韓國營養學會誌 24(4) : 356~365, 1991
Korean J Nutrition 24(4) : 356~365, 1991

Liver Plasma Membrane and Nuclear T_3 Receptor Binding in the Obese (ob/ob) Mouse

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ABSTRACT

L-Triiodothyronine(T_3) binding to purified plasma membrane and to isolated nuclei from the same liver in obese(ob/ob) mice and their lean littermates was examined. The maximal binding capacity(Bmax) for T_3 receptor of liver nuclei, as compared to lean control, was significantly lower in the obese mouse(obese 527± 80fmol/mg DNA ; lean 883± 62 fmol/mg DNA), without an apparent difference in dissociation constant(Kd). The finding that obese mice have fewer liver nuclear T_3 receptors confirms previous reports. The Bmax and Kd of liver plasma membrane T_3 receptor were not significantly different between obese and lean mouse, which suggests no defect to be occurring in the function of the plasma membrane T_3 receptor and reinforces the view that the peripherally impaired thyroid hormone action in obese mice is a post plasma membrane receptor event. These results further support the hypothesis that the major defect of thyroid hormone metabolism in genetic obesity occurs at the level of the nuclear receptor.

KEY WORDS : genetic obesity · liver · triiodothyronine.

Introduction

Genetically obese(ob/ob) mice have been extensively studied as an animal model of obesity. The ob/ob mouse has a reduced resting metabolic rate and exhibits hypothermia in response to cold, which can be observed as early as 12 days of age and precedes the development of obesity[1)2)]. However, ob/ob mice are not hypothyroid. An abnormality of the hypothalamic-pituitary-thyroid axis was not found[3)4)] and the level of T_3 in the blood of ob/ob mice is normal throughout most of their life cycle[3)5)]. Therefore, an impaired peripheral thermogenic response to the thyroid hormone has been suggested.

Although obese mice have low nonshivering thermogenesis[6)] and low diet induced thermogenesis[7)], no inherent defect in the brown adipose tissue (BAT) of obese mice was found[8)]. Since thyroid hormone has a permissive role in BAT function[9)], the reduced adaptive thermogenesis may be related to impaired thyroid hormone function in obese mice. In fact, thyroid hormone treatment improved the function of BAT in obese mice[10)]. The activity and units of Na^+, K^+-ATPase, a thyroid hormone sensitive enzyme, are reduced in several tissues of obese mice[11)12)]. This

Received July 29, 1991
Accepted September 6, 1991

also supports a partial resistance of ob/ob mice to the normal level of T_3 in its blood. This hypothesis is further supported by the finding of a decreased concentration of nuclear T_3 receptors in the thyroid hormone sensitive tissues of obese mice[2)13)14)]. Khan et al.[2)] suggested that a decrement in nuclear T_3 receptor concentration may be a primary cause of the development of obesity by demonstrating a reduced T_3 receptor concentration in the liver of preobese(ob/ob) mice pups.

It had been thought that thyroid hormone enters target cells by passive diffusion and initiates biological effects by interacting with specific nuclear receptors. However, recent reports strongly suggest that T_3 enters into the cell by receptor mediated endocytosis[15-17)], binds to different cellular sites and then exerts its biological effect[18-20)]. The binding of T_3 to plasma membrane receptors enhances substrate availability, its binding to mitochondrial receptors increases mitochondrial oxygen consumption and ATP synthesis, and its binding to nuclear receptors increases specific protein synthesis. All these effects[18-20)] of T_3 at different cellular binding sites can be related to the thermoregulatory role of thyroid hormone. Therefore, it is apparent that the entry of thyroid hormone from plasma into the cell has important influences on the overall action of thyroid hormone.

The ob/ob mice have an altered membrane fatty acid composition and fluidity when compared to lean counterparts[21)]. Membrane composition and membrane fluidity influence hormone receptor interactions[22)]. Therefore, the peripherally impaired thermogenic action of thyroid hormone in obese mice may begin at the level of the plasma membrane and may limit its effect at all other cellular parts. This study examined this possibility in liver by contrastiong T_3 binding in the plasma membrane and the nucleus.

