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Eicosapentaenoic fatty acid increases leptin secretion from primary cultured rat adipocytes: role of glucose metabolism

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Pérez-Matute, Patricia, Amelia Marti, J. Alfredo Martínez, M. P. Fernández-Otero, Kimber L. Stanhope, Peter J. Havel, and María J. Moreno-Aliaga. Eicosapentaenoic fatty acid increases leptin secretion from primary cultured rat adipocytes: role of glucose metabolism. *Am J Physiol Regul Integr Comp Physiol* 288: R1682–R1688, 2005. First published January 13, 2005; doi:10.1152/ajpregu.00727.2004.— Eicosapentaenoic acid (EPA), one of the n-3 polyunsaturated fatty acids, has been shown to stimulate leptin mRNA expression and secretion in 3T3-L1 cells. However, other studies have reported inhibitory effects of EPA on leptin expression and secretion in vivo and in vitro. To determine the direct effects of EPA on basal and insulin-stimulated leptin secretion, isolated rat adipocytes were incubated with EPA in the absence and presence of insulin. EPA (10, 100, and 200 μM) increased basal leptin gene expression and secretion (+43.8%, $P < 0.05$; +71.1%, $P < 0.01$; and +73.7%, $P < 0.01$, respectively). EPA also increased leptin secretion in the presence of 1.6 nM insulin; however, the effect was less pronounced than in the absence of it. Because adipocyte glucose and lipid metabolism are involved in the regulation of leptin production, the metabolic effects of this fatty acid were also examined. EPA (200 μM) increased basal glucose uptake in isolated adipocytes (+50%, $P < 0.05$). Anaerobic metabolism of glucose, as assessed by lactate production and proportion of glucose metabolized to lactate, has been shown to be inversely correlated to leptin secretion and was decreased by EPA in both the absence and presence of insulin. EPA increased basal glucose oxidation as determined by the proportion of ^{14}C-labeled glucose metabolized to CO_2. Lipogenesis (^{14}C-labeled glucose incorporation into triglyceride) was decreased by EPA in the absence of insulin, whereas lipolysis (glycerol release) was unaffected. The EPA-induced increase of basal leptin secretion was highly correlated with increased glucose utilization ($r = +0.89$, $P < 0.01$) and inversely related to the anaerobic glucose metabolism to lactate. EPA's effect on insulin-stimulated leptin secretion was not related to increased glucose utilization but was inversely correlated with anaerobic glucose metabolism to lactate ($r = -0.84$, $P < 0.01$). Together, the results suggest that EPA, like insulin, stimulates leptin production by increasing the nonanaerobic/oxidative metabolism of glucose.

ω-3 polyunsaturated fatty acids; obesity; adipose tissue; diabetes

DIETARY FAT IS AN IMPORTANT macronutrient in the diet of all animals (24). A growing body of literature suggests that the amount (7) and type (6, 20, 48) of fat included in the diet have roles in the development of obesity and insulin resistance. Specifically, diets high in saturated fat promote obesity and insulin resistance in rodents (25, 40), whereas diets high in fish oil appear to prevent or attenuate the development of these diseases (53). n-3 Polyunsaturated fatty acids (n-3 PUFAs) present in fish oil are known to have numerous beneficial effects on health (9, 13, 51). These include improvement of endothelial function, anti-arrhythmic effects, reductions in platelet aggregation and serum triglyceride concentrations (10), and amelioration of pathological conditions such as inflammatory diseases (23) and hypertension (39). Diets containing high levels of n-3 PUFAs have been shown to reduce white adipose tissue (3, 42) and adipocyte size (42, 43) and to prevent non-insulin-dependent diabetes in rats (27, 28, 38, 53). Indeed, eicosapentaenoic acid (EPA) [20:5 (n-3)], one of the prominent n-3 PUFAs contained in fish oil, has been reported to be useful in preventing the onset of insulin resistance and diabetes in animal models of obesity and diabetes (32, 33, 38).

The results of several studies support the hypothesis that alterations in the insulin sensitivity and metabolic activity of adipocytes have critical roles in the development of insulin resistance and type 2 diabetes in rodents (4).

