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Sub Grouping of Lipase Enzymes According to Tween Substrate in Different Organs of Rat

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Abstract
Lipase is a general term involved types of lipase enzymes that hydrolyse long chain esters. Yet, no one knows which type of lipase is specific for certain organ or tissue.Therefore the aim of this study is to highlight which type of lipase enzymes subgroup is specific for each organ, using different tween substrates.Norway rats had been used. Different tissues had been taken from these animals. Detection of lipase enzyme, for each tissue, had been done by using Gomori method using different types of tween substrates.Different organs revealed different lipase enzymes activities according to the different substrates used in this experiment.From this study, different organs elicited their own lipase enzymes subgroups since different organs utilized different types of tweens as substrate and in different intensity.
Keywords: Lipase, Gomori method, tween substrates.

1. Introduction

Lipase is a general term that applied to group of enzymes that have the ability to hydrolyse long chain esters.Lipase catalyse esters by the following reaction[1]:

$$RCOOR' + H2O \longrightarrow RCOOH + R'OH$$

In mammals, dietary lipid digestion is commonly assumed to be mediated by three main enzymes[2,3]:

- Pre-duodenal (lingual or gastric) lipase.
- Pancreatic lipase.
- Post- duodenal lipase.

Types of Lipase

Cholesterol ester lipase: also called pancreatic ester lipase.

a. Lysophospho-lipase: this enzyme could be isolated from from pancreas, liver, intestine, kidney, and muscles (including skeletal and cardiac).
b. Choline-esterase: which are distinguished from the other esterases by their ability to hydrolyse esters of choline[4,5].
c. Triacylglycerol lipase: this enzyme is generally referred to as lipase, functioning primarily in the hydrolysis of long-chain fatty acids. This hydrolyses triglycerides to form monoglycerides[6].
   This enzyme is present in adipose tissue, liver, kidney, salivary glands, stomach, intestine, brain, heart and muscle [5].
d. Lipoprotein Lipase (LPL): also called body lipase. LPL hydrolyses triglycerides in plasma lipoproteins into fatty acids available for energy production, storage, or other metabolic reactions[6,7]. LPL acts on triglycerides but has no effect on cholesterol[8].

   Lipase enzymes activities could be identified by many methods and as follows[9]:
   1. Titrimetry.
   2. Spectroscopy (photometry, fluorimetry, infra-red).
   3. Chromatography.
   4. Radio-activity.
   5. Interfacial tensiometry.
   6. Turbidimetry.
   7. Conductimetry.
   8. Immuno-chemistry.
   9. Microscopical histo-chemistry.

   Histo-chemical method for detection of lipase enzymes is done using Gomori method [by] using tweens as substrate[10].

There is no previous study to determine which of the tween substrates are suitable to elicited specific lipase enzyme in different mammalian tissues.

Aim of the study: is using different tissues to elicited lipase enzymes activities using different tween substances as a substrate in different tissue, putting in consideration sex and sexual maturity.

2. Material and method

Norway albino rats were used in this experiment. Both sexes including mature and immature rats were used. Forty animals had been used and divided into four groups and as follows:

Table1: number and criteria of rats in experimental groups

Maturity of animals	Age	Sex	Number
mature	7-10 weeks	Male	10
immature	3-4 weeks	Male	10
mature	7-10 weeks	Female	10
immature	3-4 weeks	Female	10

From each animals, different tissues had been obtained,including, skeletal muscle, pancreas, small intestine, testis, liver, kidney, and ovary.

To identify the activity of lipase enzyme, the following histo-chemical method had been used. This method is Gomori method (1952) using different tween substances as a substrates[10].

Here, different types of tween substances had been used i.e. tween 20, 40, 60, and 80.

In this work, tween substances used as substrate. Lipase will hydrolyse tween substances to form fatty acid, which in turn combined with calcium ions (from already prepared calcium chloride solution). This combination will form relatively insoluble calcium soap. This later will be treated with lead ions, then with ammonium sulphide . This will form visible dark brown-black lead sulphide deposite at the site of lipase activity within the tissue [11].

The activity of lipoprotein lipase was measured using the method of (+) as shown in the following table [9]:

Table 2: scaling of LPL activity.

