Adipose tissue compensates for defect of phosphatidylinositol 3′-kinase induced in liver and muscle by dietary fish oil in fed rats

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Submitted 5 May 2005; accepted in final form 14 July 2005

Am J Physiol Endocrinol Metab 290: E78–E86, 2006; doi:10.1152/ajpendo.00200.2005.—The present work aimed to study in rats whether substitution of a low level of fish oil (FO; 2.2% of calories) into a low-fat diet (6.6% of calories from fat as peanut-rape oil or control diet) 1) has a tissue-specific effect on insulin signaling pathway and 2) prevents dexamethasone-induced alteration of insulin signaling in liver, muscle, and adipose tissue. Sixteen rats were used for study of insulin signaling, and sixteen rats received an oral glucose load (3 g/kg). Eight rats/group consumed control diet or diet containing FO over 5 wk. Four rats from each group received a daily intraperitoneal injection of saline or dexamethasone (1 mg·kg−1·day−1) for the last 5 days of feeding. In liver, FO decreased phosphatidylinositol 3′-kinase (PI 3′-kinase) activity by 54% compared with control diet. A similar result was obtained in muscle. In both liver and muscle, FO clearly amplified the effect of dexamethasone. FO did not alter early steps of insulin signaling, and in muscle GLUT4 protein content remained unaltered. In adipose tissue, FO increased PI 3′-kinase activity by 74%, whereas dexamethasone decreased it by 65%; inhibition of PI 3′-kinase activity by dexamethasone was similar in rats fed FO or control diet, and GLUT4 protein content was increased by 61% by FO. Glycemic and insulinemic responses to oral glucose were not modified by FO. In conclusion, FO increased PI 3′-kinase activity in adipose tissue while inhibiting it in liver and muscle. The maintenance of whole body glucose homeostasis suggests an important role of adipose tissue for control of glucose homeostasis.

INeicosapentaenoic acid; docosahexaenoic acid; insulin resistance; polyunsaturated fatty acids

At first glance, the title suggests the study focuses on the role of fish oil in compensating for defects in phosphatidylinositol 3′-kinase activity in liver and muscle, specifically in fed rats. The authors present their findings on how dietary fish oil (FO) affects insulin signaling in liver, muscle, and adipose tissue. They report that FO increases PI 3′-kinase activity in adipose tissue, while decreasing it in liver and muscle. The study also indicates that FO partially prevents the effects of dexamethasone on insulin signaling. This suggests the importance of adipose tissue in maintaining whole body glucose homeostasis.

The authors conclude that FO can be an interesting model of induced insulin resistance to study the effects of a low level of LC-n-3 PUFA without altering other dietary parameters which could interfere with insulin sensitivity. Further studies are needed to confirm these findings and to understand the mechanism behind the effects of FO on insulin signaling.
ad libitum with a standard chow diet (59% of calories from carbohydrates, 7% from fat, 21% from protein, 13% from minerals and ash). Sixteen rats were killed in the fed state and not after insulin injection, in order to study insulin signaling in liver, muscle, and adipose tissue in a true physiological state. Sixteen additional rats were used for study of glycemic and insulimetic responses to an oral glucose load.

For each group, rats were randomly divided into two groups (n = 8/group) and placed on either a normolipidic diet containing n-6 PUFA (control diet) or LC-n-3 PUFA (n-3 diet) with water ad libitum. Diets were prepared by Atelier de Préparation Aliments Expérimentaux (APAE, INRA, Jouy-en-Josas, France). The control diet contained 6.6% of calories from fat as peanut-rape oil, 22% from casein, and 7% from fat, 21% from protein, 13% from minerals and ash). Diets were prepared by Atelier de Préparation Aliments Expérimentaux (APAE, INRA, Jouy-en-Josas, France). The control diet contained 6.6% of calories from fat as peanut-rape oil, 22% from casein, and 7% from fat, 21% from protein, 13% from minerals and ash).