Leptin is a hormone primarily secreted by white adipose tissue, which has been implicated in the regulation of food intake, energy expenditure, body fat stores, and insulin signaling (16, 44). Circulating leptin concentrations are correlated with adiposity in humans (14, 17) and animals (1); however, these are also altered independently of changes of body adiposity by fasting and refeeding (15). Numerous studies indicate that leptin production is increased by insulin (2, 56) and therefore is modulated by insulin responses to meals and dietary macronutrient composition (16, 19, 54). The effect of insulin to increase leptin expression and secretion (34) and the transcriptional activity of the leptin promoter (31) are mediated by insulin's effects to increase adipocyte glucose utilization. Furthermore, insulin shifts glucose metabolism from anaerobic metabolism to lactate to mitochondrial oxidation, and this effect may contribute to insulin-mediated leptin production, since anaerobic glucose metabolism does not increase leptin secretion (35).

EPA has been reported to stimulate leptin gene expression and secretion in 3T3-L1 adipocytes (37). In contrast, Reseland et al. (47) reported that dietary n-3 PUFAs decreased leptin mRNA expression in vivo and in vitro. To further address these conflicting results, we examined the effects of EPA on the leptin expression and secretion in primary cultures of isolated rat adipocytes. Adipocytes were maintained for 96 h anchored to a collagen matrix, which simulates basement membrane attachment, producing a more physiological environment and maintaining adipocyte differentiation compared with "free-

floating" adipocytes (34). Little is known about the effects of EPA on adipocyte metabolism (glucose and lipid metabolism), which has been demonstrated to be involved in the regulation of leptin production (34). Therefore, we also determined the effects of EPA on basal and insulin-stimulated adipocyte glucose utilization, lactate production, glucose oxidation, lipogenesis, and lipolysis.

MATERIALS AND METHODS

Materials. Media (DMEM), minimal essential medium amino acids, penicillin/streptomycin, fetal bovine serum, and nystatin were purchased from GIBCO (Grand Island, NY) and Invitrogen Life Technologies (Carlsbad, CA). BSA fraction V, HEPES, and insulin were from Sigma (St. Louis, MO). Collagen (Vitrogen 100) was purchased from Cohesion Technologies (Palo Alto, CA). [^{14}C]glucose was obtained from Perkin Elmer Life Sciences (Boston, MA). Type I collagenase was purchased from Worthington Biochemical (Lakewood, NJ), and EPA was from Cayman Chemical.

Animals. Male Wistar rats (250–280 g of weight) were obtained from the Applied Pharmacobiology Center (CIFA-Spain). The animals were housed in cages in temperature-controlled rooms (22 ± 2°C) with a 12:12-h light-dark cycle. All experimental procedures were performed according to National and Institutional Guidelines for Animal Care and Use and after the approval of the Ethical Committee for Animal Care and Use at the University of Navarra. Animals were euthanized, and epididymal adipose tissue was removed.

Adipocyte isolation and culture. Adipocytes were isolated from epididymal fat depots. Fat was minced with scissors in HEPES buffer (pH 7.4; containing 5 mM D-glucose, 2% BSA, 135 mM NaCl, 2.2 mM CaCl$_2$·2H$_2$O, 1.25 mM MgSO$_4$·7H$_2$O, 0.45 mM KH$_2$PO$_4$, 2.17 mM Na$_2$HPO$_4$, and 10 mM HEPES). Adipose tissue fragments were digested in the same buffer with type I collagenase (1.25 mg/ml per 0.5 g tissue) at 37°C with gentle shaking for 30 min. The resulting cell suspension was diluted in HEPES-phosphate buffer, and the isolated adipocytes were then separated from the undigested tissue by filtration through a 400-μm nylon mesh and washed three times. Isolated adipocytes were then resuspended in DMEM supplemented with 1% FBS and incubated for 40 min at 37°C. The isolated adipocytes (150 μl of 2:1 ratio of packed cells to medium) were then plated on 500 μl of a collagen matrix (Vitrogen 100, Cohesion Technologies) in six-well culture plates. After 50 min of incubation at 37°C, the culture media containing 0 or 1.6 nM insulin and the different concentrations (0, 0.1, 1, 10, 100, 200 μM) of the assayed EPA were added. Cells were maintained in an incubator at 37°C in 5% CO$_2$ for up to 96 h. Aliquots (300 μl) of the media were collected at 24, 48, 72, and 96 h and replaced with fresh medium containing the appropriate concentration of insulin and/or EPA.

Assays. Leptin concentrations in the media samples were determined by a radioimmunoassay for rat leptin (Linco Research, St. Charles, MO), as previously described (26). Glucose and lactate were measured with an Autoanalyzer (Cobas Roche Diagnostic, Basel, Switzerland) (29).