Methods and Materials

Genetically obese male mice, C57BL/6J-ob/ob, and their lean counterparts were purchased from Jackson Laboratory, Bar Harbor, Maine. The mice were maintained at 22-25℃, with controlled humidity and a 12 hour light cycle. The 911A diet which the mice are fed after weanling at Jackson Laboratory was purchased from Jackson Laboratory and was provided with tap water ad libitum. At 8-9 weeks of age, the mice were sacrificed by cervical dislocation and their livers were excised. Each liver was halved. Half of the liver was used for a plasma membrane T_3 receptor binding assay and the other half was immediately frozen at−80℃ for a later assay of nuclear T_3 receptor binding. Khan[23)] demonstrated that storing frozen liver up to 30 days did not change the nuclear T_3 receptor binding parameters.

Purified plasma membrane from fresh liver was prepared from the 1000×g pellet by the method of Ray[24)] and washed according to Lesko et al.[25)] Glucose-6-phosphatase was assayed[26)] as a measure of microsomal contamination. Succinic-INT-reductase[27)] was used to determine mitochondrial contamination. 5-nucleotidase[28)] was used as the plasma membrane marker enzyme. Table 1 provides details for liver plasma membrane purity. DNA content of the purified plasma membrane preparation was measured by the method of Burton[29)] and found to be negligible. Proteins were estimated by the Lowry method modified by Markwell et al.[30)] Prepared liver plasma membrane (50-100㎎ protein) was incubated at 23℃ for 25 minutes with increasing concentrations of ^{125}I-L-T_3 (0.5nM to 4nM) in the buffer system described by Pliam and Goldfine[31)]. In a preliminary study, it was shown that at 23℃, specific binding reached equilibrium in 20 minutes of incubation and the

Table 1. Marker enzyme activities in homogenate and purified plasma membrane prepared from lean and obese mice

Enzyme	Lean(n=10)		Obese(n=10)	
	Specific Activity (mol/mg prot./min)	Purification (fold)	Specific Activity (mol/mg prot./min)	Purification (fold)
5'-Nucleotidase				
Homogenate	0.006		0.006	
Plasma memb.	0.062	10.80	1.04	17.1
Succinic-INT-Reductase				
Homogenate	0.022		0.018	
Plasma memb.	0.006	0.27	0.001	0.27
Glucose-6-Phosphatase				
Homogenate	0.122		0.170	
Plasma memb.	0.049	0.40	0.063	0.37

steady state was stayed for up to 90 minutes. After incubation, the samples were chilled on ice and centrifuged at 1500×g for 8 min at 4℃. The pelleted membrane was washed once with ice-cold buffer and its radioactivity was measured(Tm Analytic gamma counter).

Crude nuclei from frozen tissue were prepared as described by Samuels and Tsai[32]. DNA content in isolated nuclei was determined by using the method of Burton[29]. ^{125}I-L-T_3 binding to isolated nuclei was measured by incubating isolated liver nuclei(50-100㎍ DNA) with increasing concentrations of ^{125}I-L-T_3(0.1 to 5nM) under the conditions described by Morishige and Guernsey [33]. In a preliminary study, at 37℃, specific binding reached equilibrium in less than 20 minutes of incubation and the equilibrium was maintained up to 90 minutes. The incubation reaction was stopped by washing with buffer containing polyethyleneglycol[34]. The radioactivity of the nuclear pellet was measured (Tm Analytic gamma counter).

All experimental procedures wers performed on the paired tissues from obese and lean mice at the same time, using identical reagents in order to minimze external factors that might influence interpretation of comparative results. The binding parameters (Bmax and Kd) were determined by analyzing the data by reverse Scatchard plot using the program described by Zibin and Waud[35].

