ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 45(1): 197-209, (2007).

HOMEOSTATIC ROLE OF FOOD BIOACTIVE PROTEIN IN HYPERCHOLESTEROLEMIC RAT

BY

Sayed-ahmed, A.I.*; El-Deeb, F.E.A. and Dalia M. El-Nahal*

* Special food and nutrition, FTRI, ARC,Giza, Egypt *.

** Fac. of Specific Edu., New Damietta, Mansoura, Univ.

ABSTRACT

Based on the hypocholesrolemic effect of plant proteins, soy's protein proteolytic digest (PPD) was introduced. In the previous studies, the PPD has been suggested to be as an up-regulatory agent that acts at level of gene expression. However, besides the normal hormonal balance that induced by endocrinal and nurocrinal secretions, there are what so-called emergencies alternative mechanisms that regulated by balanced diets and their medicinal constituents. Data in this study showed that this food protein preparation, i.e. plant protein digest (PPD), when injected in rats strongly controlled liver fat and plasma lipoproteins. In conjugation, some normal hormonal levels were shifted assuming the presence of this alternative mechanism, but this was capable to protect liver and keep it at the nearest normal histopathological performance. The PPD as a potent bioactive protein may also considered as an analogue to some neurotransmitter or modulator protein, or even in structure and function similar to an interlukine of great metabolic effect. This general metabolic correction for lipoproteins at level of hormonal action, liver function, tissues and liver histopathological protection were altogether in the same time confirmed. Of course, further investigation should be forwarded towered this topic of special interest since it has been observed that natural or food origin drugs do not alter the organ longevity or even reverse their estimated functional expectancy.

INTRODUCTION

Functional food, or drug of natural origin, is becoming an interesting scientific issue. The nutracuticals made of original materials are becoming the title of this era .It is anticipated that more and better vegetable ingredients will be introduced in years ahead as industry eyes on booming these foods and nutraceutical market (Sirtori, *et al.,* 2004; Manzoni, *et al.,* 2003 and Gianazza, *et al.,* 2003).

However, the proteins are known of great biological roles. Greater metabolic functions for these biological compounds are discovered nowadays. Moreover, an intracellular antifungal protein was isolated from sugar beet leaves and cloned to used in immunological studies (Kristensen, *et al.,* 2000). Scientifically, the homeostatic adaptations on organism makes to a constantly

changing environment are in large part accomplished through alterations of the activity and amount of proteins (Murray *et al.*, 2003). However, the phenomenon of hypolipemic metabolic action of plant protein was investigated in different animals as an alternative health promoter or dietary therapeutic besides the pharmaceutical drugs used against these pathological diseases (Potter, 1996). Some authors believe that plant protein itself is the most important (Xiao, *et al.*, 2004; Sirtori, *et al.*, 2004; Manzoni, *et al.*, 2003; Gianazza, *et al.*, 2003; Jenkins, *et al.*, 2002 and Adams, *et al.*, 2004). Some others have the assumption that other substances are more effective (Jenkins, *et al.*, 2002 and Kerckhoffs, *et al.*, 2002).

Anderson (2003) has stated, however, that diet is the first, then medication for hypercholesterolemia. Actually, dietary therapy is effective in most cases of hyperlipoproteinemia and has established a concept of hormonal oxidative balance theory (HOBT) for diet and dietetics (Ahmed, *et al.*, 2003). This theory can provide more reasonable biochemical definition for these metabolic processes. Further biochemical and nutritional studies is to be continued in order to elucidate the medical potential of these food and food drived industries on health as hormonal oxidative balance (HOB) protective agents (Ahmed, *et al.*, 2003).

Again, this unique plant protein digest (PPD) has been shown to effect on hypercholesterolemia in rats that need more and more effort to cover as a safe drug. The active protein fragment was extracted and identified in some legume protein concentrates and found to be of about 27kd. The PPD processing seems to be highly systemic, i.e. the duration time of proteolytic action on the raw proteins to produce PPDs has a sharp optimal point. The PPD prepared at the optimal point is of the relatively strongest effect in controlling total cholesterol both in the blood of rabbits and rats, its functions also looks like that of insulin in lowering the glucose in the alloxide or streptosotozysed rats (Ahmed, *et al.*, 2005).