Analyses of mRNA. Leptin mRNA levels were determined by Northern blotting. The leptin cDNA probe was a 388-bp fragment of mouse leptin cDNA, which was kindly provided by Dr. Charles Mobbs (Mount Sinai School of Medicine, New York, NY). The 18S ribosomal probe was obtained from Ambion (Austin, TX). RNA was extracted according to the GIBCO-Life Technologies procedure with the use of Trizol. The ultraviolet absorbance and integrity gels were used to estimate RNA. Leptin and 18S cDNA probes were labeled by random priming (Rediprime kit, Amersham, Buckinghamshire, UK) in the presence of [^{32}P]dCTP (3,000 Ci/mmol, Amersham). We removed unincorporated nucleotides using NucTrap probe purification columns (Stratagene, La Jolla, CA). For each tissue sample, 7 μg of total RNA were fractionated by electrophoresis on a denaturing 1%

agarose gel containing 2.2 M formaldehyde and 1× MOPS running buffer. One microliter of a 50 μg/ml ethidium bromide (GIBCO BRL, Gaithersburg, MD) stock solution was added to check RNA integrity and even loading. After electrophoresis, RNA was transferred to nylon membrane (Duralon-UV, Stratagene) by overnight capillary transfer and ultraviolet cross linked (Stratalinker 1800, Stratagene). Blots were then hybridized for 1 h at 68°C in the presence of the labeled cDNA probe (2 × 10^6 cpm/ml Express Hyb solution, Clontech, Palo Alto, CA, and 1 × 10^6 cpm/ml with the 18S cDNA probe). After blots were washed at high stringency, they were exposed to X-ray films with an intensifying screen at −80°C. To allow loading of equal masses of RNA in each well, after analysis of leptin mRNA using a single-stranded cDNA probe followed by quantification of bands from film, we reanalyzed the blots using a probe complementary to mouse 18S ribosomal RNA. Leptin mRNA was then normalized with respect to the 18S ribosomal signal.

Glucose incorporation into triglyceride. Glucose incorporation into triglyceride was measured after 96 h of culture by counting the ^{14}C-radiolabeled glucose incorporated into the adipocytes (52). Throughout the culture, the adipocytes were incubated in media containing 0.01 μCi/ml of [^{14}C]glucose. After 96 h, the medium was removed, along with any extracellular triglyceride in the wells, and 4 ml of methanol were added to each well. The adipocytes anchored into the collagen and methanol were then transferred into a 50-ml glass tube with screw cap. We washed the well again using an additional 1 ml of methanol, which was also transferred to the tube. Ten milliliters of chloroform were added to each tube to extract the triglyceride as described by Folch et al. (11). Twenty-four hours later, the tube was filled with deionized water to separate the methanol from the chloroform containing the triglyceride. The water-methanol layer was aspirated, and the remaining moisture was absorbed by addition of 4 g of sodium sulfate. One milliliter of the chloroform-triglyceride was pipetted into a scintillation vial, and radioactivity (dpm) was counted. Five milliliters of the chloroform-lipid was pipetted into preweighed aluminum pans. The chloroform was allowed to evaporate, and the pan was weighed again to determine the retained triglyceride (mg).

Glucose oxidation. We measured glucose oxidation using a modification of the method of Rodbell (49) and based on the culture system described by Bottcher and Furst (5). Briefly, cells isolated from rat epididymal adipose tissue were plated in 25-ml glass scintillation vials on 0.3 ml of collagen and 2 ml of medium containing different concentrations of EPA. The vials were loosely covered with a sheet of aluminum foil and incubated in a 37°C incubator (5% CO$_2$). After 48 h, 0.5 ml of medium was sampled from each vial and 0.5 ml of treatment medium, which contained 0.12 μCi/ml of the [^{14}C]glucose, was added. Vials were capped with a rubber stopper that had been fitted with a polystyrene hanging well containing a strip of Whatman filter paper. Vials were gassed with 5% CO$_2$ for 5 s using vent/delivery needles. After 48 h of incubation, we sampled media using a long needle and syringe, and benzethonium hydroxide was placed onto the paper strips and hanging well to capture CO$_2$. Concentrated sulfuric acid was added to the vials to lyse cells and liberate all CO$_2$ from the collagen matrix. After an overnight period, the hanging well and paper were transferred to another vial containing scintillation fluid and counted in a β-counter.