Results

Nuclear T_3 Receptor Binding

The estimated T_3 receptor maximum binding capacity (Bmax) and equilibrium dissociation constant (Kd) of isolated nuclei from the livers of lean and obese mice are presented in Table 2. A representative analysis is depicted in Fig. 1. There was no significant difference in the affinity (Kd) for T_3 between lean and obese hepatic nuclei. However, the apparent binding capacity (Bmax) of liver nuclei of obese mice was significantly less than their lean littermates ($p<0.001$; obese ; 883± 62 fmol/㎍ DNA, lean ; 527± 80 fmol/㎍ DNA). These results are in agreement with previous reports[12][13].

Plasma Membrane T_3 Receptor Binding

Although two binding sites were detected on the hepatic plasma membrane[31], only the first binding site was compared between obese and lean mice in this study, since only the high affinity

Table 2. Characteristics of ^{125}I-L-T_3 binding to hepatic nuclear receptor and plasma membrane receptor of obese and lean controls

		Lean(n=10)	Obese(n=10)
Nuclei	Bmax(fmol/mg DNA)	883± 62	527± 80*
	Kd(nM)	0.70± 0.23	0.45± 0.19
Plasma Membrane	Bmax(pmol/mg Protein)	3.19± 0.37	3.16± 0.41
	Kd(nM)	5.76± 1.15	4.59± 0.78

Values are means± S.E.
*$p<0.001$ compared to obese animals using t-test for paired data between obese and lean littermate controls.

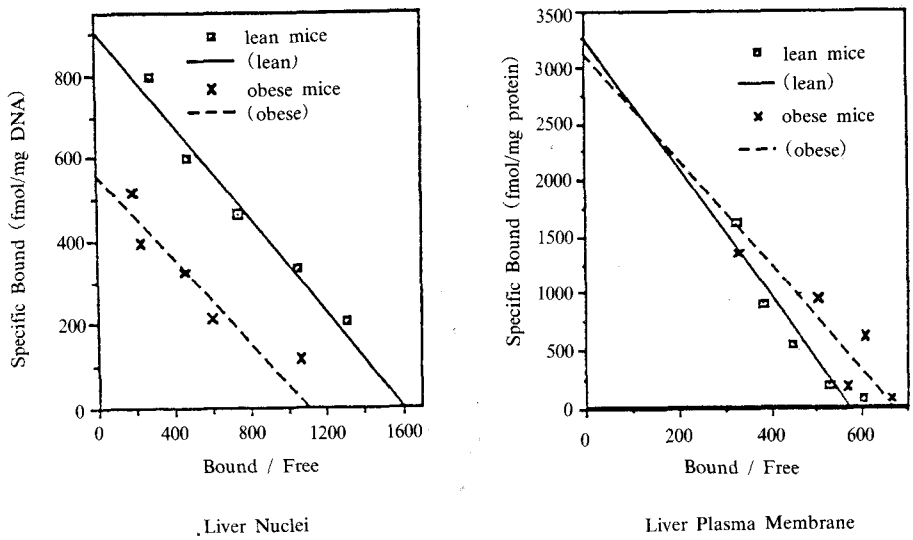

Fig. 1. Representative reverse Scatchard analysis from an experiment of ^{125}I-L-T_{13} binding to isolated nuclei and plasma membrane from liver of lean and obese mice.

binding site has been considered to be a true plasma membrane constituent[36] and only the high affinity system is energy dependent and is involved in the uptake of T_3 into the cell[37,38]. Fig. 1 is a representative reverse Scatchard plot of the high affinity binding site for T_3 on the liver plasma membrane. Table 2 provides the estimated equilibrium dissociation constant (Kd) and maximum binding capacity (Bmax) values for the liver plasma membrane of obese and lean mice. It is evident that there are no significant differences in apparent Bmax and Kd between obese and lean mice. Bmax values were 3.19 and 3.16 pmol/mg protein, and Kd were 5.76 and 4.59 nM in lean and obese, respectively. The Bmax and Kd values are in good agreement with previously reported results for rat liver plasma membrane. Pliam and Goldfine[31] reported a Bmax of 2.5 pmol/mg protein and a Kd of 3.2 nM. Gharbi-Chihi and Torresani[36] reported a Bmax of 1.3 pmol/mg protein and a Kd of 9.7 nM.

