [^{14}C]oleic acid (OA) in the presence or absence of 5 mM glucose. *Adaptability (B).* Myotubes were pretreated for 24 h with 100 μM OA, EPA, LA, PA, 40 μM BSA

(control), and thereafter underwent CO_2 trapping for 4 h with 5 or 100 µM [^{14}C]OA in the absence of glucose. *Substrate-regulated flexibility (C).* Myotubes were pretreated for 24 h with 100 µM OA, EPA, LA, PA, 40 µM BSA (control), and thereafter underwent CO_2 trapping for 4 h with 100 µM [^{14}C]OA in the absence of glucose or with 5 µM [^{14}C]OA in the presence of 5 mM glucose. Results represent means ± SEM for n=11-17 (A), n=6-12 (B), n=6-12 (C). *p<0.05 vs. control, #p<0.05 vs oleic acid (t-test).

Abbreviations: BSA, bovine serum albumin; EPA, eicosapentaenoic acid; LA, linoleic acid; OA, oleic acid; PA, palmitic acid. Reproduced from [24].

Figure 4. Adaptability of fatty acid oxidation. Myotubes were pretreated for 24 h with 100 µM OA, PA or 40 µM BSA (control), and thereafter underwent CO_2 trapping for 4 h with 5 or 100 µM [^{14}C]OA or [^{14}C]PA in the absence of glucose (A). Myotubes were pretreated for 24 h with 100 µM tetradecylthioacetic acid (TTA), and thereafter underwent CO_2 trapping for 4 h with 5 or 100 µM [^{14}C]OA in the absence of glucose. Results represent means ± SEM for n=5 (A and B). *p<0.05 vs. control.

Figure 5. Substrate-regulated flexibility after chronic hyperglycemia (HG). Myotubes were pretreated for 4 days with 20 mM glucose, and thereafter the cells underwent CO_2 trapping for 4 h with 100 µM [^{14}C]OA in the absence of glucose or with 5 µM [^{14}C]OA in the presence of 5 mM glucose. Results represent means ± SEM for n=4. *p<0.05 vs. control.

Figure 6. Carbondioxide formation after pretreatment with PPAR agonists. Myotubes were pretreated for 4 days with 100 µM tetradecylthioacetic acid (TTA), 10 nM GW501516 or vehicle (control, DMSO), and thereafter the cells underwent CO_2 trapping for 4 h with 100 µM [^{14}C]oleic acid (OA) in the absence of glucose. Results represent means ± SEM for n=3-7. *p<0.05 vs. control.

Figure 7. Suppressibility of oleic acid (OA) oxidation by glucose, adaptability of OA oxidation and substrate-regulated flexibility of OA after pretreatment with

PPAR agonists. *Suppressibility (A).* Myotubes were pretreated for 4 days with 100 μM tetradecylthioacetic acid (TTA), 10 nM GW501516 or vehicle (control, DMSO), and thereafter the cells underwent CO_2 trapping for 4 h with 100 μM [^{14}C]oleic acid (OA) in the presence or absence of 5 mM glucose. *Adaptability (B).* Myotubes were pretreated for 4 days with 100 μM tetradecylthioacetic acid (TTA), 10 nM GW501516 or vehicle (control, DMSO), and thereafter the cells underwent CO_2 trapping for 4 h with 5 or 100 μM [^{14}C]OA in the absence of glucose. *Substrate-regulated flexibility (C).* Myotubes were pretreated for 4 days with 100 μM tetradecylthioacetic acid (TTA), 10 nM GW501516 or vehicle (control, DMSO), and thereafter the cells underwent CO_2 trapping for 4 h with 100 μM [^{14}C]OA in the absence of glucose or with 5 μM [^{14}C]OA in the presence of 5 mM glucose. Results represent means ± SEM for n=8, 3, 3 for A, B, C, respectively.

Figure 8. Suppressibility of oleic acid (OA) oxidation by glucose, adaptability of OA oxidation and substrate-regulated flexibility of OA after pretreatment with eicosapentaenoic acid (EPA) and a LXR agonist T0901317. *Suppressibility (A).* Myotubes were pretreated for 24 h with 1 μM T0901317 or vehicle (DMSO), and then for another 24 h with 100 μM EPA or 40 μM BSA (control) with and without T0901317, and thereafter the cells underwent CO_2 trapping for 4 h with 100 μM [^{14}C]oleic acid (OA) in the presence or absence of 5 mM glucose. *Adaptability (B).* Myotubes were pretreated for 24 h with 1 μM T0901317 or vehicle (DMSO), and then for another 24 h with 100 μM EPA or 40 μM BSA (control) with and without T0901317, and thereafter the cells underwent CO_2 trapping for 4 h with 5 or 100 μM [^{14}C]OA in the absence of glucose. *Substrate-regulated flexibility (C).* Myotubes were pretreated for 24 h with 1 μM T0901317 or vehicle (DMSO), and then for another 24 h with 100 μM EPA or 40 μM BSA (control) with and without T0901317, and thereafter the cells underwent CO_2 trapping for 4 h with 100 μM [^{14}C]OA in the absence of glucose or with 5 μM [^{14}C]OA in the presence of 5 mM glucose. Results represent means ± SEM for n=6-17, 6-12, 6-12 for A, B, C, respectively. *p<0.05 vs. control, #p<0.05 vs EPA.