Lipolysis. Lipolysis was assessed by measuring glycerol released into the media at 96 h. We determined glycerol using an Autoanalyzer following the manufacturer's instructions (Cobas Roche Diagnostic).

Data analyses. Glucose utilization was assessed by measuring the concentration of glucose in the medium in each well at 24, 48, 72, and 96 h. Lactate production was calculated as the increase of lactate in the medium at 24, 48, 72, and 96 h. The amount of carbon released as lactate per amount of carbon taken up as glucose over 96 h was calculated as Δ[lactate]/Δ[glucose], where Δ is the difference, and expressed as a percentage. The amount of glucose incorporated to CO$_2$ was calculated as (dpm collected on Whatman 1 strip)(total glucose)/total dpm and expressed as a percentage of total glucose
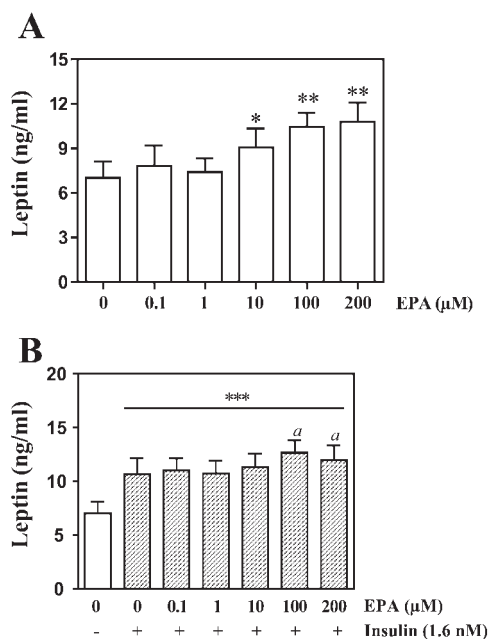
Fig. 1. Effects of different concentrations of eicosapentaenoic acid (EPA) on basal (*A*) and insulin-stimulated (*B*) leptin secretion by isolated rat adipocytes over 96 h in culture. Results (media ± SE) are representative of 10 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control cells; $^aP < 0.05$ vs. insulin alone-treated adipocytes.

utilized. The amount of glucose incorporated into lipid was calculated as [(dpm extracted in 1 ml of chloroform)(total glucose)/total dpm] × 10 ml of chloroform. This value was normalized over the amount of total lipid recovered from the well and expressed as a percentage of total glucose utilized.

Because of the high variability in basal leptin secretion between adipocyte cultures from different rats, the experimental results from each adipocyte suspension prepared from a single animal were analyzed in relation to a control well from the same suspension. For this reason, the statistical analysis of the data was performed by a repeated-measures ANOVA followed by a Dunnet's posttest or by a paired Student's *t*-test (GraphPad Prism, GraphPad Software, San Diego, CA). Results were also analyzed and expressed as the percentage of the control. Differences were considered as statistically significant at $P < 0.05$. The animals used for the glucose incorporation into lipid and glucose oxidation analysis were different from the ones used in the rest of the experiments.

RESULTS

Effects of EPA on leptin expression and secretion. Basal leptin secretion over 96 h was increased by incubation of adipocytes with EPA at 10, 100, and 200 μM compared with control (+43.8 ± 21.0%, $P < 0.05$; +71.1 ± 24.5%, $P < 0.01$; and +73.7 ± 25.6%, $P < 0.01$ for 10, 100, and 200 μM,

respectively) (Fig. 1*A*). An increase in leptin secretion was observed from 48 h of treatment (data not shown). As expected, 1.6 nM insulin induced a highly significant increase of leptin secretion (+55.6 ± 9.3%, $P < 0.001$). The stimulatory effect of EPA on insulin-stimulated leptin secretion was present but less pronounced than that observed in the absence of insulin (+26.7 ± 7.9% for 100 μM, $P < 0.05$; and +18 ± 9.1% for 200 μM, $P < 0.05$) (Fig. 1*B*).

