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INFLUENCE OF GLUCOSE ON THE LEUKOCYTE RESPONSE IN WOMEN ATHLETES DURING PROLONGED EXERCISE

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ABSTRACT

Objective. This study investigated the influence of carbohydrate versus placebo beverage intake on leukocyte and cortisol responses to 90 minutes of running on a treadmill at 70% VO_{2max} in women. Subjects. Ten moderately trained women athletes (age: 27.6±3.7 yr; VO_{2max}: 46.6±2.0ml.kg^{-1}.min^{-1}) from the local athletic club volunteered to participate in the study. Design. In a randomised, single blind, placebo-controlled crossover study, subjects received three beverages during exercise: a placebo ad lib (Pla), 6% glucose (voluntary glucose: VG) and 1 000 ml 6% glucose (forced glucose: FG). Blood samples were analysed for serum glucose ([Gluc]) and cortisol ([Cort]), as well as for total white blood cell (WBC) counts and WBC subpopulations. Results. No difference was observed in the volume of beverage intake during Pla and VG trials. [Gluc] was significantly higher in the VG and FG trials when compared with the Pla trial at 45 and 90 minutes ($p<0.05$). [Cort] was stable in the VG and FG trials, but was significantly increased in the Pla trial at 90 minutes ($p<0.05$). Total WBC counts were raised at all stages in all three trials when compared with pre-exercise values, but the significance differed between trials. In the Pla trial, the greatest increase in total WBC count ($p<0.01$) was seen at the 45-minute time point, with significant increases ($p<0.05$) in neutrophil, lymphocyte and monocyte counts. The total WBC count increased in the VG trial, but to a lesser extent than in the Pla trial ($p<0.05$). In the VG trial, only the lymphocyte subpopulation increased significantly ($p<0.05$). There were no significant differences in the total WBC count or WBC subpopulations in the FG trial. All measured parameters, except the total WBC and neutrophil counts ($p<0.01$), returned to pre-exercise levels during recovery (90-180 minutes).Conclusion. We conclude that both voluntary and forced ingestion of 6% glucose solutions prevented a rise in cortisol at the end of a 90-minute period of exercise. Although the effect of the unforced ingestion of 6% glucose was less explicit, both forced and unforced ingestion of 6% glucose had an attenuated effect on WBC counts during exercise.

Key words: Women; Exercise; Blood glucose; Cortisol; Leukocytes.

INTRODUCTION

Studies by Nieman *et al.* (1997a, 1997b, 1998a, 1998b, 1999) and contributions by Nehlsen-Cannarella *et al.* (1997) and Mitchell *et al.* (1998) investigated the influence of carbohydrates on the immune system during exercise. From these studies, it is apparent that carbohydrate ingestion has an attenuating effect on the immune response during exercise when compared to the ingestion of a placebo. This includes a lesser increase in neutrophils, lymphocytes, monocytes, cytokines, oxidative burst activity and hormones associated with the immune

response, such as cortisol, epinephrine and growth hormone (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1998b, 1999; Mitchell *et al.*, 1998). These findings and their possible beneficial effects on the immune system have been discussed in a number of review articles (Nieman *et al.*, 1999; Gleeson & Bishop, 2000a, 2000b; Nieman & Perdersen, 2000; Robson, 2000). With the exception of Mitchell *et al.* (1998), who used an exercise protocol of 72 minutes, the results of all the current investigators mentioned (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1998b, 1999) are based on extensive exercise periods of at least 120 minutes.

In addition, comprehensive carbohydrate supplementations were employed in all the current studies (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1998b, 1999; Mitchell *et al.*, 1998). Carbohydrates were supplemented before as well as during exercise periods (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1998b, 1999; Mitchell *et al.*, 1998) and, in most cases, supplementations were continued in the post-exercise period (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1999; Mitchell *et al.*, 1998). With regard to the experimental subjects, only Nieman *et al.* (1999) made use of women exclusively as subjects for their investigation. Finally, athletes often do not consume enough fluid and/or carbohydrates to maintain the hydration and blood glucose levels necessary for optimum performance (Noakes, 2001). This aspect in relation to the possible attenuating effects on the immune system was not addressed by any of the present authors (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b; Mitchell *et al.*, 1998; Nieman *et al.*, 1998a, 1998b; Nieman *et al.*, 1999).

