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Thromboelastographic Assays of the Clotting Process in Situations of Obesity and Caloric Restriction

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A study on blood clotting has been carried out in a number of obese individuals and compared to a group of non-obese persons, in order to assess if the former can be considered to be in «high risk» regarding the onset of a thromboembolic process. The technique of thromboelastography was chosen. The results point out that in obese people a series of alterations take place, both in the time of clot formation, which is enlarged, as in the organization of its nets, which appear strongly structured, favoured by the hyperfibrinogenemia and thrombocytosis detected in these subjects. Likewise, the effect of a hypocaloric diet on clotting in obese persons has been evaluated and compared with the former groups. Clotting in treated obese individuals is modified in the same way as in the untreated group when compared to the non-obese population; nevertheless, when both groups of obese people are compared, no significant difference is observed in the different parameters studied, even though constants determined in citrated whole blood are closer to normality in the subjects undergoing caloric restriction.

Key words: Coagulation, Caloric restriction, Obesity, Thromboelastography.

Changes experimented in society in recent years have favoured a more readily acquisition of larger amounts of food by its members. At the same time the technological advancement offers greater possibilities of shirking physical activity with the overall result of the apparition of a high number of obese people (16).

According to JENSEN (8), obesity is the result of an ingestion of calories in the shape of food that exceeds the expenditure of energy, i. e. less is consumed than it is ingested, which favours the apparition of a positive energy balance (16). To fight obesity different sorts of treatment have arisen, directed mainly to making these individuals lose weight, aiming before all at an improvement in

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their situation. The most widespread are the dietary ones. From different statistical studies it has been concluded that there exists a close relationship between obesity and a number of diseases (4, 8, 16) among which those affecting the cardiovascular system and hypertension, which occurs with a higher frequency in these people (1), must be pointed out.

This number of alterations taking place in human obesity, and the fact that some anomalies tend towards normalization after losing weight, drove us to center the aim of this work in the study of modifications that can be present in the process of clotting in obese individuals, both with or without caloric restrictions, in order to know if a relationship exists with the onset of a thromboembolic process, and in this case, to observe whether diet has any influence on diminishing the thromboembolic risk.

Materials and Methods

Samples of blood were obtained by venous puncture from different healthy volunteers of both sexes, aged between 15 and 50 years. Three lots were then arranged. First, the control group, made up by 15 non-obese individuals, with an average weight of 61.09 ± 3.57 kg and a medium relative weight (actual weight/ideal weight × 100) (1) of 96.04 ± 1.42 (C). Secondly, a group constituted by 10 obese people not subjected to any form of treatment, either diet or drugs, with an average weight of 84.9 ± 2.23 kg, and a medium relative weight of 128 ± ± 3.9 (O). Last, a group including 20 subjects undergoing a hypocaloric diet of approximately 500 cal daily with an average weight of 82.24 ± 4.1 kg and a medium relative weight of 135.47 ± 6.7 (TO).

Blood is collected according to RABY (12). To study blood clotting thromboelastography was chosen because it is a test that yields a global view about how the whole haemostatic process is accomplished (2, 12, 13, 15). The apparatus used was a direct-reading thromboelastograph (Clotscanner Elvi 810 model).

Three types of TEG* have been made according to RABY (12, 13): in CWB which lets us know how the clot has been structured; in PRP in order to know the functional role of fibrin and platelets without the interference of other formed elements and the hematocrit; and lastly, in PPP, which indicates the contents of fibrinogen, and when compared to PRP, the way platelets influence its structure.

On these subjects a series of analysis were carried out: cellular counts (red blood cells, leucocytes and platelets), hematocrit, hemoglobin, differential leucocyte count and sedimentation rate, according to the classical methods of CISCAR and FARRERAS (6), in order to establish whether they presented any sort of anomaly interfering with the results; if that was the case they were discarded.

Results and Discussion

In table I, for determinations of CWB, it can be seen that even though reactivity time r is a little shortened in both obese groups the difference is not significant with the control; therefore thromboplastin-formation time remains more or less constant. Clotting speed k is significantly diminished in group O so that thrombin activity and consequently formation of fibrin are increased. Nevertheless, in group TO, though the value is a little lower, it does not show any significant difference with the control group, and therefore the formation of fibrin is not increased. $r + k$, an index of the time elapsed until the apparition of the visible clot, is significantly shortened in

* Abbreviations: CWB = citrate whole blood; PPP = platelet-poor plasma; PRP = platelet-rich plasma; TEG = thromboelastograms; TPI = thrombodynamic potential index.