The patterns of EPA's effects on leptin expression at 96 h of treatment were similar to those observed for leptin secretion. Basal leptin mRNA expression was increased during incubation with EPA at 100 and 200 μM, and insulin-stimulated leptin expression was increased by EPA at the 200 μM concentration (Fig. 2).
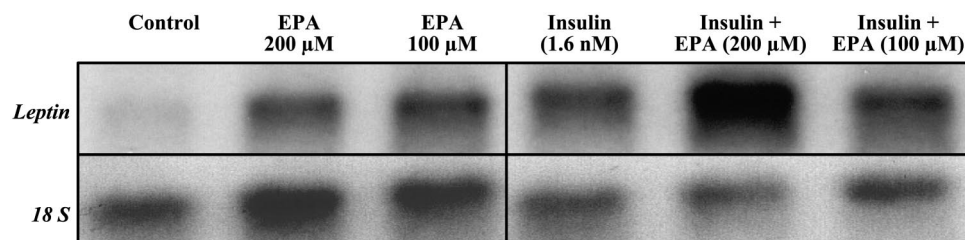
Effects of EPA on glucose utilization. EPA increased basal glucose utilization, an effect that was observed from the first 24 h of treatment (data not shown). The increase over 96 h (Fig. 3*A*) was statistically significant at the highest concentration tested, 200 μM (+50 ± 15.7%, $P < 0.05$), and was strongly related to the increase observed in leptin secretion at 96 h ($r = +0.89$, $P < 0.01$) (Fig. 3*B*). As expected, insulin (1.6 nM) significantly increased glucose uptake (+179.7 ± 52.1%, $P < 0.001$). However, EPA did not further increase insulin-stimulated glucose utilization, and the EPA-induced increase in insulin-stimulated leptin secretion was not related to glucose uptake ($r = -0.12$, not significant).

Effects of EPA on lactate production. Basal lactate production by adipocytes incubated with EPA at 100 and 200 μM was significantly decreased at 96 h (−25.9 ± 6.4%, $P < 0.05$; −36.8 ± 10.6%, $P < 0.01$, respectively). Insulin (1.6 nM) also decreased lactate production, and the cotreatment with EPA with insulin at the two highest concentrations, 100 and 200 μM, produced a further decrease in lactate production (Fig. 4*A*). The proportion of glucose carbon metabolized to and released as lactate over 96 h was decreased by EPA at the highest concentration (200 μM) in both the presence and absence of insulin (Fig. 4*B*). This effect of EPA on the anaerobic utilization of glucose was observed from 48 h of treatment (data not shown). Furthermore, as shown in Fig. 4, *C* and *D*, both basal ($r = -0.93$, $P < 0.001$) and insulin-stimulated ($r = -0.84$, $P < 0.01$) increases of leptin secretion were inversely related to lactate production.

Effects of EPA on glucose oxidation. EPA (200 μM) and insulin (1.6 nM) by themselves significantly increased ($P < 0.05$) the percentage of glucose oxidized to CO_2. This increase induced by EPA on glucose oxidation was not observed in the presence of insulin (Fig. 5).

Effects of EPA on lipogenesis. The amount of glucose incorporated into triglyceride was decreased by 200 μM EPA (−28.0 ± 5.9%, $P < 0.01$). As expected, insulin significantly

Fig. 2. Effects of different concentrations of EPA on basal and insulin-stimulated leptin mRNA expression, as assessed by Northern blots. Expression level of 18S ribosomal RNA was determined and used as an internal control to correct for minor variations in total RNA amount. Results are representative of at least 2 independent experiments.
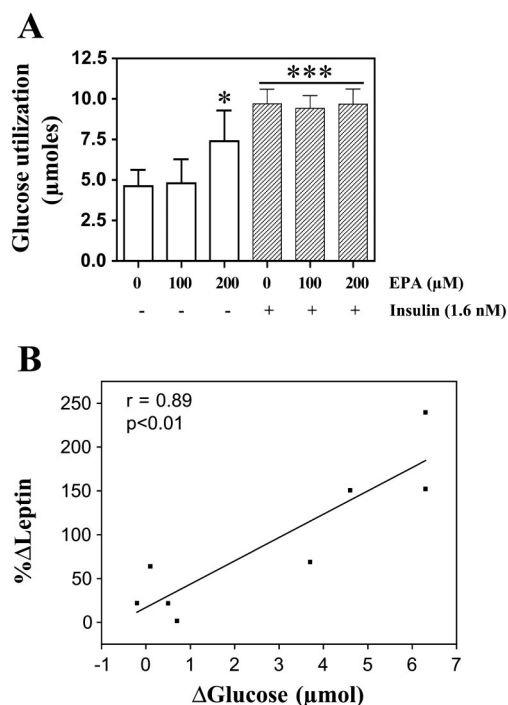
Fig. 3. *A*: effects of EPA on basal and insulin-stimulated glucose utilization by isolated rat adipocytes over 96 h in culture. Data are means ± SE of 8 independent experiments. *B*: relationship between change in basal glucose utilization and percent change of leptin production induced by 200 µM EPA compared with control over 96 h of culture. *$P < 0.05$, ***$P < 0.001$ vs. control (non-EPA- or non-insulin-treated cells).