The results do not support the hypothesis that thyroid hormone binding at the level of the plasma membrane is in any way limiting the thermo-

genic activity of thyroid hormone.

Discussion

In this study, the binding of radiolabeled L-T_3 to the liver plasma membrane and nuclei was measured and compared between lean and obese mice. Liver was used for this purpose, since liver is metabolically active, a thyroid hormone sensitive tissue, and all nuclear T_3 appears to be derived from the plasma T_3 with little contribution coming from local conversion of T_4 to T_3[39]. Moreover, Ma and Foster[40] demonstrated that liver is the major effector for diet induced thermogenesis in contrast to a general belief that BAT is the major regulatory site of diet induced thermogenesis.

There are reports which support a hypothesis of diminished T_3 transport across the plasma membrane in the obese (ob/ob) mice. The subcutaneous injection of T_3 at an amount that increases serum T_3 ten fold in lean mice increases serum T_3 twenty fold in obese mice[41]. A decreased tissue to plasma T_3 ratio in the liver of ob/ob mice as compared to the lean controls was observed after a tracer injection of T_3 in vivo[42]. The hypothesis of diminished T_3 transport was further supported by the finding that nuclear T_3 receptor coccupancy was decreased in livers of obese mice as compared to lean[43].

In the present study, no significant differences between ob/ob and lean mice were found in the Bmax and Kd of the high affinity binding site for T_3 on the hepatic plasma membrane. This result suggests that there is no defect in thyroid hormone action at the liver plasma membrane level of obese mice. However, this result does not exclude the possibility of reduced uptake of T_3 into the cell in the obese mice, since T_3 uptake is accomplished by receptor mediated endocytosis via the coated pit-receptosome pathway[16]. A defect in the endocytosis process would prevent cellular entry of T_3 even though T_3 binds to the plasma membrane T_3 receptors. Horiuchi et al.[16] demonstrated that accumulation of T_3 into nuclei could be decreased by blocking the endocytosis process, possibly because of a decreased availability of T_3 in the cytoplasm. Since the endocytosis process can be affected by membrane lipid composition[22], it is possible that the endocytosis process may be altered in obese mice which have an abnormal membrane composition[21].

Another possible rationale for reduced uptake of T_3 into the cell despite unaltered plasma membrane receptor function is that lower ATP production and lower Na^+, K^+-ATPase activity in the liver of obese mice could inhibit T_3 uptake into the cell. T_3 uptake into rat hepatocytes is dependent on cellular ATP concentration and a sodium gradient is essential for T_3 transport[37,38]. It has been suggested that obese mice have less Na^+, K^+-ATPase activity because of impaired thyroid hormone action, however, the relationship between cause and result could be the inverse. In other words, because of lowered Na^+, K^+-ATPase activity, thyroid hormone transport has decreased. In fact, Hughes and York[44] suggested that changes in membrane lipid composition might be an important factor that regulates Na^+, K^+-ATPase in ob/ob mice. Supportive evidence is that "dietary" obese rats have a significantly lower Na^+, K^+-ATPase activity with altered liver plasma membrane composition compared to lean controls[45].

In addition to transport of T_3 across plasma membrane, transport of T_3 from cytosol to nuclei might be impaired in obese mice causing impaired thyroid hormone action at the nuclear level. The findings of T_3 binding sites in cytosol[46] and in the nuclear envelope[47] suggest that these rece-

ptors are involved in the transport of T_3 to the nuclei. Oppenheimer and Schwartz[48] demonstrated the existence of an active transport system from cytosol to nuclei for T_3 in vivo. By using antimycin A in vitro, Valdivielso and Bernal[49] could inhibit transport of T_3 into the nuclei resulting in decreased affinity of nuclear T_3 receptors without effect on the entry of T_3 into the cell.