Table I. *Thromboelastographic parameters from obese (O) and treated obese subjects (TO) compared to a group of non-obese persons (C). Tracing obtained in citrated whole blood.* The treated individuals have undergone a hypocaloric diet of approximately 500 cal daily. Each value is the mean ± S.E.

Parameters (mm)	Group (C) n = 15	(O) n = 10	(TO) n = 20
r = reactivity time	12.70 ± 0.50	11,90 ± 0.64	12.57 ± 0.35
k = clotting speed	10.63 ± 0.57	8.50 ± 0.72*	9.47 ± 0.40
r + k = clotting time	23.33 ± 0.82	20.40 ± 1.16*	22.05 ± 0.55
ma = maximum amplitude	43.20 ± 0.89	50.25 ± 1.18**	48.55 ± 0.69**
Emx = maximum elasticity coefficient	76.65 ± 2.68	102.05 ± 4.85**	95.05 ± 2.66**
TPI = thrombodynamic potential index	7.65 ± 0.65	13.39 ± 2.00**	10.35 ± 0.49**

* $p < 0.05$, ** $p < 0.01$, *vs* control.

O, which shows, according to KIMCHE and EISENKRAFT (9), BELLON (3) and SULTAN (14), that there exists a certain hypercoagulability of a kinetic nature. This shortened time can be favoured as well by the biochemical activity of the formed elements present in this sort of sample (13). This kinetic hypercoagulability is an indication that the clot will be formed in a time shorter than normal. In group TO, although showing a tendency, the decrease is not significant, which means that there exists a chronometric normocoagulability.

Maximum amplitude (*ma*) thromboelastographic constant «par excellence» (6, 15) that points to the maximum intensity of the mechanical properties of the clot, experiences a considerable increase in both obese groups, being more pronounced in those not treated. This elevation may be favoured by fibrinogen and platelets, because these two are the factors that, according to LEE (10), MARCHAL (11) and RABY (13) have a greater influence on the structure of the clot and therefore, on *ma*. In fact, a hyperfibrinogenemia has been detected in the determination in PPP, which will be commented on later, and besides, a significantly increased number of platelets in both obese groups relative to control (C: 264.97 ± 15.7 × 10^3 p/mm^3, O: 328.2 ± 22.02 × 10^3 p/mm^3 and TO: 364.65 ± 26.1 × 10^3 p/mm^3; being p 0.01 among them). These results are in agreement with BUTLER (5) and HOWLAND (7) who also find that fibrinogen and platelets induce an increase in *ma*. The maximum elasticity coefficient, *Emx*, being a multiple of *ma*, is modified in the same direction and due to the same reasons as *ma* is, so that it will be increased in O and TO. At any rate they are higher in the untreated ones.

The TPI is significantly increased in both obese groups, the increase being more marked in group O. This index, proposed by RABY (12, 13), easily evaluates the dynamic aspect of clotting, i.e. how the structure of the clot has been developed, on which its greater or smaller efficiency to stop the outflow of blood depends. The increase detected is a proof of structural or dynamic hypercoagulability, which implies a high quality of structures in the clot.

When both groups of obese people are compared with each other few differences are observed regarding all the parameters; chronometric constants slightly higher than those in the treated group can be noted while the dynamic parameters in TO are lower than those in O, which makes the values encountered in diet-recipient obese people come closer

to those in the control group. Despite these small variations, the difference is not significant in any case.

Results obtained in PRP can be seen in table II. The average value of the constant r' is enlarged significantly in both obese groups, being more pronounced in the treated group. According to SUÑER (15), this could be due to a hypoactivity of the plasmatic factors that take part in the formation of active thromboplastin, which will cause a delay in the time it takes to appear. k' is also found significantly enlarged in O and TO, so that thrombin activity and therefore formation of fibrin are diminished. Due to these alterations $r' + k'$ experience a significant increase in both obese groups, which is once again more marked in the treated group. These longer times reveal a global hypocoagulability (12, 14): the clot will be formed, but it will take longer than normal.