Activity of LPL (intensity of reaction)	Presentation in (+)
No LPL activity	-
Very weak LPL activity	+/-
Weak LPL reaction	+
Weak to moderate LPL reaction	+/++
Moderate LPL reaction	++
Moderate to strong LPL reaction	++/+++
Strong LPL reaction	+++

3. Results

The intensity of activity of lipase enzyme in skeletal muscle was elicited as shown in table (3).

Table 3: the intensity of lipase activity in skeletal muscles using different types of tweens.

MATURITY AND SEX	TWEENS			
	80	60	40	20
Mature male	+	+/-	-	-
Premature male	+/-	-	-	-
Mature female	+	-	-	-
Premature female	+/-	-	-	-

The intensity of activity of lipase enzyme in intestine was elicited in table (4).

Table 4: the intensity of lipase activity in intestine using different types of tweens.

MATURITY AND SEX	TWEENS			
	80	60	40	20
Mature male	+++	-	-	-
Premature male	++	-	-	-
Mature female	+++	-	-	-
Premature female	++	-	-	-

The intensity of activity of lipase enzyme in pancreas had been shown in table (5).

Table 5: The intensity of lipase activity in pancreas in different age and sex groups using different tweens.

MATURITY AND SEX	TWEENS			
	80	60	40	20
Mature male	+++	++/+++	+++	++
Premature male	++/+++	++	++	+/++
Mature female	++/+++	++/+++	++/+++	++/+++

The intensity of activity of lipase enzyme in the testis was shown in table (6).

Table 6: the intensity of lipase activity in testis using different types of tweens.

MATURITY AND SEX	TWEENS			
	8o	60	40	20
Mature male	++	+	+	-/+
Premature male	-	-	-	-

The intensity of activity of lipase enzyme in ovary had been shown in table (7).

Table 7: the intensity of lipase activity in ovary using different types of tweens

MATURITY AND SEX	TWEENS			
	80	60	40	20
Mature female	+	+	+	-
Premature female	-	-	-	-

The intensity of activity of lipase enzyme in the liver had been shown in table (8).

Table 8: the intensity of lipase activity in the liver using different types of tweens.

MATURITY AND SEX	TWEENS			
	80	60	40	20
Mature male	-	+	+++	-
Premature male	-	-/+	++	-
Mature female	-	-/+	++	-
Premature female	-	-/+	++	-

4. Discussion

Almost all the previous studies involved in detection of lipase enzymes activities, using Gomori method, had been used tween substances as substrate[11]. Yet, no one mentioned which tween substances were specific substrate for specific tissues. This raises a question that which tween (of the four tweens used as a substrate) is specific for each tissue lipase when Gomori method has been used.

Regarding activity of lipase enzyme in skeletal muscles, this study revealed no enzyme activity in all groups of animal tissues and in all tween substrates used except tween 80 which revealed weak reaction. This result agreed with that of the Oscai et al[12]. And Levak –Frank et al[13] .

Lipase activity in intestine elicited strong lipase activity when used tween 80 only, as shown in table (4). Otherwise no reaction could be elicited using other tween substrates. This means that tween 80 is the only tween substrate that could showed activity of lipase enzyme in the intestine. This findings conicides with the findings of Montin [14] and petridou and Mougios [15].

Regarding pancreatic tissues, in both sexes, tween 80 showed strong reaction of lipase enzyme among all tissues lipase. Although among all tween substrates, tween 80 showed the strongest reaction. These results go in the same line with the finding of Winkler et al. [16], Withers-Martinez et al.[17] and Petridou et al.[18]. This strong activity is expected since pancreas is the most strongest gland responsible for lipid metabolism [17,19].

The gonads, both in male and female, showed mild to moderate activity when lipase enzymes activities had been detected using tween substrates. Yet, tween 80 showed the strongest reaction among other tween substances. However, ovaries revealed lesser lipase activity than testes. These results agreed with findings of Kuwajima et al.[3], Soterion and Cryer [20], Holst et al. [21], and Chung et al.[22].

The liver had unexpected results. Lipase enzyme activity revealed strong reaction when tween 40 had been used,while tween 80 and 20 showed no reaction. Besides, the female rat′s liver tissue showed lesser reaction when tween 40 had been used in comparisom with that of male. This difference in activity between male and female could be attributed to the differences in metabolic activities between the two sexes. Besides sex hormones could play important role. However these findings coincide with the findings of Rug et al[23], Schulz et al [24], and Elliot and Elliot[25].

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