Figure 9. Pathways involved in metabolic processes upregulated by fatty acids in myotubes. Myotubes were incubated with fatty acids (100 μM) or control (BSA 40 μM) for 24 h and harvested for RNA isolation. Gene expression was measured by Affymetrix human NuGO Genechip arrays and gene set enrichment analysis (GSEA) was performed to identify pathways regulated by OA, PA, LA, and EPA compared to control. Pathways with FDR (q-value) < 0.2, that is –log(q) > 0.7, were considered significantly regulated. Line marks –log(q) = 0.7, the cut-off for significance for the GSEA. In short, GSEA identifies pathways in which more genes are regulated than one would expect on the basis of chance.
Abbreviations: BSA, bovine serum albumin; EPA, eicosapentaenoic acid; LA, linoleic acid; OA, oleic acid; PA, palmitic acid. Reproduced from reference [24].

Figure 10. Metabolic switching of myotubes. The metabolic parameters suppressibility, adaptability and substrate-regulated flexibility can be modified by fatty acids or by chronic hyperglycemia (HG). Eicosapentaenoic acid (EPA) increased all three parameters, and the effect of EPA could be counteracted by LXR activation. The fatty acid analogue and PPAR activator tetradecylthioacetic acid (TTA) could also modify metabolic switching depending on treatment time. Treatment of myotubes with HG negatively influenced suppressibility and substrate-regulated flexibility.

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Suppressibility: the ability of the cells to decrease oleic acid (OA) oxidation by acutely added glucose: [(1-(oxidation of 100 μM OA at 5 mM glucose/oxidation of 100 μM OA at no glucose added)) × 100 %].
Adaptability: the ability to increase the OA oxidation with increasing OA concentration: [oxidation of 100 μM OA/oxidation of 5 μM OA].
Substrate-regulated flexibility: the ability to increase the OA oxidation while changing from the "fed" (low fatty acid, high glucose) to the "fasted" (high fatty acid, no glucose added) condition: [oxidation of 100 μM OA without glucose added/ oxidation of 5 μM OA at 5 mM glucose].

Figure 1

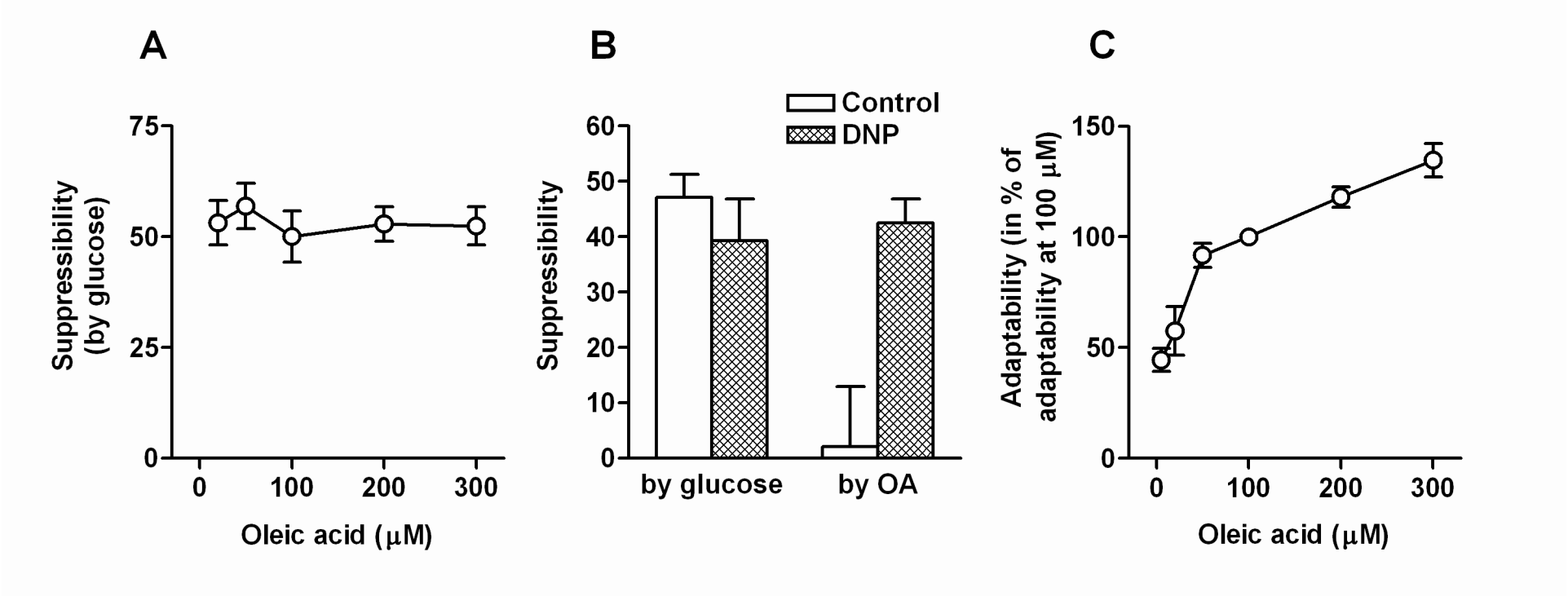
A
Suppressibility (by glucose)
75
50
25
0
0
100
200
300
Oleic acid (μM)
B
Control
DNP
Suppressibility
60
50
40
30
20
10
0
by glucose
by OA
C
Adaptability (in % of adaptability at 100 μM)
150
100
50
0
0
100
200
300
Oleic acid (μM)

