activity in the coronary arteries, in the peripheral branch in particular. It may be associated with the fact that atherosclerotic lesions are more frequent in the main branches than in the peripheral branch of the coronary artery.

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Pseudocholinesterase in obesity: Hypercaloric diet induced changes in experimental obese mice[1]

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Summary. Pseudocholinesterase activity is significantly higher in liver and serum, but lower in adipose tissue of genetically obese, diabetic and gold thioglucose treated mice. Similar enzyme changes were also observed in lean mice on a high carbohydrate diet. A marked reduction (40%) in PChE activity occurred in the liver of genetically diabetic mice when starved for 24 h. These observations suggest that pseudocholinesterase induction in the liver and repression in the adipose tissue is affected by excessive calorie intake in obesity. This provides a model to study the biological function of PChE in health and disease.

Since its discovery in human plasma 50 years ago, pseudocholinesterase (PChE, acylcholine acyl hydrolase, E.C. 3.1.1.8) has been found in many tissues and animals[2,3]. There have been, however, very few studies conducted to understand the biological function of this enzyme. Several studies suggested that PChE may have a function in the metabolism of lipids[4,5] and low density lipoprotein[6–8]. We and others have observed a significant increase in serum PChE activity in obese and hyperlipemic patients[9,10].

We now report the results of studies conducted in experimental models of obesity in order to obtain a better understanding of the relationship between PChE and obesity.

Male obese mice (ob/ob) and its lean controls (ob/+) of the strain C57BL/6J of age groups 10 and 5 months, diabetic mice (db/db) and its nondiabetic controls (db/+) of the strain C57BL/KsJ of 10 weeks old were used in this study.

The animals were all kept in individual cages under ideal conditions and fed ad libitum with regular mouse chow (Purina, protein 17.5%, fat 11%, carbohydrate 53.9% and gross energy 4.3 kcal/g) and free access to water. The mice in group II, subgroups c and d, were treated differently, as indicated in the table. They were given a high carbohydrate diet (ICN-Pharmaceuticals, protein 18%, fat 8%, carbohydrate 68% and gross energy 4.2 kcal/g) with free access to water containing 10% sucrose for a period of 3 months. The animals in subgroup d were also injected with gold thioglucose (0.5 mg/g b.wt in 2 equal doses, one at the beginning and another 30 days later) i.p. to induce obesity[11]. 4 (db/db) mice in group III were fasted for 24 h prior to sacrifice. The mean daily calorie intake was calculated on the basis of daily food intake over a period of 15 days. The group I was a preliminary study, and no measurement was made in this respect.

Before collecting the tissue and blood samples, animals were immobilized with the anesthetic Ethrane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether). Blood was collected by heart puncture and livers were first perfused with normal saline before isolation. Epididymal fat pads were dissected out very carefully avoiding major blood vessels. Sera and tissues were stored at −20 °C until ready for use. For analysis, livers were homogenized with 1% triton X-100 in 0.25 molar potassium chloride in a ratio of 1:4 and supernatant obtained by centrifuging at 3000 rpm for 15 min in the cold. Adipose tissues were homogenized with cold distilled water in ratio of 1:2 and spun at 1500 rpm for 10 min. The clear infranatant aqueous phase was used for enzyme studies.

PChE was measured[12] using the substrate propionylthiocholine iodide and the color forming reagent 5,5′ dithiobis-(2-nitrobenzoic acid) (DTNB) and recording the absorbance change at 410 nm of the thiocholine-DTNB colored complex formed. The enzyme activity was expressed in terms of the μmoles of thiocholine formed in 1 min by 1 ml of serum or g wet wt of tissue. Serum glucose was measured by the glucose oxidase method using the autoanalyzer Gilford 3500[13].

I.p. injection of tetramonoisopropylpyrophosphor tetramide (Iso-OMPA, 2.3×10^{-8} mole/g b.wt), a specific PChE inhibitor[14] almost completely abolished the enzyme activity in the liver, serum and adipose tissue.