The aim of the present study was, in the first instance, to determine whether the *ad lib* supplementation of glucose during 90 minutes of exercise would influence leukocyte and cortisol responses in women athletes and, subsequently, to ascertain whether a prescribed supplementation of glucose would have the same effect as the *ad lib* supplementation thereof. To achieve these objectives, we designed a randomised, single blind, placebo-controlled crossover study to investigate the influence of supplementary glucose (voluntary and forced) on blood leukocytes and serum cortisol in women athletes during 90 minutes of running.

METHODS

Subjects:

Ten moderately-trained women athletes who had completed at least two half-marathons (21.1km) in the previous six months were recruited for the study. Each subject was initially tested for VO_{2max} on a motor-driven treadmill (TechnoGym, Italy), using a graded protocol as described by this laboratory (Strauss *et al*., 2001). This basically entailed a period of free stretching exercises lasting 10 minutes, after which the subject was requested to get onto the treadmill, which was switched on. The monitors (MetaMax, Germany) for heart rate, oxygen consumption and carbon dioxide production were coupled and the procedure for the VO_{2max} test was explained. The treadmill was then set at a speed of $6km.h^{-1}$ and the subject had to run at this speed for 10 minutes. From this point onwards, the speed of the treadmill was continually increased every minute by $1km.h^{-1}$ until the subject was completely exhausted. The results of these tests were used to establish a target running intensity ($km.h^{-1}$) that would correspond to 70% of the VO_{2max} of the subject. This running speed was then used throughout

the test protocol. Written informed consent from each subject, as well as institutional ethical approval, was obtained prior to commencing the study.

Research design

After the initial VO_{2max} test, each subject participated in three experimental trials that were done on a randomised basis, with at least one week between trials. Subjects fasted overnight and all testing was done between 07:00 and 10:00, with individual trials starting at the same time of the day. In all three trials, the subjects had to run on a treadmill for 90 minutes at the predetermined speed (70% VO_{2max}), as described above. The laboratory was air-conditioned and a desk fan, to create airflow, was directed towards the subject. The temperature ranged from 17 to 19°C for all test sessions. Different fluid replenishments were consumed during each trial and the subjects did not know what they were drinking. They were told only that their drink contained a harmless substance. The beverages were either a sugar-free orange drink or the same drink to which a long-chain glucose polymer supplied by FAST FUEL® (Observatory, South Africa) had been added at a concentration of 6%. During the sugar-free (placebo: Pla) trial and one of the glucose trials (voluntary glucose: VG), the subjects were allowed to drink as much of the particular beverages as they wanted (*ad lib*). In a subsequent random trial, the subjects had to consume 1 000 ml of the 6% glucose beverage (forced glucose: FG), 500 ml of which had to be consumed within the first 45 minutes of the trial and the remainder in the last 45 minutes. The FG consumption of glucose was within the prescriptions of the American College of Sports Medicine (ACSM) (1996). The ACSM recommends that distance athletes should consume 600 - 1 200 ml of fluid with a glucose concentration of between 4 and 8% an hour to prevent dehydration and to maintain blood glucose levels. During the post-exercise period in each of the trials, the subjects were allowed to drink only water (*ad lib*). Blood samples were collected by venepuncture from the antecubital vein before (0 minutes), during (45 minutes) and immediately after (post) (90 minutes) exercise, as well as during the recovery period (180 minutes). Blood samples were collected in SST® Gel and Clot Activator vacutainers (Preanalytical Systems, Plymouth, UK) for serum glucose and cortisol analysis and in VACUTAINER™ K_3EDTA vacutainers (Preanalytical Systems, Plymouth, UK) for haematological analysis.