In contrast to the equivocal finding in plasma membrane T_3 receptor binding, there was a significant difference in Bmax of nuclear T_3 receptor binding between obese and lean mice in the same animal liver, confirming previous reports[2,13]. This might imply that there is no direct relationship in T_3 receptor concentration between plasma membrane and nuclei. Ortiz-Caro et al.[50] suggested that extranuclear T_3 levels and nuclear T_3 receptors are controlled separately. They reported that short chain fatty acids increased nuclear T_3 receptor concentration by changing chromatin structure in vitro without changing extranuclear T_3 level. It is known that phospholipids are associated with chromatin.[51] If there is a change in the fatty acid composition of chromatin in obese mice as there is in other cellular membranes, it may affect the nuclear T_3 receptor concentrations of obese mice. Wiersinga et al.[52] demonstrated by in vitro experiments that unsaturated fatty acid in the medium inhibited nuclear T_3 receptor binding with a resultant decreased affinity of nuclear T_3 receptors.

In our experiment, intact nuclei were incubated for nuclear T_3 receptor binding ; thus, one can speculate that an active transport mechanism from cytosol to nuclei might interfere in receptor binding capacity. However, when the nuclei are isolated and incubated in an in-vitro system, uptake of T_3 by the nuclei is no longer dependent on this energy requiring system and proceeds by simple diffusion[20].

On the contrary, Hillgartner and Romsos[43] demonstrated no reduction of nuclear T_3 receptor concentration but a reduction in T_3 receptor occupancy in solublized nuclear T_3 receptors from livers of obese (ob/ob) mice. Anselmet et al.[53] also detected no significant difference in Bmax for nuclear T_3 in differentiating preadipocytes. However, preadipocytes T_3 receptors were different from hepatic T_3 receptors as measured by their reactivity to antibodies[54]. It is apparent that different tissues may give different results. Hillgartner and Romsos used liver, however, the animals were bred in their lab and more importantly a different methodology was used ; they used soluble nuclear extracts in lieu of intact nuclei. These variables could account for the different findings of various investigators. Even though Hillgartner and Romsos could not detect decreased hepatic nuclear T_3 receptors, finding a reduced T_3 receptor occupancy implies impaired thyroid hormone action at the nuclear level.

In conclusion, obese (ob/ob) mice exhibit no defect in T_3 receptor maximal binding capacity and affinity at the level of the liver plasma membrane whereas a significantly reduced nuclear T_3 receptor binding capacity is apparent. Our equivocal results for plasma membrane in the light of nuclear changes indicates that peripheral impairment of T_3 action in the obese mice is most likely a post plasma membrane receptor event, i.e. an alteration in the endocytosis process at the membrane or at the nuclear and/or cytoplasm level.

Literature cited

1) Trayhurn P, Thurlby P, James WPT. Thermogenic defect in preobese ob/ob mouse. *Nature 266* : 60-62, 1977

2) Khan SG, Boyle PC, Lachance PA. Decreased triiodothyronine binding to isolated nuclei from

livers of preobese and obese (ob/ob) mice. *Proc Soc Exp Biol Med 182* : 84-87, 1986

3) Ohtake M, Bray GA, Azukizawa M. Studies on hypothermia and thyroid function in the obese (ob/ob) mouse. *Am J Physiol 233* : R110-115, 1977

4) Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals : an automatic and endocrine hypothesis. *Physiol Rev 59* : 719-909, 1979

5) Mobley PW, Dubec PU. Thyroid hormone levels in the developing obese-hyperglycemic syndrome. *Horm Metab Res 11* : 37-39, 1979

6) Trayhurn P, James WPT. Thermoregulation and nonshivering thermogensis in the genetically obese (ob/ob) mouse. *Pflugers Archiv 373* : 189-193, 1978

7) Trayhurn P, Johns PM, McGuckin MM, Goodbody AE. Effects of over feeding on energy balance and brown fat thermogenesis in obese (ob /ob) mice. *Nature 295* : 323-325, 1982