Figure 2

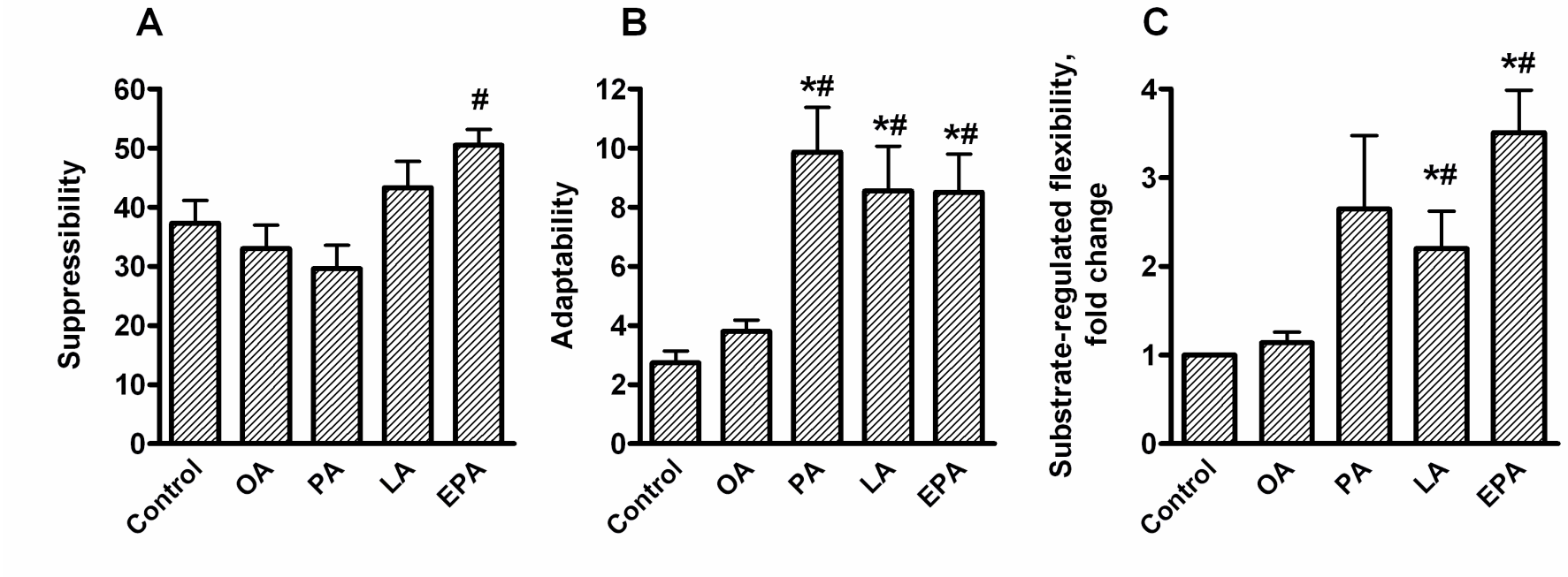
A
Suppressibility
60
50
40
30
20
10
0
#
Control
OA
PA
LA
EPA
B
Adaptability
12
10
8
6
4
2
0
*#
*#
*#
Control
OA
PA
LA
EPA
C
Substrate-regulated flexibility, fold change
4
3
2
1
0
*#
*#
Control
OA
PA
LA
EPA