Pseudocholinesterase (PChE) activity and daily calorie intake in experimental mice

Animal groups	N	PChE activity* Serum (μm/ml/min)	Liver (μm/g/min)	Adipose (μm/g/min)	Calorie intake (kcal/day)
I a) ob/+	10	12 ±0.74*	7.04±0.49	1.21± .073	–
b) ob/ob	12	32.9±1.1	14.1 ±0.97	0.6 ±0.05	–
II a) ob/+	8	15.3±0.88	7.42±0.48	1.47±0.042	13.5±1
b) ob/ob	6	32.9±3.2	14.9 ±1.14	0.66±0.03	26 ±1.3
c) ob/+ (HC)	5	21.8±0.9	10.1 ±0.43	0.79±0.03	17.8±1.3
d) ob/+ (GTG+HC)	4	38 ±3.8	15.6 ±0.68	0.71±0.04	22 ±1
III e) db/+	4	14.8±3.5	9.4 ±0.92	1.1 ±0.18	13 ±0
f) db/db (fed)	4	33.7±1.5	22.3 ±1.5	0.51±0.042	31 ±1.9
g) db/db (starved)	4	33.9±1.3	13.7 ±0.52	0.45± .02	

All mice, except for subgroups c and d, were fed with regular chow. N, number of animals. * Means±SEM. Statistical significance was analyzed by the Student's t-test. The difference in the mean value of PChE and calorie intake between tests and controls are highly significant ($p < 0.001$). PChE activity of starved db/db mice is significantly lower than fed db/db mice ($p < 0.001$).

The results in the table show that PChE activity in the liver and serum of genetically obese, diabetic and gold thioglucose treated mice is 100–150% higher than that of their respective controls. In contrast, PChE activity in the adipose tissue is 40–50% lower in the obese animals. Interestingly, lean controls (ob/+) fed with a high carbohydrate diet showed a similar pattern of PChE activity as the obese mice when compared to the same type of animals on regular chow. Diabetic mice, starved for 24 h in contrast to its fed counterparts, showed a 40% decrease in liver PChE activity. The data also indicated that all the animals, whether obese, diabetic or high carbohydrate fed, have a significantly higher calorie intake. Severe hyperglycemia (serum glucose > 500 mg%) is a consistent finding in the obese and diabetic mice. High carbohydrate fed lean mice also showed a moderate degree of hyperglycemia (serum glucose 294±34 mg%) vs a serum glucose value of 167±16 mg% in the lean controls given only regular chow.

The results in hyperphagic[15] obese animals suggest that increased PChE activity in the liver is affected by excessive calorie intake. This conclusion is supported by the fact that lean controls fed a high calorie diet showed similar changes in liver and serum PChE activity and diabetic mice, when starved for 24 h, showed a 40% reduction of liver enzyme activity but not in the serum. Serum PChE is known to be synthesized in the liver[16,17]. However, failure to observe a decrease in the serum enzyme activity might be due to its longer half life than 24 h. It has been shown[18] that human serum PChE has a half life of 10–14 days. Finally, Gerebtzoff[19] demonstrated in mice fasted for 48 h an increase in the liver PChE activity after they were refed and suggested that this enzyme has a function in food assimilation.

Pseudocholinesterase has also been demonstrated in adipose tissue of rats and suggested to be involved in lipid metabolism[20]. We have not only been able to similarly show PChE activity in the adipose tissue of mice, but also were able to demonstrate its decrease in obese and lean mice on a high calorie diet. Hyperglycemia is a common feature in all these animals. Whether hyperglycemia is a cause for PChE repression in adipose tissue of obese mice remains to be answered. Adipose tissue PChE may be related to hormone sensitive lipase, which is known to be decreased in obesity[21]. For example, inhibition of PChE activity by either eserine or iso-OMPA in rats was found to be associated with a reduction in plasma non-esterified fatty acids and aortic lipolytic activity[22].

The quantitative alteration of PChE activity in obesity appears to be secondary to overeating. The mice with either inherited obesity or diabetes do not show frank obesity until they are at least 4 weeks old[23,24]. One would expect overeating to precede the development of symptoms. If so, it may be possible from serum PChE measurement to predict the people and animals at risk for the development of obesity.

It seems clear that a hypercaloric intake is the cause for PChE changes in obesity, and it is likely that one of the intermediates in carbohydrates or lipid metabolism may be responsible for either induction or repression of the enzyme. While biological function of PChE is not yet clear, such an approach as reported here may provide a theoretical basis for further study about this enzyme in health and disease.

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