Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/poamed

Original Research Article

The moderate physical exercise significantly increases von Willebrand's factor's activity and concentration in the blood

Ryszard Paczuski[a,*], *Mirosława Cieślicka*[b]

[a]*Department of Immunology, Genetics and Microbiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Poland*

[b]*Institute of Physical Culture, Kazimierz Wielki University in Bydgoszcz, Poland*

ARTICLE INFO

Article history:
Received 8 January 2013
Accepted 20 September 2013
Available online 25 September 2013

Keywords:
Von Willebrand factor
vWf:Ag
vWf:CBA
Exercise
Von Willebrand disease

ABSTRACT

Introduction: Physical exercise causes a range of physiological changes including the release of hemeostasis proteins to blood. Among them very important is von Willebrand factor (vWf) which is involved in both platelet adhesion and coagulation cascade.

Aim: The task of the study was to evaluate the effect of moderate physical exercise on plasma concentration and activity of vWf.

Material and methods: The impact of physical effort on vWf's concentration (vWf:Ag) and activity (vWf:CBA) underwent analysis in a group of 42 people (22 men and 20 women) aged 19–22, mean 20 years, SD 8 months). The effort consisted of 35 min of swimming in an indoor swimming pool.

Results and discussion: An increase of both parameters after exercise was observed in whole group, reaching mean values 58% and 69%, correspondingly, SD 60%. A big difference in reaction after effort was observed between the male and female group. In the male group the scale of vWf:Ag increase was higher than in female group, with increase of the mean value from 105%, SD 26% before the effort to 189%, SD 81% after the effort. In the female group the same mean parameter changed from 92%, SD 18% before to 120%, SD 23% after exercise. These differences were reflected in similar manner in the increase in vWf:CBA activity, which was 96%, SD 24% before and 134%, SD 46% after the exercise.

Conclusions: Even a moderate exercise significantly increases vWf levels and changes coagulation parameters.

*Correspondence to: Department of Immunology, Genetics and Microbiology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, Jagiellońska 78, 10-357 Olsztyn, Poland. Tel.: +48 89 526 49 66; fax: +48 89 526 49 77.
E-mail address: b.r.paczuski@wp.pl (R. Paczuski).

1230-8013/$ – see front matter © 2013 Warmińsko-Mazurska Izba Lekarska w Olsztynie. Published by Elsevier Urban & Partner Sp. z o.o. All rights reserved.
http://dx.doi.org/10.1016/j.poamed.2013.09.008

1. Introduction

Physical effort causes a range of physiological changes in an organism, among which the release of hemeostasis proteins to blood is an important but not thoroughly recognized one. One of the most important among these proteins is von Willebrand's factor (vWf). It is a glycoprotein with molecular mass up to 20×10^6 D possessing complex adhesive functions, and playing at least two functions (1) enables blood platelets adhesion on site of vessels' wall damage and (2) transports the coagulation factor VIII (FVIII) and protects it against proteolysis (II).[16,17] vWf is synthesized in megacarocytes and endothelium of blood vessels and released in a continuous (I) and controlled (II) manner. Measurements of plasma concentration (vWf antigen – vWf:Ag) and vWf activity, such as ristocetin cofactor (RCof) collagen binding assay for vWf (vWf:CBA), are necessary for the diagnosis of congenital or acquired bleeding disorders, especially of the von Willebrand's disease (vWD) and thrombotic thrombopenic purpura (TTP).[2,3,5] It is also indicated that the measurements of vWf are useful in the monitoring of the endothelium activation or damage. Along with pathological processes, physiological factors such as physical effort, stress or pregnancy significantly affect concentration and activity of plasma vWf. Also various genetic factors including ABO blood group polymorphism influence it and for this reason the results of population studies on the incidence of vWf abnormalities differ significantly.[4,15,17] The impact of physical effort being probably the most common interfering factor, in spite of being listed in publications, is not researched thoroughly. Publications concerning this issue are based on, for the most part, small group of subjects, and, moreover, it is difficult to determine the reason for changes – whether they were caused by physical effort itself or perhaps tissue constriction or local circulation stasis.[7,8,9,10,20,24,27]

2. Aim

The aim of our research was to evaluate the process of releasing vWf in experimental method, which excludes or minimalizes phenomena connected with tissue constriction as a factor stimulating the release of vWf. In order to carry out these assumptions, we chose swimming as an optimal model of exercise, evaluating changes of plasma concentration (vWf:Ag) and activity (vWf:CBA) of vWf.