Figure 3

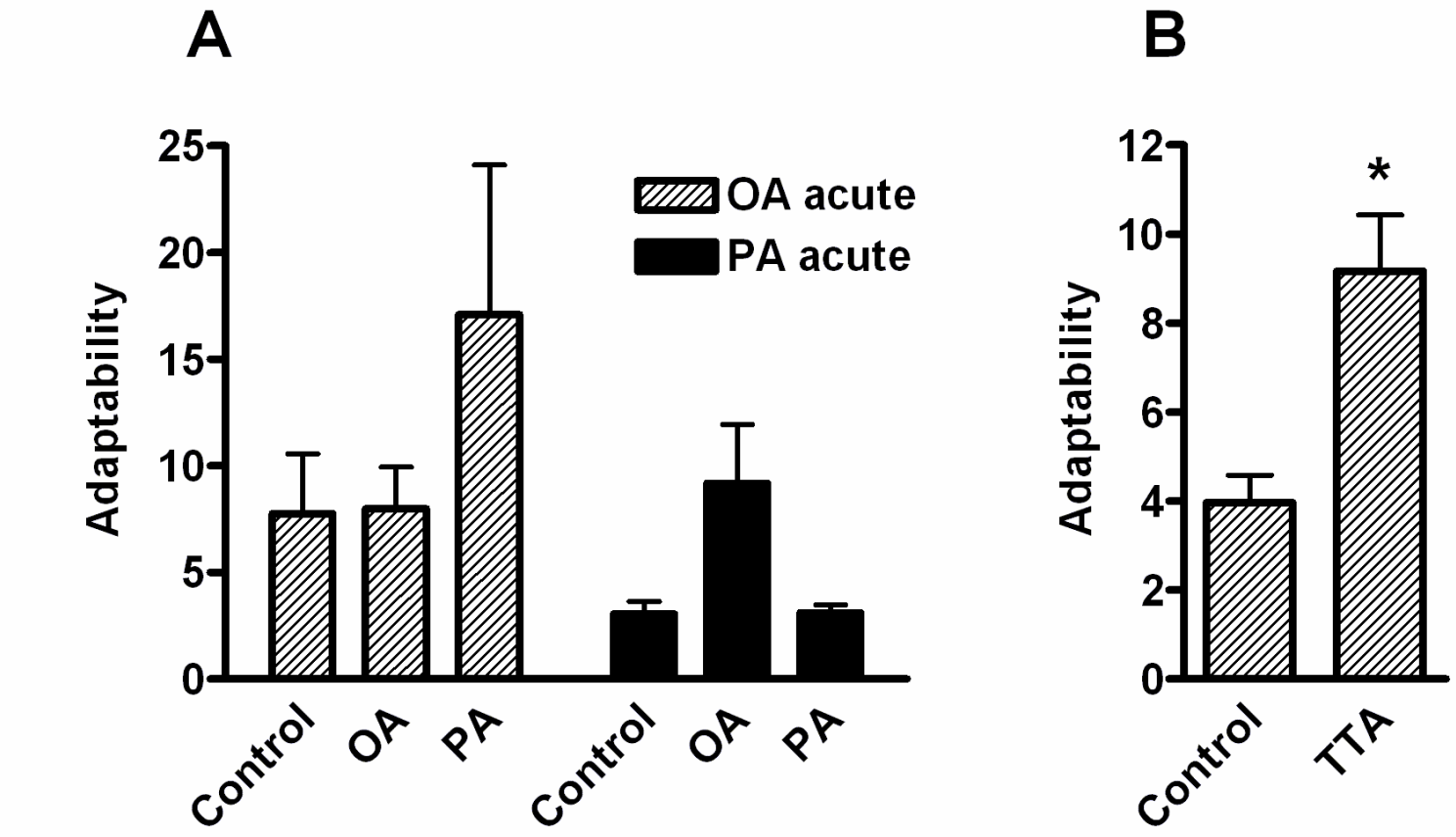
A
B
Adaptability
25
20
15
10
5
0
OA acute
PA acute
Control
OA
PA
Control
OA
PA
Adaptability
12
10
8
6
4
2
0
*
Control
TTA