Glucose, leukocytes and cortisol

Blood samples for glucose and cortisol were kept on ice and, after stasis, were centrifuged for 20 minutes at 3 000 rpm and 5°C. Serum samples were frozen at -80°C and were thawed just before analysis. Whole blood samples were analysed for haematological values within two hours on a Coulter Counter as described by the manufacturer (Coulter Counter Inc., Germany). Blood smears for a differential white blood cell (WBC) count were obtained during this time (Ham, 1965). The serum content of the glucose was determined spectrophotometrically (Hyvarinen & Nikkila, 1962). Serum cortisol was assayed using a competitive solid-phase ^{125}I radioimmunoassay technique (Diagnostic Products Corporation, Los Angeles, USA). Plasma volume changes were estimated using the method of Dill and Costill (1974).

Statistical analysis

Data are expressed as means, plus/minus the standard error of the mean (±SEM). Serum cortisol and glucose concentrations, WBC and WBC subpopulations were analysed using a

repeated measures analysis of variance (ANOVA). Changes from the baseline for the pre-, middle- and post-exercise and recovery periods for the different trials were compared using a multiple t-test with a Bonferroni adjustment, as described by Altman (1991). Significance was set at $p<0.05$.

RESULTS

Subjects

The characteristics of the subjects are summarised in Table 1. Two of the subjects had obtained regional colours for road running, while the remainder participated in road running as a recreational sport. The speed at which the subjects ran (70% VO_{2max}) during the protocol was adjusted to predict a time for a half-marathon. The average predicted time would have been 2:09±0:5 or 0:18±0:16 slower than their personal best time in the preceding six months.

TABLE 1. **SUBJECT CHARACTERISTICS**

Age (yr)	27.6±3.7
Stature (cm)	169±1
Mass (kg)	60.5±1.6
BMI ($kg.m^{-2}$)	21.3±0.5
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	46.6±2.0
RBC ($10^6.\mu l^{-1}$)	4.1±0.1
Hct (%)	37.4±0.8
Hb ($g.dl^{-1}$)	12.8±0.3
Best ½ marathon (hrs:min)	1:51±0:4

Values are means±SEM; n=10; BMI: body mass index; RBC: red blood cell; Hct: haematocrit; Hb: haemoglobin

Fluid intake

During the Pla trial, the subjects consumed a mean of 561±68 ml of the sugar-free orange beverage, while they consumed a mean of 583±59 ml of the 6% glucose orange beverage during the VG trial. These amounts did not differ from each other ($p>0.05$), but were significantly less than the 1 000 ml that the subjects were required to drink during the FG trial ($p<0.01$). Body mass remained stable during the trials and plasma volume changes were negligible (less than 1%) and did not differ between the trials ($p>0.05$).

Leukocytes

The leukocyte responses are summarised in Table 2. In comparison with the pre-exercise values, the total WBC counts were significantly ($p<0.05$) elevated throughout the measured periods of all the trials, except for in the mid-exercise period (45 minutes) of the FG trial. In the latter case, the total WBC counts were elevated, but significant elevations from pre-exercise values could not be established ($p>0.05$). An assessment of the WBC subsets indicates that it was mainly the neutrophils and, to a lesser extent, the monocytes that contributed to the observed increases in WBCs in all the trials. These cells were also responsible for the lesser increase in the total WBC count that was observed during the mid-

exercise period in both the FG and VG trials, as both neutrophils and monocytes were not significantly raised from pre-exercise values ($p>0.05$).

TABLE 2. **RESPONSE OF BLOOD LEUKOCYTE SUBSETS IN PLACEBO, FORCED AND UNFORCED CONSUMPTION OF 6% GLUCOSE TRIALS BEFORE, DURING AND IN RECOVERY FROM 90 MINUTES TREADMILL RUNNING AT 70% VO_{2MAX}**