8) Himms-Hagen J, Hogan S, Zaror-Behrrens G. Increased brown adipose tissue thermogenesis in obese (ob/ob) mice fed a palatable diet. *Am J Physiol 250* : E274-281, 1986

9) Himms-Hagen J. Thyroid hormones and thermogenesis. In : Mammaliam Thermogenesis, edited by L. Girardier and M. J. Stock. London : *Chapman & Hall,* 141-147, 1983

10) Hogan S Himms-Hagen J. Abnormal brawn adipose tissue in genetically obese mice (ob/ob) : effect of thyroxine. *Am J Physiol 241* : E436-443, 1981

11) York DA, Bray GA, Yukimura Y. An enzymatic defect in the obese (ob/ob) mouse : Loss of thyroid-induced sodium-and potassium dependent adenosine-triphosphatase. *Proc Natl Acad Sci 75* : 477-481, 1978

12) Lin MH, Romsos DR, Akera T, Leveille GA. Na^+, K^+ -ATPase enzyme units in skeletal muscle and liver of 14 day old lean and obese (ob/ob) mice. *Proc Soc Exp Biol Med 161* : 235-238, 1979

13) Guernesy D, Morishige W. Na^+ pump activity and nuclear T_3 receptors in tissues of genetically obese (ob/ob) mice. *Metabolism 28* : 629-632, 1979

14) Gilvary EG, Lachance PA, Khan SG. Decreased hindlimb skeletal muscle weight and nuclear T_3 receptor binding of obese (ob/ob) mice. *Nutr Res 8* : 949-954, 1988

15) Maxfield FR, Willingham MC, Pastan I, Dragstem P, Cheng SY. Binding and mobility of the cell surface receptors for 3,3',5 triiodo-L-thyronine. *Science 211* : 63-65, 1981

16) Horiuchi R, Cheng S, Willingham M, Pastan I. Inhibition of the nuclear entry of 3,3',5-triiodo-L-thyronine by monodansylcadaverine in GH3 cells. *J Biol Chem 257* : 3139-3144, 1982

17) Hennemann G, Krenning EP, Polhuys M, Mol JA, Bernard BF, Visser TJ, Docter R. Carrier mediated transport of thyroid hormone into rat hepatocytes is rate limiting in total cellular uptake and metabolism. *Endocrinology 119* : 1870-1872, 1986

18) Segal J. A rapid, extranuclear effect of 3,5,3'-triiodothyronine on sugar uptake by several tissues in the rat in vivo. Evidence for a physiological role for the thyroid hormone action at the level of the plasma membrane. *Endocrinology 124* : 2755-2764, 1989

19) Sterling K, Tsuboyama G, Brenner MA. Thyroid hormone action : Early calorigenic effect on dispersed rat liver cells in the absence of protein synthesis. *Endocrine Res 14* : 109-116, 1988

20) Oppenheimer JH. Thyroid hormone action at the nuclear level. *Ann Int Med 102* : 374-384, 1985

21) York DA. Alterations in membrane function, organization and composition in the ob/ob mouse. *Proc Nutr Soc 44* : 189-200, 1985

22) Spector AA, Yorek MA. Membrane lipid composition and cellular function. *J Lipid Res 26* : 1015-1035, 1985

23) Khan SG. Nuclear T_3 receptor binding in the (ob/ob) mouse and zucker rat : developmental changes in the hepatic nuclear T_3 receptors in the (ob/ob) mouse. Ph. D. thesis 1985 : Rutgers, The State Univ. of New Jersey.