In higher level of LDL, it has been supposed that free cholesterol inactivates some main bioactive protein such as insulin. However, a sort of chemical interaction between this protein and cholesterol has been *In Vitro* detected earlier (Ahmed *et al.*, 1999). In correlation of cholesterolemia and glycemia in blood elevation, a type of hormonal defect is proposed (Ahmed *et al.*, 2003). Uncontrollable cholesterol conjugates not only less insulin reactivity, but also several pathological disorders, i.e. immundysfunction, muscle growth alteration, CHD, etc (Forsyth, 1995; Keyes and Heimberg, 1979; Griffin and Wildenthel, 1978 and Beynen, 1990), assuming a clusters of metabolic defect either permitted to or caused by this TC upnormale elevation. Moreover, there are strong believed that correlation between plasma uncontrollable cholesterol and aging and/or the consumption of non-healthy food is existed. Uncontrollable cholesterol, in turn, leads to severe oxidative resistance impairment and a chronic hormonal shift, e.g. insulin inactivity. In case of pathological disorders, again, food type might act either as hormonal oxidative imbalance generator or as a preventive agent.

The role of PPD, that has been previously proposed (Ahmed *et al.*, 2003), is as a hormonal balance bioactive protein enhancer. A role that may positively affect numerous metabolic systems. This protein, which is administrated as drug but not as food, must be discussed comparing to the insulin function, the protein hormone of central effect extended to protect organs such as liver. This protection, for example, was examined for selenium as member of antioxidant system (Issa *et al.*, 2000). In fact, the survival of multicellular organisms depends on their ability to adapt to a constantly changing environment or its homeostatic capability. Recently, recombinant Insulin-like growth factor-1 (IGF-1) potency of pure homeopathic recombinant IGF-1 is clinically proven to improve muscle tone, increase energy, increase memory and positive mood and decrease cravings. For women, IGF-1 helps maintain balance in the hormonal, nervous and immune systems. It has been most recently discovered that the power of some protein microsphere technology has lead to a protein-matrix drug delivery system that supports these biological communication skills.

The communication mechanisms are necessary requirements for the overall metabolic adaptation. The nervous and endocrinal systems provide this intracellular organism-wide communication. The neural regulation of the endocrine system is important in the production and secretion of some hormones, many neurotransmitters resemble hormones in their synthesis, transport, and mechanism of action. It is a fact also that many hormones are synthesized in the nervous system. The proteins are extremely rich metabolic substances of diverse array of hormones has evolved to provide homeostatic response (Greany, *et al.*, 2004). The domain responsible for hormone recognition and signal generation has been identified in the protein polypeptide and catecholamin hormone receptors. Steroid, thyroid, and retinoid hormone receptors have several functional domains, one site binds hormone, another binds to specific DNA regions, a third is involved in the interaction with other co-regulator proteins that result in the activation or repression of gene transcription. The fourth may specify binding to one or more other proteins that influence the intracellular trafficking of the receptor (Greany, *et al.*, 2004). Is PPD acting as one or more of these proteins.

The theory of hormonal oxidative balance is based on a number of facts. Taking in account the uncontrollable cholesterol, changes in insulin, glucagon, T3, T4 and TSH were noticed (Ahmed *et al.*, 2003). The rabbit, pig, monkey and human are species in which atherosclerosis can be induced by feed cholesterol. NIDDM, IDDM, hypertension, lipid nephrosis, hypothyroidism or some other conditions of hyperlipidemia induce atheroma and coronary heart diseases (CHD) (Carroll, 1991). Dietary protein, based on what have been mentioned before, plays a specific role concerning lipoproteinemia. In general, plant protein considered being hypolipidemic agent and used in dietary therapy. Jenkins, *et al.* (2001) postulated the effect of high-protein diets in hyperlipidemia, particularly the effect of wheat gluten on serum lipids, uric acid, and renal function. There is, however, an overlap between plant and animal proteins. In some human subjects, soyprotein is effective in lowering TC and LDL, others have observed moderate reduction, and some have found little or no effect (Carroll, 1991).

Here, we are examining the use of a dietary protein fragment in special medical form (PPD). The study of that role under the light of the proposed theory of hormonal oxidative balance (HOBT) led to more accurate scientific data. The physiological case of uncontrollable cholesterol in plasma has been chosen to be main target due to its metabolic diverse. Our main objective, in another words, is to continue exploring the equipotent level action and the reality of PPD in connection with the validity and profile shape of that theory.

MATERIALS AND METHODS

In the proteoletic protein digests (PPD) preparation, a sort of soy defat flour (SDF), was enzymatically processed, i.e. using a proper sequences of poteolytic enzymes, to get a specific formal structural peptide for injection in isotonic solution. The prePPD, PPD, and postPPD were prepared in 30, 60, and 90 min of enzymatic action, respectively.