Oral glucose tolerance test. After an overnight fast, a local anesthetization with lidocaine (EMLA 5% cream; Astrazeneca, Rueil-Malmaison, France) was performed on the tail, a vein blood sample was taken first, and then a glucose solution (3 g/kg body wt) was injected intraperitoneally (BPX, 70.60 m long, 0.25 mm ID, 0.25 μm film thickness, SGE Analytical Science, Courtaboeuf, France) programmed from 55°C (for 2 min) to 150°C at 20°C/min. Fatty acid methyl esters were identified by comparison of their equivalent chain lengths with those of authentic standards, and quantification was done using marginic acid (17:0) as internal standard (4, 20).

IR density and affinity for insulin in liver membranes. Insulin binding to crude liver membranes was measured using 125I-labeled porcine insulin at 0.1 ng/ml, as previously described (36). Tracer binding was inhibited by increasing concentrations of unlabeled monoclonal porcine insulin (4–40,000 ng/ml). After centrifugation, pellets were washed with 0.2 ml of chilled sucrose (0.25 M), and incorporated radioactivity was counted. Nonspecific binding was determined in the presence of an excess of cold insulin (13.33 μg/ml) and was found to be 2–7% of total binding. The competition curves were analyzed using Cricket Graph software (version 4.00).

Immunoprecipitation and Western blotting procedures. Powdered tissues (1 g) were solubilized in ice-cold buffer A (150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% Igepal, 2 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 100 mM sodium fluoride, 10 nM sodium pyrophosphate, and 2 mM sodium orthovanadate). Tyrosine-phosphorylated proteins or IRS-1 were immunoprecipitated with specific antibodies (Upstate Biotechnology, Lake Placid, NY). Lysates or immunoprecipitates were separated by SDS-PAGE, and immunoblotting was performed using antibodies directed against either IR or the p85α subunit of PI3-kinase (Santa Cruz Biotechnology, Santa Cruz, CA), IRS-1 (Upstate Biotechnology), or GLUT4 (Chemicon International). Blots were revealed by Immun-star AP detection kit (Bio-Rad, Hercules, CA). Band intensities were quantified by optical densitometry (BioID scanning software; Vilber Lourmat, France).

Table 1. Animal characteristics during the 5-day period of dexamethasone treatment

<table>
<thead>
<tr>
<th>Diet and Parameters</th>
<th>Control</th>
<th>n-3</th>
<th>Control + Dexa</th>
<th>n-3 + Dexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>432±4</td>
<td>424±9</td>
<td>362±4*</td>
<td>369±4*</td>
</tr>
<tr>
<td>Weight gain, g/day</td>
<td>14±1</td>
<td>13±1</td>
<td>−44±2*</td>
<td>−37±3*</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>20±1</td>
<td>21±2</td>
<td>9±2*</td>
<td>11±2*</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>9.6±0.2</td>
<td>10.1±0.8</td>
<td>19.3±4.3*</td>
<td>20±0.4±2.2*</td>
</tr>
<tr>
<td>Insulin, nM</td>
<td>0.71±0.15</td>
<td>0.64±0.02</td>
<td>3.14±0.22*</td>
<td>4.76±0.72*</td>
</tr>
<tr>
<td>NEFA, nM</td>
<td>0.45±0.02</td>
<td>0.40±0.03</td>
<td>1.35±0.21*</td>
<td>1.00±0.09*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4 rats. NEFA, nonesterified fatty acids. *P < 0.05 vs. rats fed the same diet not treated with dexamethasone (Dexa).
Effect of n-3 diet on glycemic and insulineic responses to OGGT. Area under the curve (AUC) of glycemic and insulineic responses to oral glucose were higher in rats treated with dexamethasone ($P < 0.05$; Fig. 1). The n-3 diet did not alter these effects of dexamethasone (Fig. 1).

