Egyptian J. of Nutrition Vol. XXXVI No. 2 (2021)

Effect of Calcium and Vitamin D Intake on Lipid Profile, Kidney and Liver Functions in Post weaning Rats

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Abstract

The present study was conducted to determine the effects of calcium and vitamin D intakes on lipid profile, kidney, and liver functions in post weaning albino rats. A total of 66 male albino rats of Sprague Dawley Strain, 25 days of age and weighing (40 ± 5g) were used in this study. The rats were divided into two main groups: the first main group (n=6) fed on a basal diet (BD) and used as a control (-ve) group. The second main group (60 rats) was divided into (10 subgroups) one of them (6 rats) fed on (BD) and considered as control positive (+ve) group. The other (9 subgroups) were fed on (BD) containing different levels (50,150&200%) from calcium, vitamin D, and calcium plus vitamin D, for 8 weeks, followed by the second 8 weeks, rats were fed on high-fat diet (HFD) as the following. The

positive control group (+ve) shift up to feed on (HFD), while the other (9 subgroups) were fed on (HFD) containing the same previous different levels (50,150&200%) from calcium, vitamin D, and calcium plus vitamin D.

At the end of the experiment, rats were sacrificed and blood samples were collected, then serum was separated, lipid profile as well as concentration of uric acid, urea nitrogen, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT) enzymes, were determined. The obtained results revealed that feeding the early post weaning rats for the first 8 weeks on (BD) containing 50% from calcium, vitamin D & calcium plus vitamin D requirements followed by second 8 weeks fed on (HFD) containing the same low level from Ca, Vit. D & Ca plus Vit D caused a significant increase in total cholesterol (TC), Triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), AST and ALT, serum uric acid, urea nitrogen and creatinine, while showed a significant decrease in serum high-density lipoprotein cholesterol (HDL-c) level. On the other hand, our results revealed a group of early post weaning rats fed for the first 8 weeks on (BD) supplemented with 150 and 200% from calcium, vitamin D& calcium plus vitamin D, followed by second 8 weeks fed on (HFD) contained the same supplementation, induced a significant decrease in (TC), (TG), LDL-c , VLDL-c , AST , ALT , serum uric acid , urea nitrogen

and creatinine , while a significant increase in the concentration of HDL-c, occurred in post weaning rats fed on (HFD) supplemented with calcium, vitamin D and calcium plus vitamin D.

In conclusion results taken together indicated that at early post weaning supplemented diet with calcium plus vitamin D may be have a significant effect to compate later obesity and its complications such as hyperglycemia, hypercholesterolemia and non-alcoholic fatty liver disease.

Key Words: vitamin D - calcium - liver function - kidney function – early post-weaning – obesity – lipid profile – complications of obesity .

Introduction

Calcium, the most abundant mineral in the human body, is involved in various physiological processes. In addition, it has been suggested that it may assist body weight control. Two possible mechanisms of action have been proposed to explain these effects. It seems that high quantities of calcium are consumed, it binds to dietary fat forming insoluble compounds, reducing fat absorption and hence the number of calories generated from this absorption ***(Rajpathak et al., 2008).***

The combination of low serum 25- hydroxyl vitamin D and inadequate calcium intake has been associated with cardiovascular

risk factors such as hypertension, Obesity, Metabolic syndromes, and type 2 Diabetes Mellitus ***(Buyukinan et al., 2012).*** The prevalence of obesity is globally increasing and associated with a higher mortality ***(Flegal et al., 2013).*** Obesity is a major risk factor of cardiovascular disease ***(Twig et al., 2016).***

Material and Methods

Chemicals: Casein, vitamins, minerals, cellulose, choline chloride were obtained from EL- Gomhoria company, Cairo, Egypt, Starch, and soy oil were obtained from the local market.

Kits: Kits for biochemical analysis were obtained from Gamma Trade Co. for pharmaceuticals and chemicals. Dokki, Egypt.

Rats: Sixty-six male albino rats of Sprague Dawley strain, 25 days of age weighing (40 ± 5g), were obtained from the laboratory of animals colony, Ministry of Health and population, Helwan, Cairo, Egypt.