Figure 4

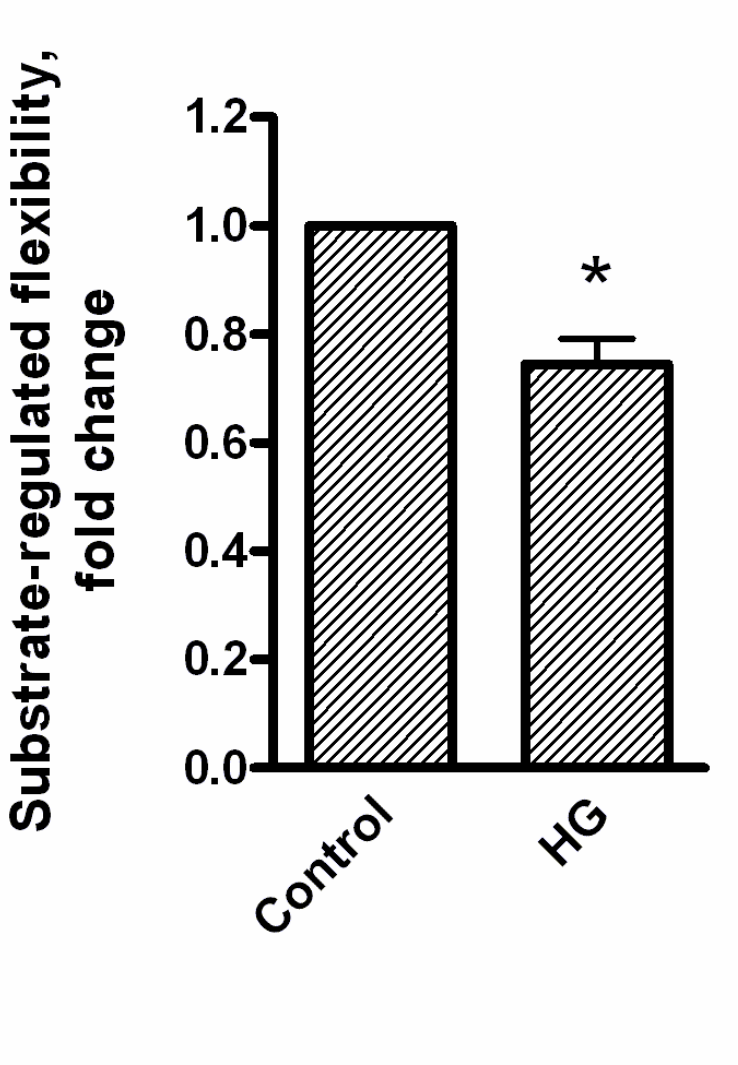
Substrate-regulated flexibility, fold change
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Control
HG

Figure 5

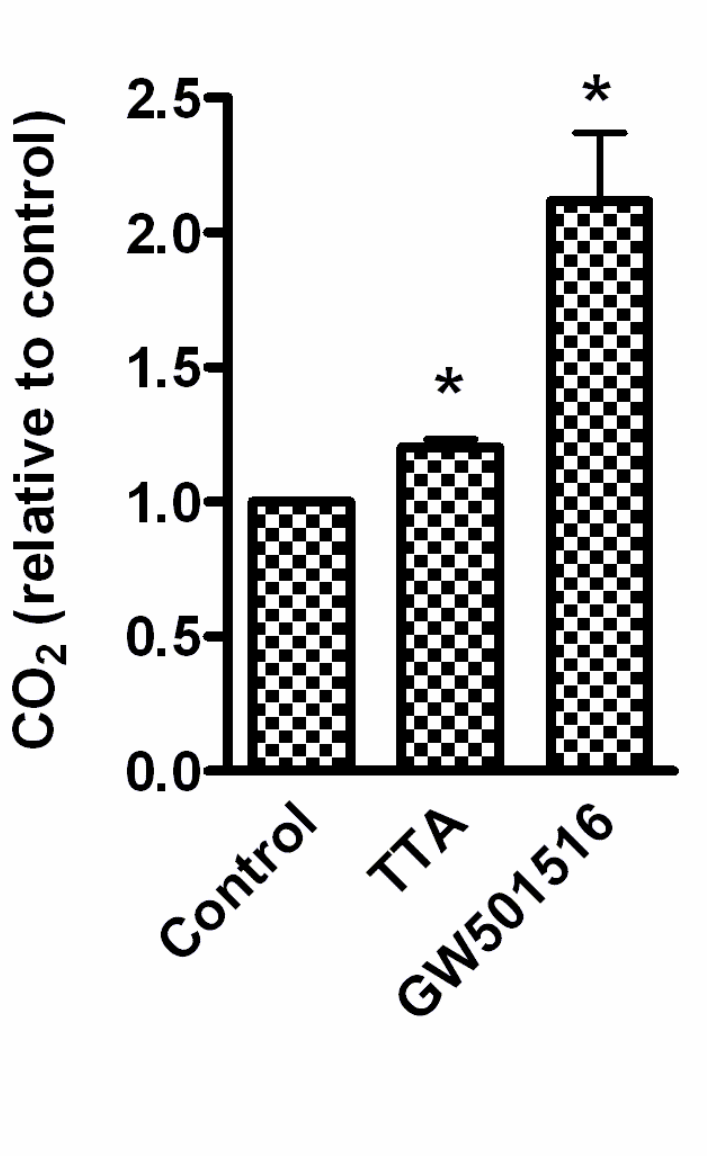
CO_2 (relative to control)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Control
TTA
GW501516