increased glucose incorporation into triglyceride ($+467.7 \pm 79.8\%$, $P < 0.01$); however, the proportion (percentage) of glucose incorporated into triglyceride of the total 96-h glucose utilization was not increased by insulin (Table 1). Treatment

with 200 µM EPA in the presence of insulin significantly decreased the amount of glucose incorporated into triglyceride ($-50.8 \pm 8.0\%$, $P < 0.01$). In addition, the percentage of glucose utilized that was incorporated into triglyceride was significantly decreased by 200 µM EPA in the absence but not in the presence of insulin (Table 1).

Effects of EPA on lipolysis. The effects of EPA on basal lipolysis were evaluated by determining the amount of glycerol released into the media over 96 h of culture. EPA (200 µM) did not significantly modify glycerol release.

DISCUSSION

Controversial results (inhibition or stimulation) regarding the in vitro effects of EPA on leptin gene expression and secretion have been previously reported (37, 47). In the present study, we demonstrated a direct concentration-dependent effect of EPA to increase basal leptin secretion and gene expression in cultured primary rat adipocytes. These results are in agreement with the report by Murata et al. (37) in which EPA induced a time- and dose-dependent increase of leptin mRNA expression and leptin secretion in 3T3-L1 cells. Several in vivo studies in rats and mice have reported that prolonged intake of diets high in n-3 PUFAs resulted in significant decreases in plasma leptin (22, 47). However, the direct effects of EPA to increase leptin expression and secretion suggest that the decrease of circulating leptin concentrations reported in animals consuming n-3 fatty acids are likely to be secondary to decreases observed in white fat adipose mass.

EPA also modestly increased leptin secretion and expression in the presence of insulin. However, Cammisotto et al. (8) reported an inhibitory effect of EPA on insulin-stimulated leptin secretion in white adipocytes. The disparity may be related to differences in the duration of the cultures (2 h vs. 96 h), the type of culture system employed (free floating vs.
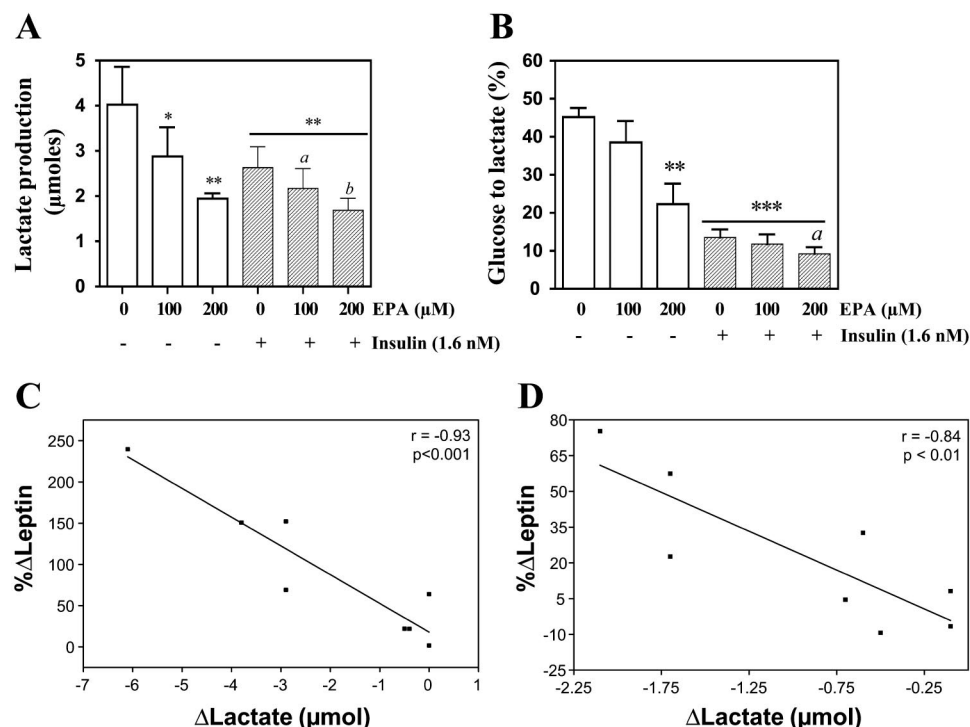