3. Material and methods

The research aimed at evaluating the process of vWf release, as a result of physical effort, excluding the impingement of injuries or tissues pressure and tourniquet. The study was conducted on the group of 42 volunteers, 22 females and 20 males aged 19–22 (20.0 ± 0.70 years) trained in swimming. The levels of swimming abilities across the group were similar and determined intermediate. Blood used to determinate vWf:Ag concentration and vWf:CBA activity was drawn approximately 10 min before a 35-min period of freestyle swimming and immediately afterwards. Blood was drawn in accordance with standard procedure from cubital vein, using sodium citrate (in 1:9 ratio) as anticoagulant, centrifuged for obtaining platelet poor plasma, which was then frozen and stored until the examination date in a refrigerator at the temperature -20°C. Concentration of vWf (vWf:Ag) was determined using enzyme-linked immunosorbent assay (ELISA) with the application of commercial DAKO (Denmark) reagents according to the company recommended procedure. The measurements of vWfs activity were performed using the test of vWf:CBA according to Falvarolo procedure taking into account own modifications of the flattening process (using Sigma, USA, human collagen type III and DAKO conjugate).[3,12] The research was carried out upon the approval of the committee of bioethics, as well as a written approval of a subject. The project was approved by regional bioethics committee and followed by confirmed agreement of all participants according to required procedure.

4. Results

The examinations conducted have shown that the physical effort of 35-min swimming caused a considerable increase of the plasma vWf concentration and activity. Both parameters evaluated in the group increased considerably – vWf:Ag by 58%, vWf:CBA by 69%. Along with the differences in ontogenetic reaction to the effort, a statistically considerable difference between the male and female group was observed (Tables 1 and 2, Figs. 1–4). In the male group vWf activity (AWF:Ag) was increasing by 81% on average, and its vWf:CBA activity by 99% ($p < .001$, Tables 1 and 2). In the female group the scale of increase was lower and reached 28% for vWf:Ag and 36% for vWf:CBA activity ($p < .001$, Tables 1 and 2). The subjects differed considerably in the scale of reaction to effort, differing both in the scale of increase of vWf:Ag plasma concentration and vWf:CBA plasma activity. Some individuals showed no reaction after effort while some others reacted with extremely high release of vWf to blood reaching almost 300%. Individual measurements of their changes are illustrated in Figs. 1–4. The change in the coefficient of correlation between vWf concentration and activity before and after the effort was also observed. The coefficient of Pearson's correlation between vWf:Ag and vWf:CBA has changed after exercise. Before the exercise its value was 0.69 ($p < .001$; Fig. 5), and after the exercise it increased to 0.92 ($p < .001$; Fig. 6).

5. Discussion

In all examined volunteers, the exercise consisting of a 35-min swim caused the increase in vWf:Ag concentration and vWf:CBA activity of vWf in blood. Observed interontogenetic differences are considerable (Figs. 1–4), yet they do not affect statistic inference. Significant statistic differences were observed in the scale of vWf concentration and activity increase between male and female groups. Physical effort has been deemed the reason for vWf increase in blood; yet, as we have determined in the introduction, literature data confirming this fact are not numerous, while for vWf:CBA activity there are none at all. The results of early experiments were not clear, and in some subjects the exercise caused the release of vWf,[20] and in it others did not.[18] At present, this fact leaves no doubt. The highest two- and threefold

Table 1 – The plasma concentration of vWf (vWf:Ag) in male group ($n=20$) and female group ($n=22$) before and after 35 min of swimming.

Study group	Before effort, %		After effort, %		P-value	Difference of vWf:Ag, %	
	Mean	SD	Mean	SD		Mean	SD
Male	105	26	189	81	<.001	84	71
Female	92	18	120	23	<.001	28	20
Total	99	23	156	70	<.005	58	60

Table 2 – The plasma activity of vWf (vWf:CBA) in male group ($n=20$) and female group ($n=22$) before and after 35 min of swimming.