Effect of n-3 diet on fatty acids content of membrane phospholipids of muscle, liver, and adipose tissue. Fatty acid content of membrane phospholipids of liver, muscle, and adipose tissue is shown in Fig. 2. The proportion of LC-n-3 PUFA into phospholipids of the three tissues was higher in rats fed the n-3 diet than in control rats, demonstrating their incorporation into membranes.

Effect of n-3 diet on IR, IRS-1, and PI 3’-kinase in liver. The n-3 diet affected neither IR membrane density nor affinity (as estimated by EC$_{50}$; control $= 12.78 \pm 1.69$ ng/ml; n-3 $= 15.29 \pm 1.03$ ng/ml) of insulin binding in liver. In contrast, dexamethasone treatment of both control and FO rats significantly reduced IR membrane density (−26%) and affinity as estimated by EC$_{50}$ (control + Dexa $= 22.54 \pm 4.36$ ng/ml; n-3 + Dexa $= 30.02 \pm 4.95$ ng/ml). IR and IRS-1 protein contents were unchanged whatever the diet and treatment (Fig. 3, A and C). In dexamethasone-treated rats, the n-3 diet induced an 89% increase in IR tyrosine phosphorylation ($P < 0.05$; Fig. 3B) and a 46% increase in IRS-1 tyrosine phosphorylation ($P < 0.05$; Fig. 3D), whereas n-3 alone or dexamethasone alone had no effect. p85α protein content and p85α association with IRS-1 remained unchanged in liver whatever the diet and treatment (Fig. 3, E and F). The n-3 diet inhibited
PI 3′-kinase activity by 54% ($P < 0.05$) (Fig. 3G) and dexamethasone alone inhibited it by 75% ($P < 0.05$). N-3 diet amplified dexamethasone-induced inhibition of PI 3′-kinase activity from 75 to 91% ($P < 0.05$; Fig. 3G).

Effect of n-3 diet on IR, IRS-1, PI 3′-kinase, and GLUT4 in muscle. In muscle, neither IR membrane density nor affinity toward insulin can be estimated using crude membranes due to the highly nonspecific binding of labeled insulin (1, 35). IR and IRS-1 protein contents were unchanged whatever the diet and treatment (Fig. 4, A and C). In dexamethasone-treated rats, n-3 diet increased IR tyrosine phosphorylation ($P < 0.05$; Fig. 4B) but had no effect on IRS-1 tyrosine phosphorylation (Fig. 4D). Dexamethasone alone or n-3 alone had no effect either on IR or on IRS-1 tyrosine phosphorylation (Fig. 4, B and D). p85α protein content and p85α association with IRS-1 remained unchanged in muscle whatever the diet and treatment (Fig. 4, E and F). The n-3 diet inhibited PI 3′-kinase activity by 54% ($P < 0.05$; Fig. 4G); dexamethasone inhibited it by 82% ($P < 0.05$). The n-3 diet amplified dexamethasone-induced inhibition of PI 3′-kinase activity from 82 to 91% ($P < 0.05$; Fig. 4G). GLUT4 content remained unchanged whatever the diet and treatment (Fig. 5A).
Effect of n-3 diet on IR, IRS-1, PI 3'-kinase, and GLUT4 in adipose tissue. In adipose tissue, IR protein content was unchanged whatever the diet and treatment (Fig. 6A). The n-3 diet increased IR tyrosine phosphorylation by 49% (not significant; Fig. 6B). Although the n-3 diet increased IRS-1 protein content by 70% (P < 0.05; Fig. 6C), IRS-1 tyrosine phosphorylation remained unchanged (Fig. 6D). Dexamethasone increased IR tyrosine phosphorylation with both control (+137%) and n-3 (+120%) diets (both P < 0.05; Fig. 6B) and had no effect on IRS-1 tyrosine phosphorylation (Fig. 6D). p85α protein content was not modified whatever the diet and treatment (Fig. 6E). In contrast to liver and muscle, the n-3 diet increased p85α association with IRS-1 by 71% (P < 0.05; Fig. 6F) and increased PI 3'-kinase activity by 74% (P < 0.05; Fig. 6G). In dexamethasone-treated rats, the n-3 diet increased p85α association with IRS-1 by 49% (P < 0.05). Dexamethasone inhibited PI 3'-kinase activity by 65% (P < 0.05). The n-3 diet did not amplify the dexamethasone-induced inhibition of PI 3'-kinase activity (Fig. 6G). GLUT4 content was increased by 61% with the n-3 diet (P < 0.05) and was unaffected by dexamethasone (Fig. 5B).