Experimental Animal Design:

Rats were housed in individual cages under hygienic laboratory conditions and were fed on a basal diet for one week for adaptation. After this week, the rats were divided into two main groups, the first main group (6 rats) was fed on the basal diet (BD) as a control negative group (-ve). The second main group: sixty rats were fed for 8 weeks on (BD) containing different levels (50, 150, and 200%) from calcium, vitamin D and calcium plus vitamin D as the following: Subgroup one, salt mix containing 100% from calcium and

vitamin D requirements and used as a positive control group. Subgroup two ,fed on (BD) salt mix containing 50% from calcium requirement, subgroup three, fed on (BD) salt mix containing 150% from calcium requirements while subgroup four, the salt mixture containing 200% calcium requirement. Subgroup five, fed on (BD) containing 50% from vitamin D requirement while subgroups, six and seven fed on (BD) vitamin mixture containing 150 and 200% from vitamin D requirements, while, subgroup eight fed on (BD) containing 50% from calcium plus 50% from vitamin D, while nine and ten subgroups fed on (BD) containing 150, 200% respectively from calcium plus vitamin D.

After the first 8 weeks all animals of the second main group, followed by the second 8 weeks, rats were fed on a high-fat diet (HFD) as the following: The positive control group shift up to feed on (HFD) containing 100% from calcium and vitamin D requirements., while the other (9 subgroups) were fed on (HFD) containing the same previous different levels (50, 150, 200%) from calcium and vitamin D.

The basal diet in the preliminary experiment consists of 14% casein (protein>85%), corn oil 4% , salt
mixture 3.5% vitamins mixture 1% choline chloride 0.25% , cellulose 5% and (72.25%) corn starch ***(Reeves et al., 1993).*** The salt mixture and vitamin mixture were prepared according to ***Hegsted et al., (1941)*** and ***Campbell (1963).*** After a period, adaptation followed by the first 8 weeks on basal diet. The second main group shift up to feed on high fat diet for another second 8 weeks containing (14% protein from casein, 20% fat, “19% saturated fat and 1% unsaturated

fat", 5% cellulose, 3.5% salt mixture, 1% vitamin mixture, 10% sucrose, 0.25% choline chloride and the reminder was corn starch ***(Min et al., 2004).*** Calcium and vitamin D were calculated as a percentage of the standard requirements. The percentage between calcium and phosphorus was 1:1 in all experimental levels.

Biochemical analysis of serum:

At the end of the experiment period, the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were collected from the hepatic portal vein by the fine capillary glass tubes ***Schermer, (1967).***

Blood samples were received into a clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m. to separate serum. Serum was carefully separated into dry clean Wasserman tubes, using a Pasteur pipette and kept frozen at (-20°C) till estimation of some biochemical parameters. Serum samples used for determination of total cholesterol ***(Allain et al., 1974)*** , triglycerides ***(Foster and Dum., 1973)*** , high-density lipoprotein cholesterol HDL-c ***(Lopes – virella et al., 1977).*** While serum low-density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated according to the equation of ***Friedwald et al., (1972).*** Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined enzymatic calorimetrically according to the method of ***Henry et al., (1974).*** Serum uric acid was determined according to

Henry et al., (1974). Serum creatinine was determined according to the method described by ***Bartels and Bohmer, (1971).***

Statistical analysis was carried out using SPSS statistical software version 11 ***(SAS., 2004).***

Results and Discussion

Effects of Feeding Early Post Weaning Rats on Basal Diet Containing Different Levels from Calcium & Vit D for 8 Weeks Followed by Second 8 Weeks Fed on High Fat Diet Containing the Same Level from Ca & Vit D on Serum Lipid Profile:

Tables (1&2) shows the effects of feeding on (BD) for 8 weeks followed by the second 8 weeks fed on (HFD) containing different levels (50, 150& 200%) from calcium, vitamin D, and calcium plus vitamin D on total cholesterol (TC), triglycerides, (TG) high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c).

Results show the effect of feeding on (BD) for 8 weeks followed by second 8 weeks on (HFD) containing low levels (50%) from calcium, vitamin D and calcium plus vitamin D. The mean values of serum (TC), (TG) (LDL-c& VLDL-c) significantly ($P< 0.05$) increased as compared to the control (+ve) group which fed on (BD) for 8 weeks followed by second 8 weeks, fed on (HFD) containing (100%) from calcium and vitamin D requirements while HDL-c recorded a significant decrease as compared to the (+Ve) control group.

Our results also revealed that groups of early post weaning rats which fed on (BD) for 8 weeks followed by second 8 weeks, fed on (HFD) supplemented with (150 or 200%) from calcium, vitamin D & Calcium plus vitamin D induced a significant decrease in (TC), (TG), (LDL-c) & (VLDL-c), while results showed a significant increase. ($P< 0.05$) in HDL-c as compared to the (+ ve) control group.