Figure 6

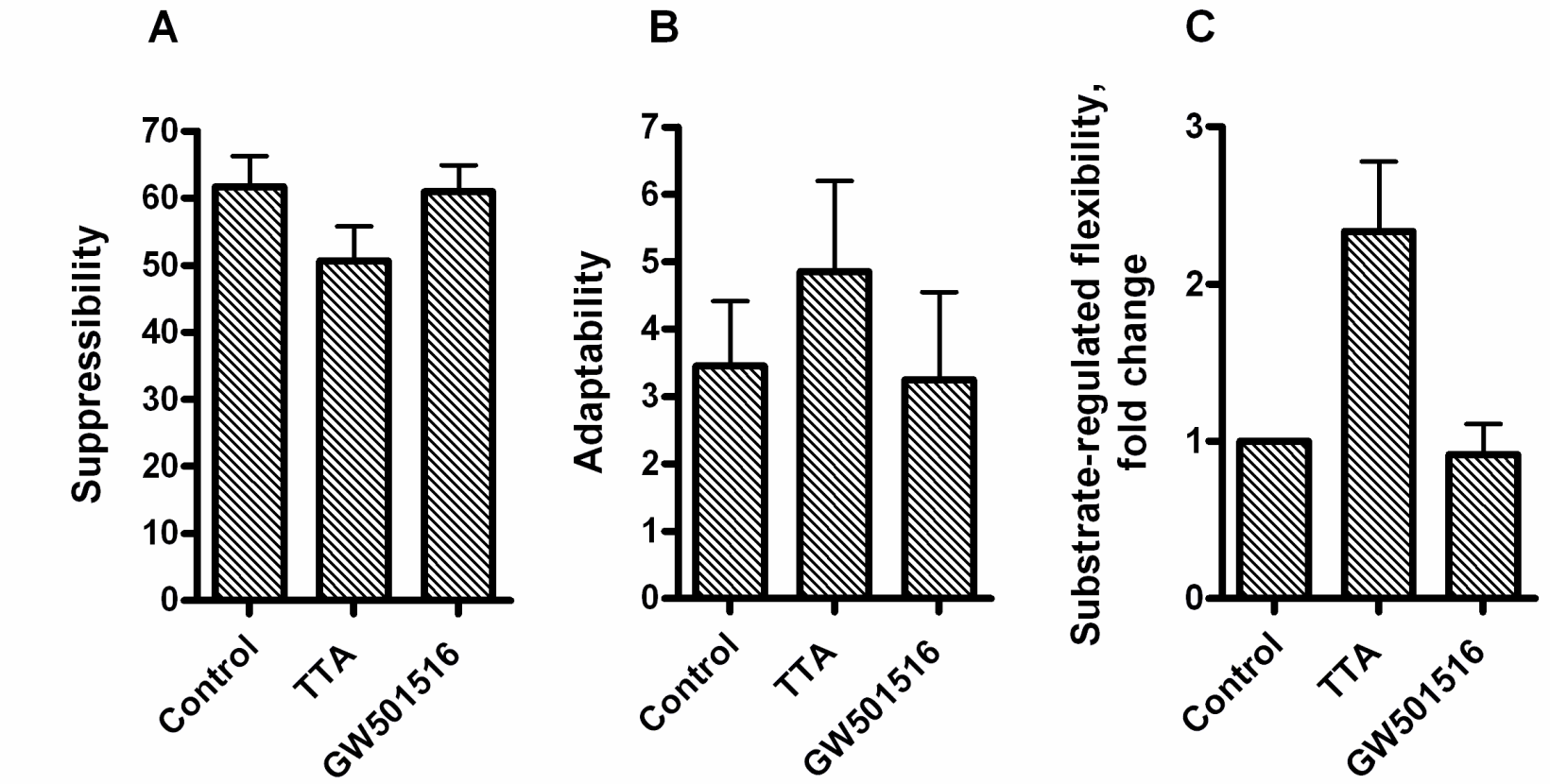
A
Suppressibility
70
60
50
40
30
20
10
0
Control
TTA
GW501516
B
Adaptability
7
6
5
4
3
2
1
0
Control
TTA
GW501516
C
Substrate-regulated flexibility, fold change
3
2
1
0
Control
TTA
GW501516