Study group	Before effort, %		After effort, %		P-value	Difference of vWf:CBA activity, %	
	Mean	SD	Mean	SD		Mean	SD
Male	96	34	195	85	<.001	99	67
Female	98	24	134	46	<.001	36	37
Total	97	29	165	74	<.001	68	63

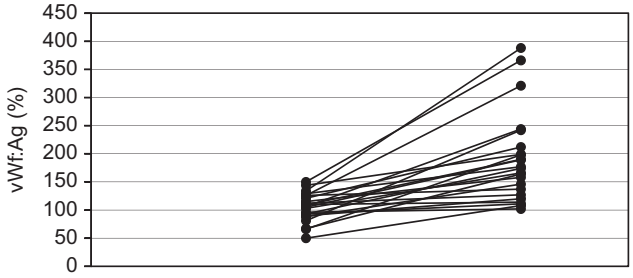

Fig. 1 – Individual changes of plasma concentration of vWf (vWf:Ag) in male group ($n=20$) after 35 min of swimming.

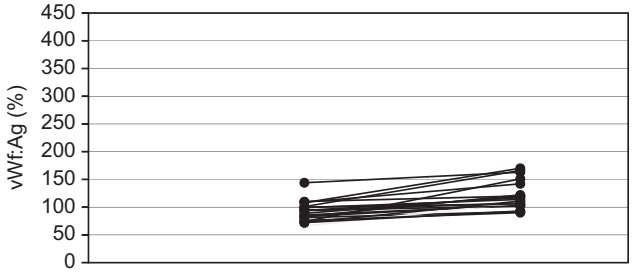

Fig. 2 – Individual changes of plasma concentration of vWf (vWf:Ag) in female group ($n=22$) after 35 min of swimming.

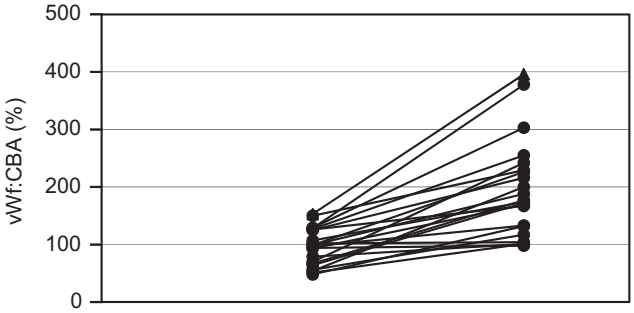

Fig. 3 – Individual changes of plasma activity of vWf (vWf: CBA) in male group ($n=20$) after 35 min of swimming.

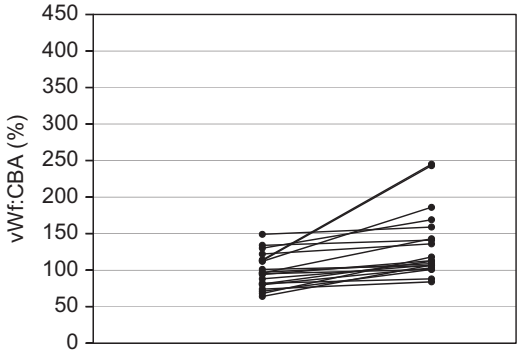

Fig. 4 – Individual changes of plasma activity of vWf (vWf: CBA) in female group ($n=22$) after 35 min of swimming.

increase of plasma concentration was observed as a result of a long-distance run.[7,27] Admittedly there is a report on vWf increase up to 800%; nonetheless, in the evaluation of probability for such a significant increase it should be treated with caution.[24] In other experiments the increase in vWf level was observed as well, but was not that significant, since it was in the range of 10%–80%.[6,8,9,10,11,14,19,22,25,26] The differences in the method of evaluation (gait, run, bike, cycloergometer) influenced the discrepancies in the scale of increase. The duration of exercise proved to be significant as well, along with the increase in the duration, vWf, activity and concentration increased as well. The increase in vWf concentration occurred for 15 min following the exercise, and then normalization was commencing.[13,23]