In this respect ***(Van Meijl et al., 2008)*** reported that adequate calcium may induce beneficial effects on lipids and lipoprotein profiles, with a decrease in LDL-c and triglycerides concentrations.

Our results are in harmony with ***(Slusher et al., 2015)*** who demonstrated the essential role of vitamin D in the regulation of skeletal metabolism as well as calcium and phosphate homeostasis, while vitamin D receptor (VDR) regulates de novo lipid synthesis. In rats early life, high calcium intake can continue to affect adulthood obesity induced by high- fat feed increase, expression level UCP2 m RNA, improve the disorder of blood fat metabolism **(*Xu et al., 2014).***

In this concern ***(Vozarova et al., 2002)*** suggested that there is a correlation between lipoprotein cholesterol changes and obesity in obesity cases there is often a decrease in HDL-c level, and elevation of HDL-c is one of the targets for anti- obesity treatment ***(Vozarova et al., 2002).***

Effects of Feeding Early Post Weaning Rats on Basal Diet Containing Different Levels from Calcium &Vitamin D for8 Weeks Followed by Second 8 Weeks Fed on High Fat Diet (HFD)Containing The Same Levels from Ca & Vit D on Kidney Functions:

Table (3) shows the effects of feeding on (BD) for 8 weeks followed by the second 8 weeks fed on (HFD) containing different levels (50, 150& 200%) from calcium, vitamin D & calcium plus vitamin D on Kidney functions. Statistically, results reveal a significant increase ($P < 0.05$) in serum levels of uric acid, urea nitrogen and creatinine of the (+Ve) control group fed on (HFD) as compared to control (-ve) fed on (BD).

On the other hand, results of the positive groups which fed for 8 weeks on (BD) containing low level 50% from calcium, vit D &Ca plus vit D followed by second 8 weeks fed on (HFD) containing the same low level from calcium, vitamin D& Calcium plus vitamin D, results recorded a significant increase in serum levels from uric acid, urea nitrogen, and creatinine as compared to the positive control group which fed on (HFD) containing (100%) from calcium and vitamin D requirements. On the other side, our results showed that groups of post weaning rats fed on (BD) supplemented with (150 or 200%) from calcium , vitamin D & calcium plus vitamin D induced a

significant decrease ($p < 0.05$) in serum levels of uric acid, urea nitrogen and creatinine as compared to the positive control group.

In this respect ***(Marotte et al., 2014)*** demonstrated that the negative effect of a low Ca diet on fat mass accumulation and lipid profile, may be more evident in rats predisposed to obesity low Cal interferes with the normal glucose homeostasis leading to an increase in insulin resistance.

Effects of Feeding Early Post Weaning Rats on Basal Diet Containing Different Levels from Calcium and Vitamin D for 8 Weeks Followed by Second 8 Weeks Fed on High Fat Diet Containing The Same Levels From Ca And Vit D on Liver Enzymes:

Table (4) illustrate the effects of feeding (BD) for 8 weeks followed by the second 8 weeks fed on (HFD) Containing different levels (50, 150 & 200%) from calcium, vitamin D & calcium plus vitamin D on Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) levels in serum of early post weaning rats. Statistically results revealed that the mean values of serum (AST& ALT) for the control (+ ve) group and the (-ve) control group recorded (157.032 ± 4.464 and 83.480 ± 5.953 VS 58.416 ± 2.789 and 20.512 ± 1.910 respectively).

Statistically, results recorded a significant increase ($P < 0.05$) of AST & ALT enzymes of the (+ ve) control group fed on (HFD) as compared to the (-ve) control group fed on (BD).

Concerning early post weaning rats (+ve) groups which fed for 8 weeks on (BD) containing low level from Ca, vit D & Ca plus vit D, followed by second 8 weeks fed on (HFD) containing the same low level from Ca, vit D &Ca plus vit D. Our results revealed that a significant increase ($P< 0.05$) in serum liver enzymes AST & ALT as compared to the (+ve) control group fed on (HFD) Containing 100% of Ca and vit D requirements.