10^3.cells.μl^{-1}	Pre-exercise	Mid-exercise (45 min)	Post-exercise (90 min)	Recovery (180 min)	P (trial x time)
Total WBC					
Placebo (ad lib)	5.78±0.45	8.51±0.85**	10.37±1.37**	10.49±1.40**	<0.001
6% Glucose (1 000 ml)	5.49±0.65	6.77±0.89†	10.10±1.75**	9.92±1.62**	
6% Glucose (ad lib)	5.49±0.65	7.53±0.96*	10.11±1.32**	9.54±1.03**	
Neutrophils					
Placebo (ad lib)	3.45±0.34	5.07±0.54*	7.32±0.98**	9.22±1.30**	<0.0001
6% Glucose (1 000 ml)	3.24±0.46	4.09±0.57	7.57±1.42**	7.92±1.55**	
6% Glucose (ad lib)	4.02±0.48	5.12±0.75	7.42±1.17**	7.15±.1.04**	
Lymphocytes					
Placebo (ad lib)	1.83±0.25	2.58±0.40*	2.39±0.36	1.43±0.14	<0.01
6% Glucose (1 000 ml)	1.98±0.30	2.33±0.41	2.14±0.31	1.49±0.13	
6% Glucose (ad lib)	1.58±0.20	2.27±0.32*	2.10±0.31	1.72±0.21	
Monocytes					
Placebo (ad lib)	0.32±0.06	0.53±0.07*	0.63±0.10**	0.41±0.11	<0.001
6% Glucose (1 000 ml)	0.41±0.05	0.37±0.05†	0.65±0.12**	0.48±0.05	
6% Glucose (ad lib)	0.39±0.04	0.37±0.06†	0.56±0.05*	0.49±0.09	

Values are means±SEM; n=10
Significant change from pre-exercise: *p<0.05; **p<0.01
Significant difference from placebo within time period: †p<0.05

Glucose and cortisol

The response of both serum glucose and cortisol during the different trials is summarised in Table 3. In the Pla trial, serum glucose levels did not change significantly during any stage of the trial ($p>0.05$). In both the FG and VG trials, serum glucose levels were raised during and immediately post-exercise ($p<0.01$), but returned to pre-exercise levels in the recovery period ($p>0.05$). Serum cortisol levels, when compared with pre-exercise concentrations, were elevated only in the immediate post-exercise period in the Pla trial ($p<0.01$). In both the FG and VG trials, cortisol levels were unchanged ($p>0.05$) in comparison with pre-exercise levels throughout all stages of the trials.

TABLE 3. **SERUM GLUCOSE AND CORTISOL RESPONSE IN PLACEBO, FORCED AND UNFORCED CONSUMPTION OF 6% GLUCOSE TRIALS BEFORE, DURING AND IN RECOVERY FROM 90 MINUTES TREADMILL RUNNING AT 70% VO_{2MAX}**

	Pre-exercise	Mid-exercise (45 min)	Post-exercise (90 min)	Recovery (180 min)	p (trial x time)
Glucose ($mmol.l^{-1}$)	5.1±0.1	6.3±1.4	5.7±0.1	4.9±0.1	<0.001
Placebo (ad lib)	5.0±0.2	7.6± 0.7**††	7.8±0.8**††	4.5±0.3	
6% Glucose (1 000 ml)	5.0±0.2	7.1.±0.96**†	6.4±0.2**†	4.7±0.3	
6% Glucose (ad lib)					
Cortisol ($nmol.l^{-1}$)	724±78	735±87	970±103**	722±103	<0.01
Placebo (ad lib)	757±102	746±120	641±88†	719±141	
6% Glucose (1 000 ml)	650±72	608±79	609±102†	573±69	
6% Glucose (ad lib)					

Values are means±SEM; n=10
Significant change from pre-exercise: *p<0.05; **p<0.01
Significant difference from placebo within time period: †p<0.05; ††p<0.01

DISCUSSION

The approach of this study, namely to examine the influence of glucose on immune cells, is unconventional when compared with other studies, given that the subjects personally regulated their fluid intake. The main outcomes of the study were that both VG and FG ingestion prevented a rise in cortisol at the end of long-duration exercise, and that only a forced quantity of glucose completely attenuated the rise in WBC counts during exercise.