24) Ray TK. A modified method for the isolation of the plasma membrane from rat liver. *Biochim*

Biophys Acta 196 : 1-9, 1970

25) Lesko L, Donlon M, Marinetti GV, Hare JD. A rapid method for the isolation of rat liver plasma membranes using an aqueous two-phase polymer system. Biochim Biophys. *Acta 311* : 173-179, 1973

26) DeDuve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Tissue fractionation Studies. *Biochem 60* : 604-617, 1955

27) Sottacasa GL, Kuylenstierna B, Ernster L, Bergstrand A. An electron-transport system associated with the outer membrane of liver mitochondria. *J Cell Biol 32* : 415-438, 1967

28) Michell R. The site of diphosphoinositide synthesis in rat liver. *Biochem Biophys Res Comm 21* : 333-345, 1965

29) Burton K. A study of the conditions and mechanism of the diphenylamine reaction of the colorimetric estimation of deoxy-ribonucleic acid. *Biochem 62* : 315-323, 1956

30) Markwell MAK, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem 87* : 206-210, 1978

31) Pliam NB, Goldfine ID. High affinity thyroid hormone binding sites on purified rat liver plasma membranes. *Biochem Biophys Res Comm 79* : 166-172, 1977

32) Samuels HH, Tsai JS. Thyroid hormone action. Demonstration of similar receptors in isolated nuclei of rat liver and cultured GH_1 cells. *J Clin Invest 54* : 656-659, 1974

33) Morishige WK, Guernsey DL. Triiodo-thyronine receptors in rat lung. *Endocrinol 102* : 1628-16 32, 1978

34) Darling D, Dickhoff W, Gorbman A. Comparison of thyriod hormone binding to hepatic nuclei of the rat and Teleost(Oncorhynchus Kisutch). *Endocrinology 111* : 1936-1943, 1982

35) Zivin JA, Wand DR. How to analyze binding, enzyme and uptake data : the simplist case, a single phase. *Life Sci 30* : 1407-1422, 1980

36) Gharbi-Chihi J, Torresani J. Thyroid hormone binding to plasma membrane preparations : studies in different thyroid states and tissues. *J Endocrinol Invest 4* : 177-183, 1981

37) Krenning EP, Doctor R, Bernard HF, Visser TJ., Hennenmann G. Active transport of triiodothyronine (T_3) into isolated rat liver cells. *Febs Letter 91* : 113-320, 1978

38) Krenning EP, Docter R, Bernard HF, Visser TJ, Hennemann G. Characteristics of active transport of thyroid hormone into rat hepatocytes. *Biochim Biophys Acta 676* : 314-320, 1981

39) van Doorn JD, van der Heide, Roelfsema F. Sources and quantity of 3,5,3'-triiodothyronine in several tissues of the rat. *J Clin Invest 72* : 1778-1792, 1983

40) Ma SW, Foster DO. Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. *Can J Physiol. Phamacol 67* : 376-381, 1988

41) York DA., Otto W, Taylor TG. Thyroid status of obese (ob/ob) mice and its relationship to adipose tissue metabolism. *Comp Biochem Physiol 59B* : 59-65, 1978

42) Kaplan MM, Young JB, Shaw EA. Abnormal thyroid hormone binding to serum proteins in ob/ob and db/db genetically obese mice. *Endocrinology 117* : 1858-1863, 1985

43) Hillgartner F, Romsos D. Nuclear triiodothyronine receptor binding characteristics and occupancy in obese ob/ob mice. *Am J Physiol 252(Endocrinol. Metab. 15)* : E414-419, 1987

44) Hughes S, York, DA. $[Na^+ + K^+]$ATP-ase in liver and brain of obese mice. *Horm Etabol Res 15* : 335-339, 1983

45) Izpisua JC, Barber T, Cabo J, Hrelia S, Rossi CA, Castelli GP, Lercker G, Biagi PL, Bordoni A, Lenaz G. Lipid composition, fluidity and enzymatic activities of rat liver plasma and mitochondrial membranes in dietary obese rats. *Int J Obese 13* : 531-542, 1989

46) Hashizume K, Miyamoto T, Nishii Y, Kobayashi M. Evidence for the presence of active and inactive forms of cytosolic triiodothyronine binding protein in rat kidney : cooperative action of Ca^{++}in NADPH activation. *Endocrinol Japan 34* : 479-