On the other hand, results revealed that all groups of early post weaning rats which fed for 8 weeks on supplemented (BD) with 150 Or 200% from Ca, Vit D &Ca plus vit D, followed by second 8 weeks fed on (HFD) supplemented with the same levels from Ca, vit D &Ca plus vit D. Results recorded a significant decrease ($p < 0.05$) in serum liver enzymes (AST & ALT) as compared to the (+ve) control group which fed on (HFD) Contained 100% from Ca or vit D. The best results recorded by groups which fed on (HFD) supplemented with 200% from Ca plus vit D followed by level 150%.

In this concern ***(panchal et al., 2013)*** suggested that high-carbohydrate, high-fat diet fed rats developed impaired glucose tolerance, non alcoholic fatty liver disease with an increased protein level of NrF2 and CPTI in the heart and liver.

Conclusion

In conclusion, the results taken together indicated that at early post weaning age supplemented diet with calcium plus vitamin D may have a significant effect to compete for later obesity and its complications such as hyperglycemia, hypercholesterolemia, and non-alcoholic fatty liver disease.

Table (1): Effects of Feeding Early Post Weaning Rats on BD Containing Different Levels from Calcium and Vitamin D for 8 Weeks Followed by Second 8 Weeks on HFD Containing the Same Levels from Ca and Vit D or Serum Cholesterol and Triglycerides:

Groups \ Parameters		Cholesterol	Triglycerides
		mg / dl	
Control (-ve) fed on *BD All over the experiment		73.873^{g}± 3.357	39.864^{h}±2.963
Control (+ve) fed on **HFD all over the experiment		165.852^{c}± 6.122	78.779^{c}± 3.918
Groups fed on (BD) for 8 weeks containing different levels from Ca&vit D followed by second 8 weeks on (HFD) containing the same levels from Ca&vit D	50% Ca	180.258^{b}± 6.755	98.091^{b}± 4.883
	150 % Ca	148.643^{d}± 3.145	66.325^{de}± 7.098
	200% Ca	136.581^{e}± 2.083	57.825^{f}± 5.666
	50 % Vit. D	177.236^{b}± 6.948	92.992^{b}± 4.443
	150% Vit. D	146.347^{d}±5.144	96.635^{d}± 4.064
	200% Vit. D	135.391^{e}± 4.341	61.225^{ef}± 4.015
	50 % Ca&Vit. D	187.380^{a}± 3.538	107.076^{a}± 5.045
	150% Ca&vit. D	134.574^{e}± 4.177	58.497^{f}± 3.559
	200% Ca&vit.D	117.923^{f}±5.037	47.962^{g}± 4.955

*(BD) Basal Diet

**(HFD) High Fat Diet

***(LSD) Least significant differences at P<0.05

Means Values in each column which have different litters are significantly different (P<0.05)

Table (2): Effects of Feeding Early Post Weaning Rats on BD Containing Different Levels from Calcium and Vitamin D for 8 Weeks Followed by Second 8 Weeks on High Fat Diet Containing the Same Levels from Ca &Vit D on Serum Lipoproteins :

Parameters / Groups		HDL –c	LDL- c	VLDL –c
		mg /dl		
Control (-ve) fed on *BD All over the experiment		46.961^{a}±2.754	18.923^{g}± 1.205	7.972h±0.592
Control (+ve) fed on **HFD all over the experiment		19.354^{e}±0.781	130.742 c±5.964	15.755c±0.783
Groups fed on (BD) for 8 weeks containing different levels from Ca & vit D followed by second 8 weeks on (HFD) containing the same levels from Ca & vit D	50% Ca	17.385f^{g}±0.424	143.254b± 6.953	19.618b± 0.956
	150% Ca	26.859^{d}±1.625	108.151d± 1.659	13.264de±1.419
	200% Ca	32.613^{c}± 1.491	92.403 e± 2.416	11.564 f± 1.133
	50% Vit. D	18.55 $^{e\,f}$±0.946	140.079b± 7.181	18.598b± 0.888
	150% Vit. D	27.272^{d}± 1.366	105.148d± 4.114	13.926d± 0.812
	200% Vit D	34.424^{c}± 2.278	88.722e± 3.411	12.244ef± 0.802
	50% Ca&Vit D	16.016^{g}± 0.853	149.948a±3.915	21.414a± 1.008
	150% Ca&Vit. D	33.345^{c}± 0.904	89.629e± 3.520	11.699f± 0.711
	200% Ca&Vit. D	38.520^{b}± 1.594	96.793f± 3.719	9.592g± 0.991

*(BD) Basal Diet

**(HFD) High Fat Diet

***(LSD) Least significant differences at P<0.05

Means Values in each column which have different litters are significantly different (P<0.05)