Figure 7

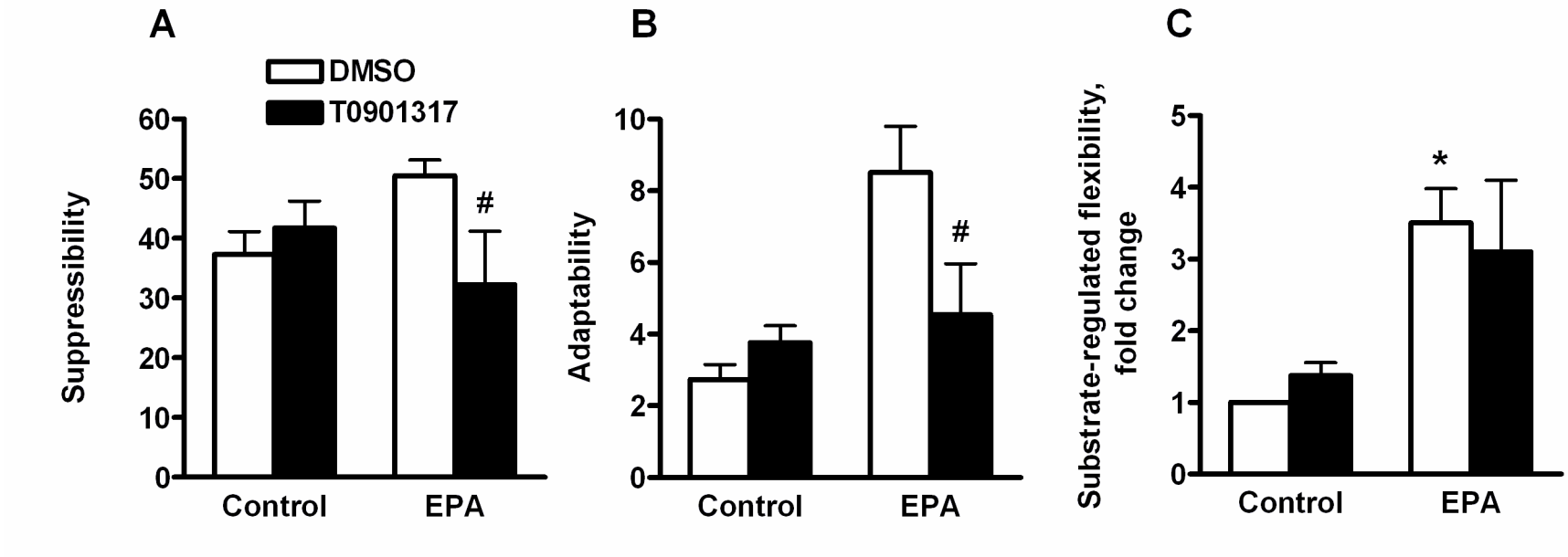
A
DMSO
T0901317
Suppressibility
60
50
40
30
20
10
0
#
Control
EPA
B
Adaptability
10
8
6
4
2
0
#
Control
EPA
C
Substrate-regulated flexibility, fold change
5
4
3
2
1
0
*
Control
EPA

Figure 8

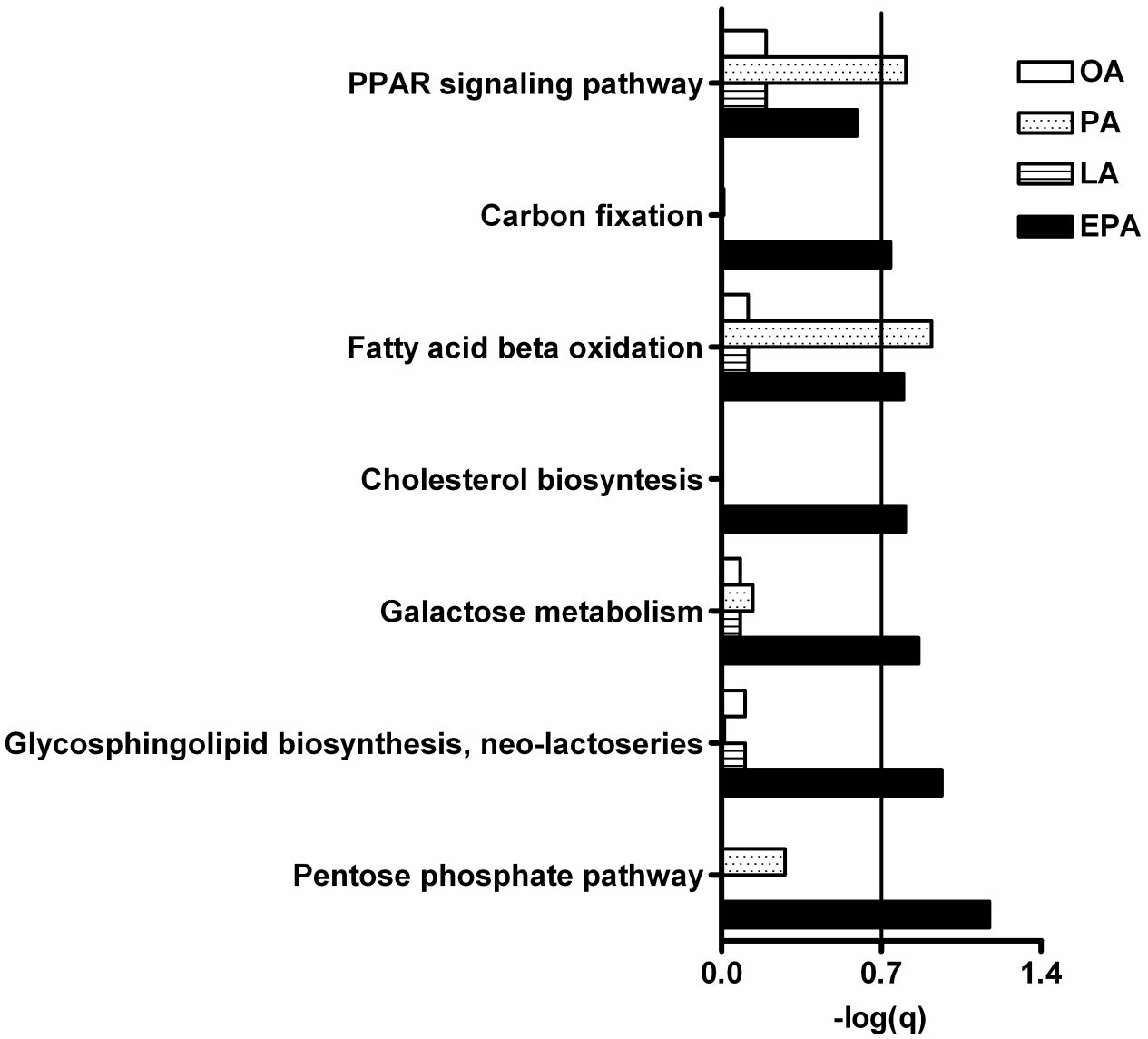
PPAR signaling pathway
Carbon fixation
Fatty acid beta oxidation
Cholesterol biosyntesis
Galactose metabolism
Glycosphingolipid biosynthesis, neo-lactoseries
Pentose phosphate pathway
OA
PA
LA
EPA
0.0
0.7
1.4
-log(q)