487, 1987

47) Lefebvre YA, Venkatraman JT. Characterization of a thyroid-hormone-binding site on nuclear envelopes and nuclear matrices of the male-rat liver. *Biochem J 219 :* 1001-1007, 1984

48) Oppenheimer JH, Schwartz HL. Stereospecific of triiodothyronine from plasma to cytosol and from cytosol to nucleus in rat liver, kidney, brain, and heart. *J Clin Invest 75 :* 147-154, 1985

49) Valdivielso L, Bernal J. Inhibition of nuclear binding of triiodothyronine by antimycin A in cultured human fibroblasts. Biochem Biophys Res Comm 147 : 1241-1244, 1987

50) Ortiz-caro JO, Montiel F, Yusta B, Pascual A, Aranda A. Short chain fatty acids modulate nuclear receptor and extranuclear L-triiodothyronine levels in Glial C6 cells by different mechanism. *Endocrinology 122 :* 2597-2603, 1988

51) Awad AB, Spector AA. Modification of the ehrlich ascites tumor cell. *Biochim Biophys Acta 450 :* 239-251, 1976

52) Wiersing WM, Chopra IJ, Teco GNC. Inhibition of nuclear T_3 binding by fatty acids. *Metabolism 37 :* 996-1002, 1988

53) Anselmet A, Gharbi-chichi J, Torresani J. Nuclear triiodothyronine receptor in differentiating preadipocytes cloned from obese and lean mice. *Endocrinology 114 :* 450-456, 1984

54) Torresani J, Ghysdael J, Bismuth J, Ghiringhelli O, Savary J. Heterogeneous distrbution of nuclear triiodothyronine receptors in liver and preadipose cells as evidenced by their reactivity to antibodies against different protein products of erb A oncogenes. *Febs Letters 250 :* 251-256, 1989

비만 쥐(ob/ob mouse)의 간 세포막과 핵에 있는 T_3 수용체의 결합능력에 관한 연구

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= 국 문 초 록 =

유전에 의해 비만증을 타고나는 비만쥐 (ob/ob mouse)의 간으로 부터 유리된 세포막과 핵의 L-triiodothyronine(T_3) 결합 능력이 그들과 한 배에서 태어난 정상 체중의 쥐들의 것들과 비교되었다. 세포막이 hypotonic 용액과 discontinuous sucrose density를 사용하여 원심분리기로 분리되었으며, 세포 각 부분의 marker enzyme들의 activity로 세포막의 순도가 측정되었다. 핵은 Triton×100를 사용하여 원심분리기로 얻어졌다. T_3 수용체의 Bmax(최대결합용량)와 Kd(dissociation constant)가 세포막 혹은 핵을 여러 농도의 ^{125}I-T_3와 함께 일정시간 incubation 시킨 후, 그 binding 이 reverse Scatchard analysis에 의하여 계산하여 얻어졌다. 모든 실험과정은 비만쥐와 정상쥐에 대하여 평행으로 진행되었다.

간 핵의 T_3 수용체의 최대결합용량은 비만쥐가 정상 체중의 쥐 보다 유의적으로 적었으나($p < 0.001$), T_3에 대한 친화력에는 차이가 없었다. 이는 이전의 보고들의 결과를 확인해 주는 것이다. 세포막에 있는 T_3 수용체의 최대결합 능력과 친화력은 비만쥐와 정상쥐 간에 유의적인 차이가 없는 것으로 밝혀졌다. 이는 비만쥐의 세포막에 있는 T_3수용체의 기능에는 결함이 없음을 나타내며, 비만쥐의 말초조직에서 손상된 갑상선 홀몬의 작용은 세포막 수용체에 결합한 이후에 일어나는 과정에 원인이 있다는 것을 의미하고, 따라서 핵에 있는 T_3 수용체의 결함이 비만쥐 (ob/ob mouse)의 비만증의 근본적인 원인일 수 있다는 제안을 뒷받침하여 주고 있다.