**DISCUSSION**

The present study aimed to determine the impact of a very low level of LC-n-3 PUFA (0.42% of calories) on insulin
signaling in fed rats. We deliberately chose to study rats in the fed state instead of after insulin injection (the usual way to activate the insulin-signaling pathway in vivo) to be able to evaluate the effect of LC-n-3 PUFA in a true physiological state and to avoid the use of high insulin doses that may not discriminate between insulin and IGF-I receptors. It was clearly demonstrated by Ito et al. (15) that, in the fed state, with exactly the same insulinemia as in the present study, insulin signaling was markedly activated from IR to PI 3'-kinase.

Moreover, GLUT4 translocation and transport are activated in rat muscles after an oral glucose load, demonstrating also the effect of a physiological state on this downstream part of insulin signaling (22). Thus, for these reasons, we can reasonably take for granted that the insulin-signaling pathway was activated solely by the conditions of the fed state in tissues from our rats.

Our study suggests an important contribution of adipose tissue to whole body insulin sensitivity. We demonstrate that LC-n-3 PUFA (0.42% of calories) given as FO (2.2% of calories) increased PI 3'-kinase activity in adipose tissue, whereas it markedly and specifically decreased it in liver and muscle. Furthermore, FO increased GLUT4 content in adipose tissue alone. Plasma glucose and insulin responses to OGTT, a method used to estimate glucose tolerance and whole body insulin sensitivity, remained unaffected with regard to the LC-n-3 PUFA-induced inhibition of PI 3'-kinase activity in liver and muscle. This contrasts with the marked deterioration of glucose tolerance and insulin sensitivity induced by dекса-

Fig. 5. GLUT4 protein content in muscle (A) and adipose tissue (B). Lysates (100 μg) were electrophoresed onto 10% SDS polyacrylamide gel and blotted with anti-GLUT4. Immunoblots are representative of 4 experiments; n = 4 rats. *P < 0.05 vs. control.

Fig. 6. IR, IRS-1, and p85α protein contents (A, C, E), IR and IRS-1 tyrosine phosphorylation (B and D), p85α association with IRS-1 (F), and PI3K activity (G) in adipose tissue. To quantify protein contents, lysates (100 μg) were electrophoresed onto 10% SDS polyacrylamide gel and blotted with anti-IR to identify the β-subunit of IR (A), with anti-IRS-1 (C) or with anti-p85α (E). For tyrosine phosphorylation, lysates (1 mg) were immunoprecipitated with anti-PY20 and then blotted with anti-IR (B) or anti-IRS-1 (D). To identify p85α associated with IRS-1 (F), lysates (1 mg) were immunoprecipitated with anti-IRS-1 and then blotted with anti-p85α. Results were quantified by densitometry. Blots are representative of 4 experiments. G: PI3K activity was assayed in PY20 immunoprecipitates by quantification of 32P incorporated into PI(3)P. Results are expressed as means ± SE; n = 4 rats. *P < 0.05 vs. control.
methasone, which depressed PI 3′-kinase activity not only in liver and muscle but also in adipose tissue. This difference between LC-n-3 PUFA and dexamethasone effects strongly suggests that, in rats consuming 2.2% of calories from fat as FO, the enhancement of PI 3′-kinase activity and GLUT4 content in adipose tissue was able to compensate for the impairment of insulin signaling in liver and muscle.