Animal and Rationales:

Male Albino rats western strains aged animals were conducted. The animal trail was 6 animals each group fed on basal diet as negative control (NC). For the positive control (PC) and the rest of the other groups: same + 0.1% cholesterol challenge were used. Protein Proteolytic Digests (PPD) freeze-dried has been desolved in saline solution before the injection intramuscular at level of 0.2 ml each time day by day was administrated at the concentration of g / L saline solution

Methods:

Total cholesterol and triglycerides were measured by the methods of (Allain *et al.*, 1974 and Wahlefeld,1974)respectively. Amino transferase (AST and ALT) activities in serum were according to the method described by Reithman and Frankel (1957) .The activity of Alkaline Phosphatase enzyme (AP) was determined following that of Kind and King(1954). Serum insulin was adapted to that of *DeFronzo et al. (1979).* Glucagon was measured according to the method of Ghatei *et al.* (1983), meanwhile, that method of Avruskin *et al.* (1976) has been used for testing the T4 and T3.

The histopathological examination for liver have been performed according to that of Lillie (1969) after fixing in formalin 10% for subsequent paraffin processing, and sections were stained with Masson's trichrome stain.

Statistical analysis of the data was carried out according to Kalton (1983) .

RESULTS AND DISCUSSION

This work has been conducted to study the effect of a prepared structural protein (PPD) on the overall metabolic function against cholestrolemia. This protein was intramuscularly injected in that special form. The hypercholestolemic aged rats metabolic response is recorded in Tables (1) to (4).

In Table (1), when compare positive control (PC) to negative control (NC), one can figure out how it is risky to consume this level of cholesterol in diet. Liver TC, for instance, as well as plasma one is significantly increased. It is clear that PPD, but not either pre or post PPD, makes the best positive response in the potential of cholestrolemic correction. Even in TG rate, PPD recorded the nearest one to the NC.

Moreover, the PPD keeps the HDL similar to that of NC as seen in Table (1), meanwhile, the ratio LTC/PTC, which tells something about the biological homeostasis system for lipidemia, was similar. It seems that the fat metabolism in liver has been successfully maintained by PPD injection. Prifly, pre or postPPD are always coming after that of PPD in controlling lipemia or liver performance assuming a specific role of structural peptide or even the presence or absence of specific group or groups of those proteins.

The hormonal situation in association to this pathological allegation is depicted in Table (2). Data may correlate hypocholesterolemic function to a sort of hyperthyroidism one. Forsyth (1995), for instance, stated that soy protein enhances the thyroid regulation for the cholesterol metabolism. In some other animals, this findings is conserved (Forsyth, 1986; Potter, 1996; Scholz-Ahren, *et al.*, 1990 and Wolfe, *et al.*, 1979).

It is extracted also from Table (2) that the PPD, in its unique form, seems to preserve insulin and glucagon secretion. A diverse array of hormones, each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport and metabolism has evolved to provide homeostatic response (Murray *et al.*, 2003).

This means that in the presence of PPD better condition for their action is existed. This protein action is similar to that maintained for PC but not that of the NC. In another words, what appears in a T3/T4 ratio properly induced a metabolic condition of hormonal balance. The later normally acts to control PTC in a metabolic way that may preserve in the mean time the organs, mainly the liver (Harper *et al.*, 1979). Surprisingly, the farest hormonal level to this is that of NC assuming the presence of another hormonal balance action to control the cholestrolemia.

The differences in liver function as seen in Table (3), which markedly influenced, may support this hypothesis. However, stimulus generate hormone signals at or within target cells, regulate a variety of biologic processes which provide for a coordinated response to the stimulus or challenge including gene expression, transporter channels, protein translocation or protein modification (Murray *et al.*, 2003).

In the main time, Table (4) shows that the two metabolic mechanisms, if any, are positively affected the liver, but differently responded in fat tissues and thyroid gland. It is obvious that the proposed alternative mechanism mainly depends on a remarkable shift in thyroxin activity. Wolfe *et al.* (1979) pointed out that there is a role for thyroid gland on lipid metabolism in the perfused rat liver.

Table (1): Lipoprotein in rats under effect of PPDs.

Items PPD	LIVER				PLASMA						
	TG		TC		TG		TC		HDL		LTC/ PTC
	mg/100g	%	mg/100g	%	mg/100ml	%	mg/100ml	%	mg/100ml	%	
NC	524±13.9*	100	312±10.2*	100	76.42±3.32*	100	33.25±4.7*	100	40.63±3.9*	100	9.38
PC	770±15.1	147	536±9.0	172	92.47±4.10	121	66.17±7.9	199	36.13±3.5	89	8.10
PPD	655±10.0*	125	327±8.0*	105	77.95±3.33*	102	35.25±4.1*	106	40.34±4.1*	99	9.26
PRE PPD	681±11.0*	130	462±9.1*	148	95.53±4.20	125	63.18±6.3*	190	35.10±3.6	86	7.31
POST PPD	760±13.2	145	452±9.1*	145	84.83±3.59*	111	57.19±5.9*	172	35.91±3.4	88	7.90

Average of 6 rats * Values were significantly different from positive control ($p < 0.05$)

Table (2): Hormonal status in rats injected with PPD.