Fig. 4. Effects of EPA on lactate production (*A*) and percentage of glucose carbon released as lactate (*B*) in absence and presence of 1.6 nM insulin by adipocytes over 96 h in culture. Data are means ± SE of 8 independent experiments. Also shown are the relationships between change in lactate production and percent change of leptin production induced by 200 µM EPA compared with control over 96 h of culture in absence (*C*) and presence (D) of 1.6 nM insulin. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control. $^{a}P < 0.05$ and $^{b}P < 0.01$ vs. insulin alone treated cells.
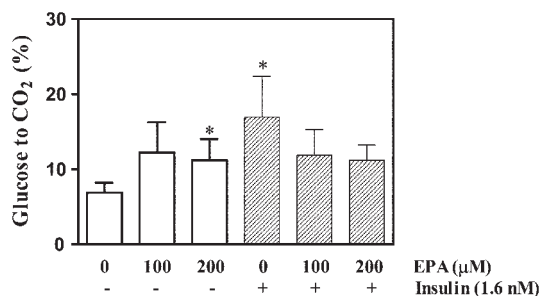
Fig. 5. Effects of EPA on glucose oxidation as determined by the proportion of ^{14}C-labeled glucose metabolized to CO_2. Data are means $\pm$ SE of 4 independent experiments. Results were determined in cultured cells obtained from different animals than used for the other parameters. *$P < 0.05$ vs. control cells (non-EPA- or non-insulin-treated cells).

collagen fixed) (34), the concentration of insulin (10 nM vs. 1.6 nM), and the concentrations of EPA (1 mM vs. 200 μM). As reported by Murata et al. (37), 100 μM EPA is comparable to its plasma concentrations after intake of dietary EPA. These authors also demonstrated that 1 mM EPA was less potent than 100 μM EPA in its effect to increase basal leptin secretion and expression in 3T3-L1 cells (37).

The results of the present study suggest that there are similarities in the effects of EPA and insulin to stimulate leptin expression and secretion. First, EPA is more effective in increasing basal leptin secretion than it is in the presence of insulin, suggesting that insulin has already activated some of the signaling pathways activated by EPA. Furthermore, both insulin and EPA increase glucose utilization, decrease anaerobic metabolism of glucose to lactate, and increase glucose oxidation. Finally, the actions of both insulin and EPA to increase leptin production are highly correlated with their effects to decrease anaerobic glucose metabolism to lactate [present results and Mueller et al. (34)]. EPA has been shown to activate intracellular insulin signaling pathways in hepatoma cells (36). EPA treatment for 24 h stimulated tyrosine phosphorylation of insulin receptor substrate-1, binding of phosphatidylinositol 3-kinase to insulin receptor substrate-1, and Akt kinase activity and downregulated the amount of phosphoenolpyruvate carboxykinase mRNA expression. These insulin-like actions (36) suggest that EPA and insulin might share and compete for a common intracellular pathway.

We have demonstrated (31, 34) along with others (57) that insulin-stimulated glucose metabolism rather than insulin per se is a major determinant of leptin production in both rodents and humans. In fact, leptin secretion by cultured adipocytes is more sensitive to the amount of glucose utilized during the culture period than to the extracellular insulin concentration. A