Glucose and cortisol

The release of corticotropin and cortisol during exercise has been linked, in part, to decreases in blood glucose concentrations (Mitchell *et al*., 1990; Murray *et al*., 1991). These studies showed that, during and after prolonged exercise, blood glucose concentrations are higher and cortisol concentrations are lower in carbohydrate-supplemented than in placebo-supplemented subjects. This tendency was also observed in the present study. Serum cortisol concentrations were unaltered during and immediately after exercise in both the FG and VG trials, in which glucose concentrations were elevated ($p<0.01$). In comparison, no change in blood glucose concentrations was observed at any stage of the protocol in the Pla trial, although cortisol concentrations were significantly elevated in the immediate post-exercise period ($p<0.01$). Elevated cortisol levels observed in the Pla trial may indicate that, although hypoglycaemia was not observed, serum glucose concentrations may have been at the stage where the demand for glucose exceeded the supply thereof. In an attempt to sustain the demand for glucose, cortisol secretion was accelerated to stimulate gluconeogenesis, which is one of the key functions of cortisol (Ganong, 1998). In contrast, in the FG and VG trials cortisol was actually reduced, though not significantly ($p>0.05$), during and post-exercise. Under these circumstances, blood glucose concentrations were elevated and therefore a cortisol stimulus for gluconeogenesis would not be a factor.

Cortisol has a circadian rhythm and irregular bursts occur throughout the day (Ganong, 1998). The bursts are most frequent in the early morning and 75% of the daily production of cortisol occurs between 4 and 10 a.m. (Ganong, 1998). Notwithstanding, pre-exercise cortisol concentrations were currently somewhat higher (712±75 $nmol.l^{-1}$) than the normal range of 140 - 690 $nmol.l^{-1}$ (a.m., fasting) as specified by Ganong (1998). Nieman *et al.* (1998a) reported an average pre-exercise (a.m., fasting) cortisol concentration of 723±62 $nmol.l^{-1}$ in 10 subjects (eight male and two female) that were tested on four separate occasions. Henson *et al.* (1998), Nehlsen-Cannarella *et al.* (1997) and Nieman *et al.* (1997a, 1997b) reported even higher pre-run cortisol values in two groups of marathon runners (n=17; 749±54 $nmol.l^{-1}$ and n=13; 798±67 $nmol.l^{-1}$). No explanation for these higher than normal resting cortisol concentrations was given by any of the mentioned authors. Nonetheless, the question still remains why current pre-exercise cortisol levels were somewhat raised. Since the subjects had fasted overnight, a probable reason could be gluconeogenesis (cortisol-stimulate glucose production from non-carbohydrate sources - Ganong, 1998). This is unlikely as the glucose concentrations were within the normal fasting range of 3.9 - 6.1 $mol.l^{-1}$ as prescribed by Ganong (1998). The only feasible answer from the data on hand is that the moderately elevated cortisol levels could be ascribed to pre-exercise anxiety. Stress is associated, among other things, with increased secretions of ACTH, which is almost exclusively mediated by hypothalamic CRH release. ACTH, in turn, stimulates cortisol release from the adrenal cortex (Ganong, 1998).

Leukocytes

The changes observed in the WBC counts in all the trials were in accordance with reports from numerous authors (Moorthy & Zimmerman, 1978; McCarthy & Dale, 1988; Oshida, *et al*., 1988; McCarthy *et al*., 1991; Gabriel *et al.*, 1992; Pyne, 1994; Nieman *et al*., 1998a; Robson, 2000). These authors indicated that the circulating leukocytes should increase during exercise of any duration (Moorthy & Zimmerman, 1978; McCarthy & Dale, 1988; Pyne, 1994; Nieman *et al*., 1998a; Robson, 2000) and/or intensity (McCarthy & Dale, 1988; Oshida,

et al., 1988; McCarthy *et al*., 1991; Gabriel *et al.*, 1992; Pyne, 1994) and that that this may even continue in the recovery period (McCarthy *et al*., 1991; Pyne, 1994; Nieman *et al.*, 1998a; Robson, 2000). An analysis of the type of WBC indicates that all the major subpopulations contributed to the increase, with the neutrophils being the most predominant at all time points during the trials.

Recent research indicates that supplementary glucose prior to and during exercise, as well as during recovery following exercise, can lessen the increase in leukocytes associated with prolonged exercise (Nehlsen-Cannarella *et al.*, 1997; Henson *et al*., 1998; Mitchell *et al*., 1998; Nieman *et al*., 1998a).