Items PPDrug	T3		T4		T3/T4	Insulin		Glucagon	
	mmol	%	mmol	%		Ug/l	%	Pmol/l	%
NC	4.29±0.96*	100	40.83±4.24*	100	0.105	14.63±1.32*	100	16.22±1.42*	100
PC	4.43±1.02	103	46.67±4.01	114	0.095	10.34±1.00*	71	12.32±1.11	76
PPD	4.51±1.11*	105	41.24±4.61*	101	0.110	8.91±0.82*	61	11.18±1.03*	69
PRE PPD	4.33±0.98*	101	43.11±4.00*	106	0.100	10.16±1.01	69	13.29±1.32*	82
POST PPD	4.20±0.87	98	46.16±4.56	113	0.091	11.25±1.11*	77	12.81±	79

Averge of 6 rats * Values were significantly different from positive control ($p < 0.05$)

Table (3): Liver function in rats under the effect of PPD injection.

Items	AP		AST		ALT	
PPDrug	u/l	%	u/l	%	u/l	%
NC	2.90±0.30*	100	22.90±2.22*	100	32.61±2.31*	100
PC	2.80±0.27	97	41.93±4.21	183	35.13±3.21	108
PPD	3.88±0.39*	134	23.58±2.33*	103	56.09±4.56*	172
PRE PPD	3.13±0.29*	108	42.02±3.99	184	20.37±1.99*	63
POST PPD	2.92±0.28*	101	27.13±2.81*	119	52.28±3.47*	160

Averge of 6 rats * Values were significantly different from positive control ($p < 0.05$)

In this concern, hormonal balance study is important metabolic issue both during age cycle and physiologic status. Estrogen, for instance, is of major interest and importance to a woman's health. Women use birth-control pills and estrogen-replacement therapy to assure health and quality of life. However estrogen application with hormonal imbalance raises the risks for breast cancer, heart attacks, stroke and blood clots, all of which shorten quality and length of life. The supplementation of estrogens, however, whether through birth control pills or through estrogen-replacement therapy, can result in unbalanced feedback signals, thus heightening risks for cancer and other adverse events. In this regard, IGF-1 signaling found through use of natural menopause relief brings a woman into greater hormonal balance and a sense of relief from physical and mental symptoms that arise when hormones are out of balance.

Table (4): Rat's liver, adipose tissues and thyroid gland weight under the effect of PPD injection

Items	Liver		Adipose Tissues		Thyroid	
PPDrug	(g)	%	(g)	%	(g)	%
NC	8.23±0.90	100	1.89±0.20	100	0.296±0.058	100
PC	9.69±1.00	118	2.01±0.21	106	0.286±0.067	97
PPD	8.39±0.84	102	1.95±0.20	103	0.322±0.094	109
PRE PPD	9.73±1.00	118	2.39±0.22	127	0.282±0.079	95
POST PPD	9.31±0.43	113	2.35±0.20	124	0.293±0.090	99

Means of six values; initial body weight of 235±7 to final body weight of 261±9.

The liver tissue histopathology in addition to tests its weight and function is of great diagnostic role. In Table (5), the hepatocyte cells were greatly influenced, meanwhile, PPD in its optimal processing point was comparable to the control, assuming that the second proposed mechanism of regulation mediated by this bioactive protein has been directed towered the protection of this important organ. As a matter of fact, this organ in a special antagonistic cooperation of those hormones mediates this control of fat homeostasis (Murray *et al.*, 2003).

Table (5): Correlation of cholestrolimia with liver histopathology.