previous study has demonstrated that dietary fish oil increased GLUT4 (insulin-dependent glucose transport) gene expression levels in skeletal muscle (32). In the present study, we observed that 200 μM EPA induced an increase in adipocyte basal glucose uptake and that there was a strong relationship between basal glucose utilization and leptin production in 200 μM EPA-treated cells. These results suggest that increased glucose uptake could contribute to the actions of EPA to stimulates leptin production. However, the EPA-induced increases of leptin secretion were not related to changes of glucose uptake in the presence of insulin, suggesting that glucose uptake per se is not the main determinant of the effects of EPA. Our previous report (30) showed that glucose transport is not a regulatory step in leptin production by adipocytes. Rather, glucose transport is necessary for glucose to be further metabolized. Inhibitors of glucose metabolism inhibit insulin-stimulated leptin secretion in a concentration-dependent manner (34). In addition, the β_3-adrenergic agonist Trecadrine, which stimulates adipocyte glucose uptake, inhibits leptin secretion (30). Thus we have also reported that the metabolism of glucose beyond pyruvate is involved in the action of glucose and insulin to stimulate leptin secretion (35). Mueller et al. (35) showed that leptin secretion is inversely related to the proportion of glucose that is metabolized to lactate. In fact, metformin, an antidiabetic drug that stimulates adipocyte glucose uptake, appears to inhibit leptin secretion by increasing the anaerobic metabolism of glucose to lactate (35). Our data showed that EPA decreases lactate production by isolated adipocytes and therefore the percentage of glucose carbon released as lactate, suggesting that the increase in basal leptin production by EPA is mediated, at least in part, by a decrease in anaerobic glucose metabolism.

Compared with the basal state, the effects of EPA on insulin-stimulated leptin secretion and expression and inhibitory effects on lactate production were more modest. A possible explanation for the lack of synergetic or additive effects of EPA in the presence of insulin is that adipocyte glucose metabolism was already markedly increased by insulin; thus EPA was only able to induce smaller additional effects on adipocyte metabolism and leptin production. However, although the effects of cotreatment with insulin and EPA on lactate production were lower than in the absence of insulin, they were still significant. Indeed, the increase on insulin-stimulated leptin secretion by 200 μM EPA was strongly related to the measured decrease of lactate production, further supporting the hypothesis that the decreased anaerobic metabolism of glucose to lactate is a major contributor to the actions of EPA to increase leptin secretion.

Table 1. *Effects of EPA on lipid metabolism in absence and presence of 1.6 nM insulin*

	0 μM EPA	200 μM EPA	Insulin (1.6 nM) 0 μM EPA	200 μM EPA
Lipogenesis,* nmol Glu/mg TG ($n = 6$)	51.3±3.3	37.3±4.7‡	281.3±31.6‡	136.5±22.5†§
Glucose to TG,* % ($n = 6$)	32.4±3.1	18.4±3.5†	30.3±3.8	26.6±1.4
Glycerol, mmol ($n = 8$)	1,107.0±182.9	1,331.0±288.5		

Values are means $\pm$ SE; n = no. of experiments. EPA, eicosapentaenoic acid. Lipogenesis [assessed as amount and percentage of glucose (Glu) carbon incorporated into triglyceride (TG)] and lipolysis (assessed as the amount of glycerol released to the media) were analyzed over 96 h in culture. *Determined in cultured cells obtained from different animals than used for the other parameters. †$P < 0.05$, ‡$P < 0.01$ vs. corresponding control. §$P < 0.01$ vs. insulin-treated cells.

Furthermore, our previous studies (18, 35) also suggest that glucose utilization stimulates leptin production by directing the metabolism of glucose to a fate other than anaerobic lactate production, most likely to oxidation. In the present study, 200 μM EPA increased the percentage of glucose that was oxidized to CO_2. These data suggest that the stimulatory effects of EPA and insulin on basal leptin secretion and gene expression may also involve an increase of glucose oxidation.

Finally, it has been suggested that n-3 PUFAs limit fat cell hypertrophy in adipose tissue by decreasing lipogenesis and increasing lipolysis by induction of hormone sensitive lipase expression (43, 45). Our results indicate that EPA decreased lipogenesis and induced a slight (nonsignificant) rise on basal lipolysis. It is possible that these effects of EPA could be secondary to the increase in leptin production, since leptin is known to directly inhibit lipogenesis (46) and to stimulate lipolysis in white adipose tissue (50).

In conclusion, we have demonstrated that EPA, like insulin, stimulates leptin gene expression and secretion, and this effect is strongly related to a decrease in the anaerobic conversion of glucose to lactate. An increase in basal glucose uptake and oxidation may also contribute to the effects of EPA on basal leptin production. In addition, this increase in leptin production may be responsible for the changes observed in lipogenesis. Because, like EPA, leptin has multiple beneficial effects (both systemic and local) on energy homeostasis and glucose (12, 16, 21, 41, 55), it is possible that some of described actions of EPA to limit obesity and improve insulin sensitivity could be mediated indirectly via increased leptin production. Further experiments are needed to better understand the molecular mechanism involved in EPA's action on adipose tissue and their relationship with insulin signaling and the endocrine function of adipose tissue.

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