Maximum amplitude (*ma'*) presents average values significantly larger in O and TO, this number being practically the same for both groups. This is favoured, as the *ma* of the tracing in CWB, by fibrinogenemia and platelets, which is in agreement with the observations by MARCHAL *et al.* (11) who found that, even though an exaggeration of platelet functions induces an increase in *ma'*, this is due in most cases to hyperfibrinogenemia.

The ratio $ma'/r'+k'$ proposed by AUDIER and SERRADIMIGNI (2) is found to be a little diminished in both obese groups, although the difference is not statistically significant. Low values of this ratio are suggestive of hypocoagulability so that this slight diminution is related to the overall hypocoagulability detected by the measurement of $r' + k'$. This value alone, however, does not indicate any anomaly as $r'+k'$ and ma' vary in the same direction, instead of in opposite directions as it usually happens. Thus, the ratio is compensated and yields normal values (2, 12).

Comparison between both obese groups does not reveal any significant difference among any of the different parameters. Chronometric constants in group O are less prolonged than those in group TO. Nevertheless, *ma'* shows practically the same value in both groups, which means that in these individuals the structure of the clot is very similar. Regarding the ratio $ma'/r'+k'$, it also presents values much alike in both groups, even though the lowest numbers observed in TO seem to be related to the lengthening in chronometric con-

Table II. *Thromboelastographic parameters from obese (O) and treated obese subjects (TO) compared to a group of non-obese persons (C). Tracing obtained in platelet-rich plasma.* The treated individuals have undergone a hypocaloric diet of approximately 500 cal daily. Each value is the mean ± S.E.

Parameters (mm)	Group (C) n = 15	(O) n = 10	(TO) n = 20
r' = reactivity time	13.83 ± 0.76	16.45 ± 0.63*	17.80 ± 1.16*
k' = clotting speed	4.70 ± 0.20	5.60 ± 0.39*	6.35 ± 0.68*
$r' + k'$ = clotting time	18.53 ± 0.90	22.05 ± 0.91*	24.15 ± 1.66*
ma' = maximum amplitude	57.36 ± 0.64	60.10 ± 1.12*	60.05 ± 0.59**
$\frac{ma'}{r' + k'}$ = ratio	3.18 ± 0.16	2.76 ± 0.10	2.72 ± 0.20

* $p < 0.05$, ** $p < 0.01$, vs control.

Table III. *Thromboelastographic parameters from obese (O) and treated obese subjects (TO) compared to a group of non-obese persons (C). Tracing obtained in platelet-poor plasma.* The treated individuals have undergone a hypocaloric diet of approximately 500 cal daily. Each value is the mean ± S.E.

Parameters (mm)	Group (C) n = 15	(O) n = 10	(TO) n = 20
ma″ = maximum amplitude	26.86 ± 1.10	31.75 ± 2.02*	35.25 ± 1.14**
ma′ — ma″ = platelet thrombodynamic action	30.50 ± 1.21	28.35 ± 2.08	25.30 ± 1.29**

* $p < 0.05$, ** $p < 0.01$, vs control.

stants, found to be more marked in this same group.

In table III results obtained in PPP can be examined. These tracings, in some authors' opinions (2, 11, 12, 16), are defined only by their maximum amplitude, *ma″*. Longitudinal constants are hardly valuable because their reproducibility is low (14). *ma″* experiences a significant increase in both obese groups, being greater in group TO, which is indicative of hyperfibrinogenemia (2, 12). This fact is confirmed by SUÑER (15) who states that a linear relationship exists between fibrinogen levels and *ma″*. The difference in amplitude between the tracings in PRP and PPP, *ma′—ma″*, which provides information about the platelet performance (2, 11, 14), shows lower mean values in both obese groups and it is significant in the treated group. This means that even though platelet activity in group O remains normal, it is decreased in group TO.

Comparing both obese groups, no significant difference appears although a higher fibrinogenemia and less active platelets are noticed in the treated group.