Factor PPDrug	HDL/TC	Lymphocyte Infiltration	Vascular Degeneration	Degeneration of Hepatocytes	Fatty Granules	Bile Duct Hyperplasia
NC	0.45	-	-	-	-	-
PC	0.24	+++	+++	+++	+++	++
PPD	0.50	-	+	+	+	-
PRE PPD	0.24	+++	+++	+++	+++	+
POST PPD	0.28	+++	+++	+++	++	++

In general, PTC tended to be decreased with increasing protein intake, in particular when diets contained cholesterol (Okita and Sugano, 1990). This must be due to the capability of synthesizing these specific bioactive proteins with increasing food protein intake. However, Lovati *et al.* (2000) discovered that soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. The activation of LDL receptors in a human hepatoma cell line (Hep G2) exposed both to subunits from 7S soy globulin and to Croksoy^{R}70, a commercial isoflavone-poor soy concentrate. To assess the final identity of the peptide(s) putatively responsible for the biochemical effect, experiments were performed in Hep G2 cells, exposed either to synthetic peptides corresponding to specific sequences of 7S soy globulin or to peptides from the *in vitro* digestion of Croksoy^{R}70. Moreover, the ability of the whole 7S globulin, its subunits and whole Croksoy^{R}70 to interfere in the apolipoprotein B (apo B) secretion in the medium as well as in sterol biosynthesis was evaluated in the same model. Increased ^{125}I-LDL uptake and degradation vs. controls were shown after Hep G2 incubation with a synthetic peptide (10^{-4} mol/L, MW 2271 Da) corresponding to positions 127–150 of the 7S globulin. Cells exposed to Croksoy^{R}70 enzyme digestion products showed a more marked up-regulation of LDL receptors vs. controls, compared with vs. Hep G2 cells incubated with undigested Croksoy^{R}70. Among soy-derived products, only the 7S globulin inhibited apo B secretion and ^{14}C-acetate incorporation when tested in Hep G2 cells at a concentration of 1.0 g/L. These findings support the hypothesis that if one or more peptides can reach the liver after intestinal digestion, they may elicit a cholesterol-lowering effect. Moreover, the protein moiety, devoid of isoflavone components, is likely to be responsible for this major biochemical effect of soy protein. Similar advanced studies at this point was most recently carried out (Klopotek *et al.*, 2006 and Duranti, *et al.*, 2004)

From the above studies and many more others, oxidative and hormonal balance control seems to be a vital tool in PTC regulation of lipoprotein profile at an acceptable metabolic level. Possibly, the PPD biological function most likely differs than the pathways of hormonal metabolic role of insulin, the hormone with great metabolic regulation even to many other hormones. The up-regulating capability of PPD, but not any other nonprotein component (Dewell, *et al.*, 2006; Ma, *et al.*, 2005; Greany, *et al.*, 2004 and Mullen *et al.*, 2004), may work as

neurotransmitters or interlukines that may cooperates the endocrine system with a total metabolic effect on liver expectancy.

Another feature that may accounts for PPD is its proposed antioxidant role. Pena-Ramos and Pena-Ramos and Xiong (2002) discovered an antioxidant activity of soy protein hydrolysates in liposomal system that may has greatest inhibitory effect on lipid oxidation. Finally, normal positive effect on PTC whatever its level of effect is important to keep liver at its degree of organ expectancy (Eldkak, 2004).

In this respect, the cholestrolemic revise effect of this preparation is referred to one or more of a specific group of structural peptides as the main effective agent (Ahmed *et al.*, 2003). However, comparing injection to ingestion, the later processed protein fragment in the stomach that moves in a long pathway from gastrointestinal (GI) to the target tissues or glands. This makes those individual peptides or peptide either low or lesser effective metabolites. Isoenergetic diet, for instance, was infused intraduodenally in an observation. During the slow infusions (8hr – 10hr) shows a significant decrease in plasma total cholesterol (PTC) most greatly by soyprotein (Wolfe and Greace, 1987). The PPD administration has positively affected the whole lipoproteins. In a human study, Anderson, *et al.* (1995) rolled up a metas-analysis for the effects of soy protein intake on serum lipids. They concluded that the consumption of soy protein rather than animal protein significantly decreased serum concentrations of total cholesterol, LDL cholesterol, and triglycerides.

Concerning TG reduction under the effect of PPD coincidence with regulation of PTC is more balanced than the use of mechanical cholesterol reduction through entirohepatie circulation (EHC). In EHC mostly, hypertriglyceridemia is induced. Therefore, sequestrantes, especially the artificial ones, are not safe in all cases of hyperlipoproteinemia.

REFERENCES

Adams, M.R; Golden, D.L.; Franke, A.A.; Potter, S.M.; Smith, H.S.; and Anthony, M.S. (2004): Dietary Soy {beta} - Conglycinin (7S Globulin) Inhibits Atherosclerosis in Mice J. Nutr., 134(3): 511 - 516.

Ahmed, A.I.S. Fathia, K.S. Abo Zeid and El-Bagoury A. A.(2003): Hormonal Oxidative Balance of Modified Diets and Uncontrollable Cholesterol. Egypt. J. Biomed. Sci.13,106-115.