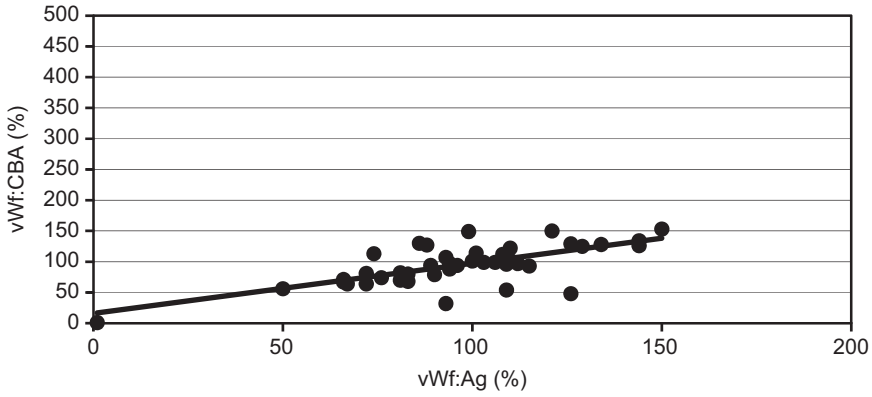

Fig. 5 – Correlation between plasma concentration of vWf (aWf:Ag) and its activity (vWf:CBA) in group of 42 volunteers before effort (Pearson's correlation coefficient $r=0.69$; $p<.001$).

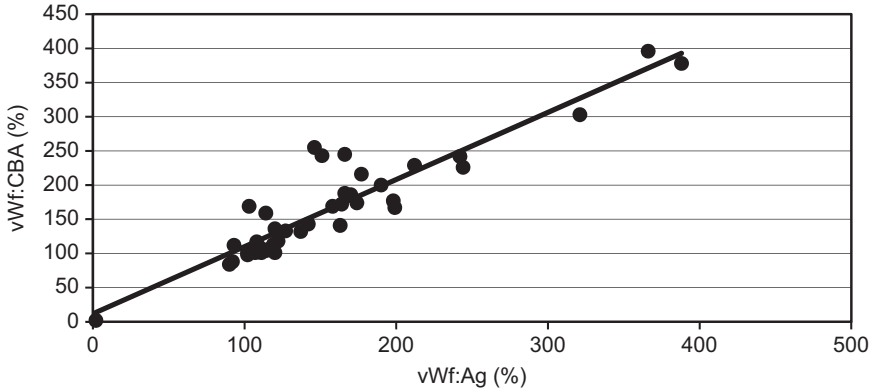

Fig. 6 – Correlation between plasma concentration of vWf (aWf:Ag) and its activity (vWf:CBA) in group of 42 volunteers after 35 min of swimming (Pearson's correlation coefficient $r=0.92$; $p<.001$).

The subject's age turned out to be significant as well, as the phenomenon apparently manifested itself in persons aged 20–30 years.[22,23] Simultaneous application of desmopressin, a medication stimulating the release of vWf in people under exercise, showed that the effect of their simultaneous interaction did not sum up.[26] The differences in correlation coefficient between the concentrations of vWf activities above observed before and after the effort should be indicated as well (Fig. 5). Higher correlation coefficient after exercises than before them indicates that the main constituent of the phenomenon is releasing active vWf molecules, and the discrepancies of vWf:CBA to vWf:Ag ratio observed between subjects should rather be accounted for by means of differences between individuals in proteolysis and vWf elimination from blood.

In spite of the facts above, there can be some reservations about the methodology of research conducted and interpretations of their results, indicating other possible reasons for vWf release, e.g. recurring local tissue constriction or circulation stasis. The report that even the kind of shoes worn or their lack can influence test results indicate that special caution in the evaluation of examinations on exercises should be preserved. The rate of feet tissue endothelium excitation was responsible for these differences.[21] The sort of exercise applied in our research, consisting of swimming in swimming pool is an optimal model of an experiment, as it excludes the factors similar to those cited above and eliminates other reasons for releasing vWf into blood. However, it should be assumed that in most situations the mechanism of releasing vWf as a result of exercise is complex, entailing both local and systemic factors.

The most surprising results of our experiment are the individual differences in reaction to physical effort (Figs. 1–4). Probably they are responsible for the confusing differences in conclusions after early studies on the effect of exercise on vWf concentration.[18,20] The individual differences of reaction after exercise may significantly affect various aspects of diagnostics and therapy, especially including the diagnosis of vWD and application of exercise therapy in arteriosclerosis.