Table (3): Effects of Feeding Early Post Weaning Rats on BD Containing Different Levels from Calcium and Vitamin D for 8 Weeks Followed by Second 8 Weeks on High Fat Diet Containing the Same Levels from Calcium and Vitamin D on Serum Uric Acid, Urea Nitrogen and Creatinine :

Parameters / Groups		Uric Acid	Urea Nitrogen	Creatinine
		mg /dl		
Control (-ve) fed on *BD All over the experiment		$1.362^{i}\pm 0.085$	$24.345^{i}\pm 1.243$	$0.556^{g}\pm 0.059$
Control (+ve) fed on **HFD all over the experiment		$2.803^{c}\pm 0.062$	$60.550^{cd}\pm 3.423$	$1.852^{b}\pm 0.069$
Groups fed on (BD) for 8 weeks containing different levels from Ca & vit D followed by second 8 weeks on (HFD) containing the same levels from Ca & vit D	50% Ca	$3.158^{a}\pm 0.098$	$61.745^{bc}\pm 3.688$	$1.833^{b}\pm 0.038$
	150% Ca	$2.310^{e}\pm 0.130$	$53.252^{e}\pm 2.100$	$1.462^{d}\pm 0.060$
	200% Ca	$2.575^{d}\pm 0.078$	$57.592^{d}\pm 2.428$	$1.693^{c}\pm 0.048$
	50% Vit. D	$2.992^{b}\pm 0.078$	$64.685^{b}\pm 3.790$	$1.683^{c}\pm 0.087$
	150% Vit. D	$2.163^{f}\pm 0.106$	$51.187^{ef}\pm 1.93$	$1.417^{d}\pm 0.097$
	200% Vit D	$1.880^{h}\pm 0.084$	$44.087^{h}\pm 2.312$	$1.074^{f}\pm 0.110$
	50% Ca&Vit D	$3.222^{a}\pm 0.116$	$70.992^{a}\pm 4.103$	$2.001^{a}\pm 0.086$
	150% Ca&Vit. D	$2.007^{g}\pm 0.087$	$45.897^{gh}\pm 2.513$	$1.133^{f}\pm 0.119$
	200% Ca&Vit. D	$2.154^{f}\pm 0.105$	$48.047^{fg}\pm 2.489$	$1.257^{e}\pm 0.093$

*(BD) Basal Diet

**(HFD) High Fat Diet

***(LSD) Least significant differences at P<0.05

Means Values in each column which have different litters are significantly different (P<0.05)

Table (4): Effects of Feeding Early Post Weaning Rats On BD for 8 Weeks Containing Different Levels from Calcium and Vitamin D Followed by Second 8 Weeks On High Fat Diet Containing the Same Levels from Cal &Vit D On Liver Enzymes:

Parameters / Groups		AST	ALT
		U/L	
Control (-ve) fed on *BD All over the experiment		$58.416^{i}\pm 2.789$	$20.512^{h}\pm 1.910$
Control (+ve) fed on **HFD all over the experiment		$157.032^{d}\pm 4.464$	$83.480^{cd}\pm 5.953$
Groups fed on (BD) for 8 weeks containing different levels from Ca & vit D followed by second 8 weeks on (HFD) containing the same levels from Ca & vit D	50% Ca	$165.094^{c}\pm 4.428$	$87.349^{c}\pm 4.158$
	150% Ca	$145.887^{f}\pm 3.670$	$74.228^{e}\pm 3.764$
	200% Ca	$150.060^{e}\pm 4.602$	$79.377^{d}\pm 3.893$
	50% Vit. D	$169.716^{b}\pm 3.765$	$92.423^{b}\pm 4.748$
	150% Vit. D	$145.244^{ef}\pm 4.006$	$73.191^{e}\pm 4.744$
	200% Vit D	$136.296^{g}\pm 3.875$	$65.777^{f}\pm 3.733$
	50% Ca&Vit D	$177.444^{a}\pm 2.654$	$98.022^{a}\pm 3.609$
	150% Ca&Vit. D	$132.535^{g}\pm 2.135$	$63.091^{fg}\pm 3.204$
	200% Ca&Vit. D	$124.450^{h}\pm 30699$	$59.266^{g}\pm 3.509$

*(BD) Basal Diet

**(HFD) High Fat Diet

***(LSD) Least significant differences at P<0.05

Means Values in each column which have different litters are significantly different (P<0.05)

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تأثير المأخوذ من الكالسيوم وفيتامين د على صورة دهون الدم ووظائف الكلى والكبد في الفئران بعد فطامها.