It is quite probable that the increase in PI 3′-kinase activity and GLUT4 content in adipose tissue was associated with an increase in glucose transport in this tissue. Indeed, Shepherd et al. (29) showed that GLUT4 overexpression in adipose tissue increased glucose transport in this tissue. Moreover, Rizkalla et al. (26) demonstrated that dietary FO (30% of calories) led to a marked increase in insulin-stimulated glucose utilization in adipose tissue. Whether an increase in GLUT4 solely in adipose tissue is able to compensate for whole body insulin resistance has also been demonstrated by Tozzo et al. (38), who showed that overexpression of GLUT4 in adipose tissue ameliorated insulin resistance in rats treated with streptozotocin. Conversely to adipose tissue, a decrease in muscle glucose transport is very likely in rats fed an n-3 diet and/or treated by dexamethasone. Indeed, a specific inhibition of muscle PI 3′-kinase by wortmannin or LY-294002 (30) has been demonstrated to lead to a severe impairment of glucose transport. Thus, in rats fed the n-3 diet, the lack of alteration of glucose tolerance and insulin response with regard to the marked inhibition of PI 3′-kinase in liver and muscle can be explained by a compensatory increase in glucose transport in adipose tissue due to both increases in PI 3′-kinase activity and GLUT4 content. This highlights the important contribution of adipose tissue to whole body glucose metabolism.

In adipose tissue, the increase in PI 3′-kinase activity can be explained by its increased association with IRS-1. The basic mechanisms of this increased association remain unclear. It was not explained by an increase in IRS-1 tyrosine phosphorylation or in p85α subunit protein content. The alteration of compartmentalization of IRS-1 and PI 3′-kinase by LC-n-3 PUFA may have played a role. Indeed, intracellular compartments have been demonstrated to play an important role in the generation of downstream insulin-signaling events in liver (2) and in adipocytes (14, 41). In a murine adipocyte cell line (3T3-L1), insulin redistributes mainly p85 to intracellular low-density microsome membranes to stimulate GLUT4 translocation (41). In the present study, LC-n-3 PUFA could have altered p85α trafficking and/or its association with IRS-1. The lack of aggravation of dexamethasone-induced inhibition of PI 3′-kinase activity in adipose tissue, which contrasts with what was observed in liver and muscle, could be explained by a resultant opposite effect of dexamethasone and LC-n-3 PUFA on PI 3′-kinase activity, the effect of dexamethasone remaining predominant.

In liver, LC-n-3 PUFA reduced PI 3′-kinase activity without significant changes in upstream signaling. This strongly suggests that the inhibitory effect of LC-n-3 PUFA on PI 3′-kinase activity exerted at the level of its catalytic subunit. We could not exclude the possibility that the p50 or p55 subunit of PI 3′-kinase is modulated by n-3 PUFA and could thus affect p85α/p110 activity in a tissue-specific manner (16). Finally, whether the p85α subunit redistributes between endosomal membranes and plasma membranes as in liver (2) and adipocytes (14, 41), LC-n-3 PUFA may have interfered with such a process.

In muscle, LC-n-3 PUFA also inhibited PI 3′-kinase activity without alteration of upstream insulin signaling and amplified dexamethasone-induced inhibition of PI 3′-kinase activity, whereas GLUT4 content was affected neither by dexamethasone treatment as previously reported (12, 23) nor by LC-n-3 PUFA. This does not exclude that GLUT4 content into plasma membranes was decreased secondarily to decreased translocation of GLUT4, itself being the consequence of decreased PI 3′-kinase activity. Indeed, it has been clearly shown that specific inhibition of muscle PI 3′-kinase by wortmannin or LY-294002 inhibited both GLUT4 translocation and then glucose transport.