In this study, an attenuated leukocyte response was observed only for the duration of exercise in the FG and VG trials, and not in the post-exercise or recovery periods in any of the trials. A possible explanation for this difference could be that all the previous reports (Nieman *et al*., 1997a, 1997b, 1998a, 1998b, 1999; Henson *et al*., 1998), except that of Mitchell *et al.* (1998), utilised a more extended exercise period than the current test protocol. Secondly, glucose and/or carbohydrate supplementations were given prior to, during and post-exercise, compared with the present investigation, where supplementations were given only during exercise (Nehlsen-Cannarella *et al*., 1997; Henson *et al*., 1998; Nieman *et al*., 1998a, 1998b, 1999). Henson *et al.* (1998) and Nieman *et al.* (1997b), for example, used a 6% glucose supplement in an exercise protocol on the treadmill with a duration of 150 minutes. The subjects had to consume 750 ml of the supplement prior to the run, 1 000 $ml.hr^{-1}$ during the run, 500 ml during the first 90 minutes of the recovery period and another 1 125 ml, at a rate of 250 $ml.hr^{-1}$, in the remaining period of recovery (Nieman *et al.,* 1997b; Henson *et al.,* 1998). Nieman *et al.* (1998a, 1999) used a similar protocol with tri-athletes who ran or cycled for 150 minutes at 75% VO_{2max} and elite female rowers who exercised for 120 minutes, also at 75% VO_{2max}. Again, a 6% carbohydrate drink was supplemented before and during exercise, as well as during the recovery period, at almost the same rate as mentioned above (Nieman *et al.,* 1997b; Henson *et al.,* 1998). Mitchell *et al.* (1998) used subjects in a crossover designed study involving a low and a high carbohydrate diet, after which the subjects had to cycle for 60 minutes at 75% VO_{2max}, and then had to do six one-minute sprints with one minute's rest in-between. During the high carbohydrate trial, a beverage was consumed that delivered 5 g carbohydrate, and an equivalent amount of water without carbohydrates was consumed in the low carbohydrate trial.

Although the glucose supplementation and exercise duration used in this trial were not as extensive as those utilised previously (Nehlsen-Cannarella *et al.,* 1997; Nieman *et al.,* 1997a, 1997b, 1998a, 1998b, 1999; Henson *et al*., 1998;), the question still remains why attenuations of leukocytes were observed. Currently it can be attributed only to the concentration of glucose, as this was the only variable that was altered deliberately. The question then is whether glucose has a direct influence on the observed attenuated leukocyte counts or whether it mediates its influence indirectly via other factors. Previous studies have shown that the glucorticoids, such as cortisol, have powerful effects on the immune cells and the inflammatory response to tissue injury. These effects include an increase in both neutrophils and monocytes (Pyne, 1994; Gleeson *et al*., 1998; Robson, 2000). Cortisol levels did not differ significantly at the mid-exercise period between the trials for this study and therefore the increase in neutrophils and monocytes that were observed in the Pla trial cannot be attributed to cortisol. Epinephrine may possibly have had an influence, as it has a known demargination

effect on neutrophils (Pyne, 1994; Gleeson *et al*., 1998; Robson, 2000). Future studies could include the measurement of epinephrine, since an increase in this hormone could explain the increase in neutrophils. However, it still would not explain the observed increase in monocytes. A clear answer to this question cannot be formulated from the current data.

In summary, in comparison with the Pla trial, the consumption of 6% glucose during exercise in the FG (666±0 $ml.hr^{-1}$) and VG (374±45 $ml.hr^{-1}$) trials had a significant effect in raising serum glucose levels, blunting cortisol release post-exercise and attenuating increases in neutrophils and monocytes during exercise. The key finding is the fact that the VG trial had almost the same effect as the FG trial. The only difference was that the forced consumption of 6% glucose also attenuated the lymphocyte increase during exercise, an effect not seen in the unforced 6% glucose trial. We therefore conclude that the voluntary intake of a 6% glucose beverage has the same effect on blood glucose and cortisol levels and causes similar neutrophil and monocyte attenuations as a forced consumption thereof during exercise.

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