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الملخص العربي

أجريت هذه الدراسة لمعرفة تأثير المأخوذ من الكالسيوم وفيتامين د في الفئران بعد فطامها على صورة دهون الدم ووظائف الكلى والكبد. استخدم لإجراء هذه الدراسة عدد ٦٦ فأر من ذكور الالبينو فصيلة اسبراجو دولي عمر ٢٥ يوم تتراوح أوزانهم (٤٠ ± ٥ جرام). ثم تقسيم الفئران على مجموعتين رئيستين. المجموعة الرئيسية الأولى (٦ فئران) ثم تغذيتهم على الغذاء الأساسي وتم استخدامهم كمجموعة ضابطة سالبة، المجموعة الرئيسية الثانية مكونة من (٦٠ فأر) تم تقسيمها إلى عشر مجموعات فرعية واحده منهم (٦ فئران) تم تغذيتها على الغذاء الأساسي بينما التسعة مجموعات الأخرى تم تغذيتها على الغذاء الأساسي مع احتوائه على نسب (٥٠ أو ١٥٠ أو ٢٠٠%) من الاحتياجات لكلا من الكالسيوم، فيتامين د والكالسيوم مع فيتامين د وذلك لمدة ٨ أسابيع أعقبها٨ أسابيع ثانية تم فيه تغذية العشرة مجموعات الفرعية على غذاء عالي الدهون يحتوي على نفس التركيزات السابقة من الكالسيوم، فيتامين د ، الكالسيوم مع فيتامين د وفي نهاية التجربة تم تخدير الفئران كما تم تجميع عينات الدم وفصل السيرم لتقدير الكوليسترول الكلي، والجلسريدات الثلاثية، الليبوبروتينات، حمض اليوريك، اليوريا نيتروجين، الكرياتين كذلك تقدير إنزيمات الكبد وقد أوضحت النتائج أن مجموعات الفئران المفطومة التي تغذت لمدة ٨ أسابيع الأولى على الغذاء الأساسي المحتوى على ٥٠ % من الاحتياجات من الكالسيوم، وفيتامين د & الكالسيوم مع فيتامين د قد أدى ذلك إلى زيادة معنوية في مستوى كلا من الكوليسترول الكلي، الجلسريدات الثلاثية، الليبوبروتينات منخفضة الكثافة، الليبوبروتينات منخفضة الكثافة جدا. كذلك أدى إلى ارتفاع مستوى حمض اليوريك، اليوريانيتروجين والكرياتين كذلك ارتفاع إنزيمات الكبد

(AST & ALT) بينما أدى ذلك أيضا إلى انخفاض الليبوبروتينات عالية الكثافة. ومن ناحية أخرى أوضحت النتائج أن المجموعات الفرعية الأخرى التي تغذت لمدة ٨ أسابيع أولى على الغذاء الأساسي والمدعم بنسبة (١٥٠% أو ٢٠٠%) من الكالسيوم، فيتامين د، الكالسيوم مع فيتامين د. ثم تلي ذلك التغذية لمدة ٨ أسابيع ثانية على غذاء عالي الدهون يحتوي على تدعيم بالكالسيوم، فيتامين د، الكالسيوم وفيتامين د. بذات النسب. وقد أدى ذلك انخفاض معنوي في مستوى السيرم لكل من الكوليسترول الكلي، الجلسريدات الثلاثية ، الليبوبروتينات منخفضة الكثافة، الليبوبرتينات منخفضة الكثافة جداً، حمض اليوريك، اليوريا نيتروجين، الكرياتين وانزيمات الكبد (AST & ALT) بينما أظهرت النتائج ارتفاع في مستوى الليبوبروتينات عالية الكثافة وذلك كنتيجة للتدعيم بالكالسيوم وفيتامين د. ونستخلص من النتائج أنه في مرحلة الفطام المبكر التغذية على غذاء مدعم بالكالسيوم وفيتامين د ربما يكون له تأثير مؤكد لمنع حدوث السمنة فيما بعد ومضاعفاتها مثل ارتفاع سكر الدم، ارتفاع الكوليسترول الضار والإصابة بالكبد الدهني.

الكلمات المفتاحية : فيتامين د – الكالسيوم – الفطام المبكر – السمنة – مضاعفات السمنة - دهون الدم – وظائف الكبد – وظائف الكلى .