Ahmed, A.I.S.; Habbib, G M; Hammad, A.A. and El-Deeb, F.E. (2005): Effect of natural plant protein digest on hyperlipemia and hyperglycemia in some animal models. Egypt. J. Biomed. Sci, 17, 85-94.

Ahmed, A.I.S; Thabet, H.A. and Shallan, M.A. (1999): On the interaction of fat and food metabolites with insulin. Cont. Med. Edu. Conf. Ain Shams Univ., Cairo, Egypt.

Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W. and Fu, P.C. (1974): "Enzymatic determination of total serum cholesterol" Clin. Chem. 20: 470-475.

Anderson, J.W. (2003): Diet First, Then Medication for Hypercholesterolemia. JAMA, 23, 290(4): 531 - 533.

Anderson, J.W.; Johnstone, B.M. and. Cook-Newell, M. E. (1995): Meta-analysis of the effects of soy protein intake on serum lipids. N. Engl. J. Med. 333:276–282

Avruskin, T.W.; Mitsuma, T.; Shenkman, L.; Sau, K.; and Hollander, C.S .(1976): Measurements of free and total serum T3 and T4 in pregnant subjects in neonates. Am. J. Med. Sci. 271(3): 309-15.

Beynen, A.C.(1990): Comparison of the mechanisms proposed to explain the hypocholesterolemic effect of soybean protein versus casein in experimental animals. J. Nutr. Sci. Vitaminology, 36: 587-593.

Carroll, K.K. (1991): Review of clinical studies on cholesterol lowering response to soyprotein. J. Am. Assoc. 91, 820-7.

DeFronzo R, Tobin J, Andres R (1979): "Glucose clamp technique: a method for quantifying insulin secretion and resistance" Am. J. Physiol. 237 (3): E214-23.

Dewell, A; Hollenbeck, P.L.W. and Hollenbeck, C.B. (2006): A Critical Evaluation of the Role of Soy Protein and Isoflavone Supplementation in the Control of Plasma Cholesterol Concentrations. J. Clin. Endocrinol. Metab., 91(3): 772 - 780.

Duranti, M.; Lovati, M.R.; Dani, V.; Barbiroli, A.; Scarafoni, A.; Castiglioni, S.; Ponzone, C. and Morazzoni, P. (2004): The {alpha}' Subunit from Soybean 7S Globulin Lowers Plasma Lipids and Upregulates Liver {beta}-VLDL Receptors in Rats Fed a Hypercholesterolemic Diet . J. Nutr., 134(6): 1334 - 1339.

Eldkak, A.N.H. (2004): Natural antioxidants and cholesterol level in rats. Ph D thesis Fac. of Agric. Cairo Univ.

Forsyth, W. A. (1995): Soy protein thyroid regulation and cholesterol metabolism. J. Nutr., 125: 6195-6235.

Forsyth, W. A.(1986): Comparison of dietary casein or soy proteins effects on plasma lipids and hormone concentrations in the gerbil (Meriones unguiculatous). J. Nutr. 116:1165-1171.

Ghatei, M.A.; Uttenthal, L.O.; Christofides, N.D.; Bryant, M.G. and Bloom, S.R. (1983): Molecular forms of human enteroglucagon in tissue and plasma: plasma responses to nutrient stimuli in health and in disorders of the upper gastrointestinal tract. J. Clin. Endocrinol Metab. 57:488 –495.

Gianazza, E.; Eberini, I.; Arnoldi, A.; Wait, R. and Sirtori, C. R. (2003): A Proteomic Investigation of Isolated Soy Proteins with Variable Effects in Experimental and Clinical Studies J. Nutr., 1, 133(1): 9 - 14.

Greany, K.A; Nettleton, J.A.; Wangen, K.E.; Thomas, W. and Kurzer, M.S. (2004): Probiotic Consumption Does Not Enhance the Cholesterol-Lowering Effect of Soy in Postmenopausal Women .J. Nutr., 134 (12); 3277-3283.

Griffin, E.E. AND Wildenthel K.(1978) Insulin interactions. Am. J. physiol. Endocrinol Metab. 234: 306-313.

Harper, H.A.; Rodwell, V.W.; and Mayes, P.A. (1979): Review of physiological chemistry, 17th ed., Lang Medical, Los Altos, California 9422, pp 434-456.

Issa, H.F.; Aza, A. Bakry; Bassemet G.F. and Fathia, K.S. Abo Zeid (2000): Experimental study of the effects of selenium deficiency. J. Egypt. Soc. Path., 20, 1: 205-13.