Another interesting observation that we have not come across analyzing literature data is the assertion of a significant difference in men's and women's reaction to exercise. In men both vWf:Ag concentration and its vWf:CBA activity increased threefold in comparison with women (Tables 1 and 2, Figs. 1–4). This difference might have been overlooked by other researchers since only the representatives of one sex underwent scrutiny or there have been disproportions within the group.[6,10,13,19,23,25] Usually only men were examined[7,9,14,23] or they dominated the group. The fact that even small, 5- or 7-person groups underwent examination is probably of significance as well.[1,7] Both physical

conditions (pool size, temperature of water and air), exercise duration, and swimming skills level in both groups did not differ, thus they should not be considered the reason. Admittedly, subjective factors cannot be excluded, e.g. a more ambitious attitude towards the set task, and so putting more effort into it in male groups, but it is not very likely that it might be the reason for threefold increase scale. The effect observed in all probability reflects the differences in physiology in both sexes. It might also reflect old evolutionary differences in behavior, in which female sex is more apt to injuries during the effort. It is worth underlining that the increase is a temporary phenomenon, followed by normalization. Even a long-term training of several months has no bearing on plasma concentration of vWf, if we should assess them a couple of days following the training.

The insensitivity of vWf increase after exercise explains problems with vWD diagnosis and indicates the necessity of verifying the blood sampling procedure. On the other hand the big difference in recorded parameters of vWf:Ag and VWf:CBA between the male and female group may be partially responsible for higher incidence of atherosclerosis and myocardial infarction among men. And there consists a strong argument on significance of vWf in patomechanism of these diseases.

6. Conclusions

The experimental model of the moderate physical exercise based on 30 min of swimming induced significant increases in the levels vWf activity and concentration in the blood. For these reasons even moderate exercise can change the coagulation parameters. This fact indicates the need for verifying blood sampling procedures for measurements of vWf.

Conflict of interest

None declared.

REFERENCES

[1] Andrew M, Carter C, O'Brodovich H, Heigenhauser G. Increases in factor VIII complex and fibrinolytic activity are dependent on exercise intensity. *J Appl Physiol*. 1986;60(6):1917–1922.

[2] Cruz MA, Whitelock J, Dong J. Evaluation of ADAMTS-13 activity in plasma using recombinant von Willebrand Factor A2 domain polypeptide as substrate. *Thromb Haemost*. 2003;90(6):1204–1209.

[3] Favaloro EJ. Collagen binding assay for von Willebrand factor (VWF:CBA): detection of von Willebrands disease (vWD), and discrimination of vWD subtypes depends on collagen source. *Thromb Haemost*. 2000;83(1):127–135.

[4] Gill JC, Endres-Brooks J, Bauer P, Marks WJ, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*. 1987;69(6):1691–1695.

[5] Ginsburg D, Bockenstedt PL, Allen EA, Fox DA, Foster PA, Ruggeri ZM, et al. Fine mapping of monoclonal antibody epitopes on human von Willebrand factor using a recombinant peptide library. *Thromb Haemost*. 1992;67(1):166–171.

[6] Gonzales JU, Thistlethwaite JR, Thompson BC, Scheuermann BW. Exercise-induced shear stress is associated with changes in plasma von Willebrand factor in older humans. *Eur J Appl Physiol*. 2009;106(5):779–784.

[7] Hansen JB, Wilsgard L, Olsen JO, Osterud B. Formation and persistence of procoagulant and fibrinolytic activities in circulation after strenuous physical exercise. *Thromb Haemost*. 1990;64(3):385–389.

[8] Jern C, Eriksson E, Tengborn L, Risberg B, Wadenvik H, Jern S. Changes of plasma coagulation and fibrinolysis in response to mental stress. *Thromb Haemost*. 1989;62(2):767–771.

[9] Jilma B, Dirnberger E, Eichler HG, Matulla B, Schmetterer L, Kapiotis S, et al. Partial blockade of nitric oxide synthase blunts the exercise-induced increase of von Willebrand factor antigen and of factor VIII in man. *Thromb Haemost*. 1997;78(4):1268–1271.