The 4-wk consumption of an amount of FO of 2.2% of energy intake was sufficient to induce a marked increase in incorporation of 20:5 n-3 and 22:6 n-3 into phospholipids of liver, muscle, and adipose tissue crude membranes. It is of interest to note that incorporation of LC-n-3 PUFA into membranes was very similar in liver and muscle, whereas inhibition of PI 3′-kinase activity was identical. We can speculate that the alteration of lipid composition of membranes induced by the n-3 diet altered the redistribution of the p85α subunit of PI 3′-kinase to the plasma membrane (2) and/or the catalytic activity of the p110 subunit or its association with the p85α subunit through conformational changes or lipid-protein interaction. Recently, it has been shown that ether-linked phospholipids composing phosphatidylcholine stimulated PI 3′-kinase activity in murine pre-B lymphocytes (19). Even though the effect was observed in pre-B lymphocytes and PI 3′-kinase was stimulated, these data reinforce the hypothesis of the possible contribution of the alteration of phospholipid composition by n-3 diet to the inhibition of PI 3′-kinase in liver and muscle in the present study.

The 5-day dexamethasone treatment induced both a marked weight loss and a decrease in energy intake as previously reported (28). Dexamethasone also induced a higher glycemic and insulinemic response to the OGTT, consistent with the development of insulin resistance (28). FO did not prevent these alterations. This contrasts with the observation of a preventive effect of highly saturated or n-6 PUFA diet-induced insulin resistance by a high level of FO (20% of calories; for a review, see Ref. 9). This different effect could be due to the fact that the very low levels of FO given to rats (2.2% of calories) in the present study inhibited per se PI 3′-kinase activity in liver and muscle, whereas a high level of FO (20% of calories) prevented the decrease in PI 3′-kinase activity and GLUT4 content in muscle of rats fed a high n-6 PUFA diet (35). Holness et al. (13) observed that a low level (7% of calories) of dietary FO substituted into a high-fat diet (20% of calories from saturated fat) induced liver insulin resistance while preventing muscle insulin resistance (13), also demonstrating a tissue-specific effect of LC-n-3 PUFA. It is also possible that dexamethasone had a too-potent effect to be alleviated by a low dose of LC-n-3 PUFA.

The present data also bring some insight about effects of LC-n-3 PUFA in patients with type 2 diabetes. In such patients, dietary FO supplementation, given at a very similar level as in the present study on a body weight basis, ameliorates neither insulin resistance nor glycemic control (21, 24, 25). Luo et al. (18) showed that 2 mo of moderate FO intake (1.8 g/day
EPA + DHA) did not restore the low level of GLUT4 mRNA in adipose tissue. Thus the lack of effect of LC-n-3 PUFA toward insulin resistance in patients with type 2 diabetes might be explained by their inability to reverse the defect of insulin signaling in adipose tissue and muscle, as shown in rats treated by dexamethasone in the present study.

In conclusion, the present study demonstrates that, in a standard low-fat diet (6.6% of calories from fat), LC-n-3 PUFA given at a very low level (0.42% of calories) exerts a tissue-specific regulation on PI 3’-kinase activity, e.g., a potent inhibitory effect in liver and muscle contrasting with a potent stimulatory effect in adipose tissue in normo-insulin-sensitive rats. The maintenance of whole body glucose homeostasis despite profound inhibition of PI 3’-kinase activity in liver and muscle suggests an important role of adipose tissue for control of glucose homeostasis. Such a low dose of LC-n-3 PUFA was unable to prevent whole body insulin resistance induced by dexamethasone, which can be explained by its inability to prevent the decrease in PI 3’-kinase activity at once in liver, muscle, and adipose tissue.

ACKNOWLEDGMENTS

Technical help from Nicole Hourmant was deeply appreciated. We thank Danièle Lucas and Marie-Pierre Moineau for the assays of glucose and insulin. We thank Prof. Pascal Ferré (INSERM U465) for helpful discussion of our results.

GRANTS

This work was supported by a grant from Région Bretagne and Région Pays de la Loire.

REFERENCES