Jenkins, D.J.; Kendall, C.W.; Vidgen, E.; Augustin, L.S.; Van Erk, M.; Geelen, A.; Parker, T.; Faulkner, D.; Vuksan, V.; Josse, R. G.; Leiter, L. A. and Connelly, P.W. (2001): High-protein diets in hyperlipidemia: effect of wheat gluten on serum lipids, uric acid, and renal function. Am. J. Clin. Nutri, 74,(1) 57–63.

Jenkins, D.J.;. Kendall, C.W.; Jackson, J.C.; Connelly, P.W; Parker, T.; Faulkner, D.; Vidgen, E.; Cunnane, S. C.; Leiter, L. A. and Josse, R. G. (2002): Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am. J. Clin. Nutr., 76(2): 365 - 372.

Kalton, G. (1983): Models in the Practice of Survey Sampling. International Statistical Review, 51, (8): 175-188

Kerckhoffs, D; F. Brouns, G. Hornstra, and R. P. Mensink (2002) Effects on the Human Serum Lipoprotein Profile of {beta}-Glucan, Soy Protein and Isoflavones, Plant Sterols and Stanols, Garlic and Tocotrienols J. Nutr., 132 (9): 2494 - 2505.

Keyes, W.G. and Heimberg, M. (1979): Influence of thyroid status on lipid metabolism in the perfused rat liver. J. Clin. Invest. 64:182-190.

Kind, P.R.N. and King, E.J. (1954): J. Clin.- phath., 1,322.[c.f.] Varley, H.; Gowenlock, A.H. and Bell, M. (1980): Practical Clinical Biochemistry 5th ed., vol. 1, pp.892.

Klopotek, A; Hirche, F. and Eder, K. (2006): PPAR {gamma} Ligand Troglitazone Lowers Cholesterol Synthesis in HepG2 and Caco-2 Cells via a Reduced Concentration of Nuclear SREBP-2. Exp. Biol. Med., 231(8): 1365 -1372.

Kristensen, A.K.; Brunstedt, J.; Enielsen, J. and Mikkelsen J.D. (2000): Partial characterization and localization of novel type of antifungal protein. Plant Sci.159,29-38.

Lillie, R. D. (1969): Mechanisms of chromation hematoxylin stains. J. Histochem. and Cell Bio. 20 (4):338-354

Lovati, M R; Manzoni, C; Gianazza, E; Arnoldi, A; Kurowska, E ; Carroll K K and Sirtori, CR (2000) Soy Protein Peptides Regulate Cholesterol Homeostasis in Hep G2 Cells. J. Nutr. 130:2543-2549.

Ma, Y.; Chiriboga, D.; Olendzki, B.C.; Nicolosi, R.; Merriam, P. A. and Ockene, I.S. (2005): Effect of Soy Protein Containing Isoflavones on Blood Lipids in Moderately Hypercholesterolemic Adults: A Randomized Controlled Trial J. Am. Coll. Nutr., 24(4): 275 -0 285.

Manzoni, C; Duranti, M.; Eberini, I.; Scharnag, H.; Marz, W.; Castiglioni, S. and Lovati, M.R. (2003): Subcellular Localization of Soybean 7S Globulin in HepG2 Cells and LDL Receptor Up-Regulation by Its {alpha}' Constituent Subunit. J. Nutr.,133(7): 2149 - 2155.

Mullen, E.; Brown, R. M.; Osborne, T. F. and Shay, N. F. (2004): Soy Isoflavones Affect Sterol Regulatory Element Binding Proteins (SREBPs) and SREBP-Regulated Genes in HepG2 Cells. J. Nutr., 134(11): 2942 - 2947.

Murray, R. K.; Granner, D. K.; Mayes, P. A. and Rodwell, V. W.(2003): Harpers; Illustrated Biochemistry, 26th Ed., Lang Medical, MC-Graw -Hill Professional, West Lafayette, Indiana.

Okita, T. and Sugano, M. (1990): Effects of dietary protein levels and cholesterol on tissue lipids of rats. J. Nutr. Sci. Vitaminology, 36: S151-S156.

Pena-Ramos, E.A. and Xiong Y. I.(2002): Antioxidant activity of soy protein hydrolysates in liposomal system. J. Food Sci. 67, 8: 2952.

Potter, S.M. (1996): Soyprotein and protein isolate similarly lower blood cholesterol but differently affect thyroid hormones in hamsters. J. Nutr. 126, 2007-2011

Reithman, S. And Frankel, S. .(1957):"A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyrovic transaminase" Am. J. Clin. Path. 28,56.

Scholz-Ahren, K.; Hagemeister, H.; Unshelm, J.; Agergaard, N. and Borth, C. (1990): Response of hormones modulating plasma cholesterol to dietary casein or soy protein in minipigs. J. Nutr., 120:1387-1392.