In short, it can be said that the group of not-treated obese people presents a dissociated coagulability: against a plasmatic hypocoagulability there appears a whole blood mixed hypercoagulability (chronometric and dynamic) related to hyperfibrinogenemia and elevation in the number of platelets. Regarding the obese people undergoing caloric restriction a dissociated coagulability is also observed characterized by the existence of plasmatic hypocoagulability and whole blood dynamic hypercoagulability, favoured by hyperfibrinogenemia and an increase in the number of platelets.

In both obese groups the plasmatic clot is formed in a time longer than normal. However, when it appears, its dynamic properties are increased, which could raise the risk of onset of a thromboembolic process. Despite the fact that this situation is detected in both groups studied, in the treated group a tendency to normality can be observed, as can be assessed in the TEG's in CWB which show closer to normal values probably related to the caloric restriction they undergo.

Resumen

Se estudia la coagulación por la técnica de la tromboelastografía, en una serie de personas obesas respecto a un grupo control formado por individuos no obesos, para conocer si pueden ser consideradas personas de alto riesgo en cuanto a la instauración de un proceso tromboembólico. Los resultados indican que en los obesos se produce una serie de alteraciones, tanto a nivel del tiempo de aparición del coágulo, que se alarga, como de la organización de sus redes, que aparecen fuertemente estructuradas, favorecido por la hiperfibrinogenemia y la trombocitosis detectadas

en estos sujetos. También se evalúa el efecto de una dieta hipocalórica sobre la coagulación de personas obesas, comparándolas con los dos grupos anterores. La coagulación de los obesos tratados se modifica de la misma manera que la del grupo que no recibe tratamiento, cuando se compara con la población no obesa; sin embargo, cuando se comparan los dos grupos de obesos no se aprecia ninguna diferencia significativa en los distintos parámetros del estudio, si bien las constantes determinadas en sangre total citratada se acercan más a la normalidad en los sujetos sometidos a restricción calórica.

References

1. Albrink, M. J.: In «Enfermedades del Metabolismo» (Bondy, Ph. K. and Rosemberg, L. E., eds.), Salvat, Barcelona, 1979, Vol. 1, pp. 453-483.
2. Audier, M. and Serradimigni, A.: Cómo Interpretar un Tromboelastograma, Daimon, Barcelona, 1964.
3. Bellon, J. L., Bores, C., Jorba, F. J., De Miguel, G. and Towse, J.: *Sangre*, 25, 1082-1110, 1980.
4. Bierman, E. L. and Glomset, J. A.: In «Textbook of Endocrinology» (Williams, R. H., ed.), W. B. Saunders Co., Philadelphia, 1974, pp. 904-916.
5. Butler, M. J.: *Thromb. Haemost.*, 39, 488-495, 1978.
6. Ciscar, F. and Farreras, P.: Diagnóstico Hematológico. Jims, Barcelona, 1972, Volume 1/2, pp. 51-107, 1345-1771.
7. Howland, W. S., Castro, E. B., Fortner, J. B. and Gould, P.: *Arch. Surg.*, 108, 605-608, 1974.
8. Jensen, D.: Fisiología. Nueva Editorial Interamericana, México, 1979, pp. 1167-1174.
9. Kimche, D. and Eisenkraft, S.: *Ann. Surg.*, 173, 164-172, 1971.
10. Lee, B. Y., Trainor, F. S., Kavner, D. and McCann, W. J.: *Surg. Gynecol. Obstet.*, 149, 843-846, 1979.
11. Marchal, G., Leroux, M. E. and Samama, M.: *Presse Medicale*, 68, 1919-1921, 1960.
12. Raby, C.: Hemorragias y Trombosis, Toray-Masson, Barcelona, 1968, pp. 190-210.
13. Raby, C.: Coagulaciones Intravasculares Diseminadas y Localizadas, Toray-Masson, Barcelona, 1976, pp. 52-60, 128-135.
14. Sultan, C., Priolet, G., Beuzard, Y., Rosa, R. and Josso, F.: Técnicas en Hematología, Toray, Barcelona, 1979.
15. Suñer Casadevall, F.: Temas de Hemostasia, Elvi Española, S. A., Barcelona, 1979, pp. 98-104.
16. Tepperman, J.: Fisiología Metabólica y Endocrina, Interamericana, México, 1975, pp. 206-232.