[10] Krzysiek J, Milewicz T, Dybkowski R, Janczak-Saif A, Dembinska-Kiec A, Guevara I, et al. Aktywacja płytek i wybrane parametry funkcji śródbłonka w trakcie standardowego wysiłku fizycznego u kobiet w okresie okołomenopauzalnym. *Przegl Lekarski*. 2001;58:419–425 [in Polish].

[11] Musumeci V, Cardillo C, Baroni S, Zuppi C, Zappacosta B, Tutinelli F, et al. Effects of calcium channel blockers on the endothelial release of von Willebrand factor after exercise in healthy subjects. *J Lab Clin Med*. 1989;113(4):525–531.

[12] Paczuski R. Determination of von Willebrand factor activity with collagen binding assay (vWf:CBA) and diagnosis of von Willebrand disease: the effect of collagen source and coating conditions. *J Lab Clin Med*. 2002;140(4):250–254.

[13] Paton CM, Nagelkirk PR, Couglin AM, Cooper JA, Davis GA, Hassouna H, et al. Changes in von Willebrand factor and fibrinolysis following a post-exercise cool-down. *Eur J Appl Physiol*. 2004;92(3):328–333.

[14] Ribeiro JL, Salton GD, Bandinelli E, Oliveira AR, Roisenberg I. The efffect of ABO blood group on von Willebrand response to exercise. *Clin Appl Thromb Haemost*. 2008;14(4):454–458.

[15] Rodeghiero F, Castman G, Dini E. Epidemiological investigation of the prevalence of von Willlebrand's disease. *Blood*. 1987;69(2):454–459.

[16] Ruggieri ZM. Structure and function of von Willebrand factor. *Thromb Haemost*. 1999;82(2):576–584.

[17] Sadler JE. Von Willebrand factor. *J Biol Chem*. 1991;226(4): 22777–22780.

[18] Sano T, Motomiya T, Yamazaki H. Platelet release reaction in vivo in patients with ischaemic heart disease after isometric exercise and its prevention with dipyridamole. *Thromb Haemost*. 1980;42(5):1589–1597.

[19] Small M, Tweddel AC, Rankin AC, Lowe GD, Prentice CR, Forbes CD. Blood coagulation and platelet function following maximal exercise: effects of beta-adrenoceptor blockade. *Haemostasis*. 1984;14(3):262–268.

[20] Stibbe J. Effect of exercise on F VIII-complex: proportional increase of ristocetin cofactor (Von Willebrand factor) and F VIII-AGN, but disproportional increase of F VIII-AHF. *Thromb Res*. 1977;10(1):163–168.

[21] Takashima N, Higashi T. Change in fibrinolytic activity as a parameter for assessing local mechanical stimulation during physical exercise. *Eur J Physiol Occup Physiol*. 1994;68 (5):445–449.

[22] Van den Burg PJ, Hospers JE, Mosterd WL, Bouma BN, Huisveld IA. Aging, physical conditioning, and exercise-induced changes in hemostatic factors and reaction products. *J Appl Physiol*. 2000;88(5):1558–1564.

[23] Van den Burg PJ, Hospers JE, van Vliet M, Mosterd WL, Bouma BN, Huisveld IA. Changes in haemostatic factors and activation products after exercise in healthy subjects with different ages. *Thromb Haemost*. 1995;74(6):1457–1464.

[24] Van Loon BJ, Heere LP, Kluft C, Briet E, Dooijewaard G, Meinders AE. Fibrinolytic system during long-distance running in IDDM patients and in healthy subjects. *Diabetes Care*. 1992;15(8):991–996.

[25] Van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, Pajkrt D, van-Genderen PJ, et al. Von Willebrand factor propeptide in vascular disorders: a tool to distinguish between acute and chronic endothelial cell perturbation. *Blood*. 1999;94(1):179–185.
[26] Vicente V, Alberca I, Mannucci PM. Reduced effect of exercise and DDAVP on factor VIII-von Willebrand Factor and plasminogen activator after sequential application of both the stimuli. *Thromb Haemost*. 1984;51(1):129–130.
[27] Woodburn KR, Rumley A, Murtagh A, Lowe GD. Acute exercise and markers of endothelial injury in peripheral arterial disease. *Eur J Vasc Endovasc Surg*. 1997;14 (2):140–142.