Sirtori, C. R.; Lovati, M. R.; Manzoni, C.; Castiglioni, S.; Duranti, M.; Magni, C.; Morandi, S.; Agostina, A. D and. Arnoldi, A (2004): Proteins of White Lupin Seed, a Naturally Isoflavone-Poor Legume, Reduce Cholesterolemia in Rats and Increase LDL Receptor Activity in Hepg2 CellsJ. Nutr., 134(1): 18 - 23.

Wahlefeld, A. (1974): Triglycerides: determination after enzymatic hydrolysis. In: Methods of Enzymatic Analysis(Bergmeyer, H. U., ed.),2nd ed., pp. 1831-1835. Academic Press, New York, NY.

Wolfe, B.M. and Greace, D.M. (1987): Substitution of mixed amino acid resembling soy protein for mixed amino acid in slowly, but not rapidly fed not fasted baboons. Metabolism, 36 (3): 223-229.

Wolfe, B.M. and Keyes, W.G. and Heimberg, M. (1979): Influence of thyroid status on lipid metabolism in the perfused rat liver. J. Clin. Invest. 64: 182-190.

Xiao, CV; L'Abbe, M.R.; Gilani, G.S.; Cooke, G.M.; Curran, I.H. and Papademetriou; S.A. (2004): Dietary Soy Protein Isolate and Isoflavones Modulate Hepatic Thyroid Hormone Receptors in Rats J. Nutr., 134(4): 743 - 749.

دور التوافق البيولوجى لبروتين الغذاء فى خفض كولسترول الدم فى الفئران

على إبراهيم سيد احمد*، فضل السيد عبده الديب، داليا النحال***

* قسم الأغذية الخاصة والتغذية، معهد تكنولوجيا الأغذية، مركز البحوث الزراعية- مصر.

** كلية التربية النوعية بدمياط - جامعة المنصورة.

استنادا الى العديد من النظريات المقترحة لتفسير العلاقة العلاجية لخفض نسبة كولسترول الدم بواسطة البروتينات النباتية،تم فى هذه الدراسة استخدام بروتين الصويا القابل للهضم(PPD) كمصدر مدروس للبروتينات النباتية الفعالة فى هذا المجال.

في دراسات سابقة ،تم افتراض أن العامل التنظيمي لهذا المستخلص البروتيني (PPD)يرجع لتأثيره على مستوى التعبير الجيني. حيث يؤدى ذلك الى

مستوى من التوازن الهرموني الطبيعي الذى يحدث نتيجة للتحكم فى الإفرازات الخاصة بالغدد الصماء و الأطراف العصبية،و قد يكون هناك مستوى تنظيمى ميتابوليزمى بديل فيما يسمى بحالات الطوارئ ترتكز على وجود آليات (ميكانيكيات) بديلة ينظمها تأثير الغذاء المتوازن أو التأثير العلاجي له.

أظهرت نتائج هذه الدراسه ان حقن فئران التجارب بمستخلص هذا البروتين (PPD)أدى الى تحسن ملحوظ في الشكل الظاهرى ووظائف الكبد وليبوبروتينات الدم.كذلك وجد ان ارتباط مستويات بعض الهرمونات للمعدل الطبيعي كانت مقترنة بافتراض وجود هذه الآليات البديلة ، ولكن هذه الاليه كانت قادرة على حمايه الكبد والحفاظ علي الصفات التشريحية له الى اقرب مستوى من الحالة الطبيعية. أيضا قد يكون لوجود هذه التراكيب الغذائية البروتينية(PPD) فعالية حيوية التى نستطيع معها ان نعتبره شكلا من اشكال التنظيم المعتمد على الناقلات العصبية،أو حتى يكافئ تأثير الانترليوكين في الشكل والوظيفة على التمثيل الغذائى. هذا التصحيح لعمليات التمثيل الغذائى بصفة عامة والتى أثرت ايجابيا على كل من الصفات المورفولوجية و التشريحية للكبد والأنسجة،و ظائف الكبد ، ليبوبروتينات الدم والتى ادت الى حمايتها كليا الى الحد الطبيعى المعترف به.

وعلى ذلك، فقد أظهرت نتائج هذه الدراسة أهمية اجراء المزيد من الدراسات على هذا الموضوع نظرا لانه قد لوحظ ان الاغذيه الطبيعيه التى لها تأثير علاجي لا تؤثر سلبيا في طول عمر العضو(الكبد)،كما يحدث عند استخدام الأدوية الكيميائية المعالجة للكولسترول.