Figure 9

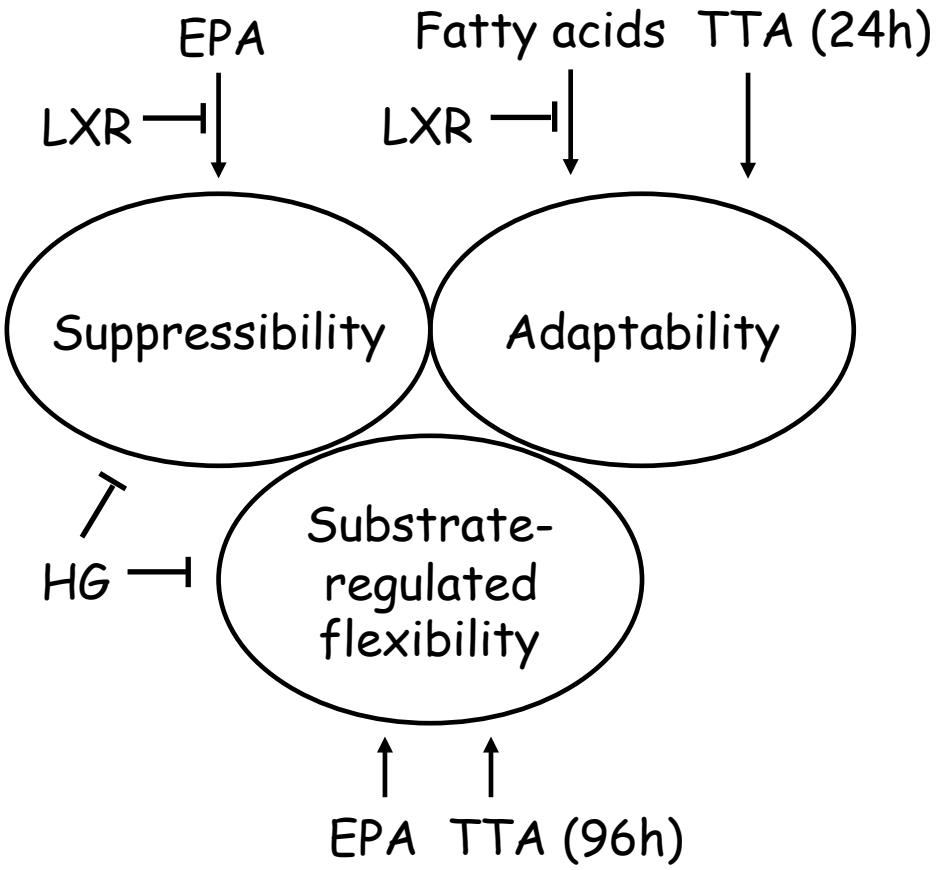
EPA
Fatty acids
TTA (24h)
LXR
LXR
Suppressibility
Adaptability
HG
Substrate-
regulated
flexibility
EPA
TTA (96h)

Figure 10