CHAPTER 3: RESULTS

1. General Results

1.1 Physical characteristics of athletes

Table 6: Physical characteristics of athletes as determined by anthropometric evaluation. Mean values are indicated.

PHYSICAL CHARACTERISTICS	
Age (years)	25.1 (5.46)
Body mass (kg)	56.2 (6.10)
Body height (cm)	165.8 (7.99)
Percentage body fat (%)	9.8 (2.24)
Lean body mass (kg)	51.3 (4.46)
Basal metabolic index (kg/m^2)	20.6 (2.23)
Basal surface area (m^2)	1.6 (0.17)
SOMATOTYPE: Endomorph	1.8 (0.42)
Mesomorph	4.1 (0.86)
Ectomorph	3.2 (0.53)

Standard deviation (SD) is indicated in brackets

1.2 Training distances

The mean training distance per week (km/week) of each individual athlete, over the two seven week trial periods (SI, SII), are shown in Table 7. League road running events, in which the athletes competed during the experimental period, are included in these training distances.

Table 7: Training distance per week (km/week), of individulal athletes, per seven week trial period (Trial period 1 - SI; Trial period 2 - SII). Mean values are indicated.

	TRAINING DISTANCE (km/week)	
Athlete	**Trial period 1 (SI)**	**Trial Period 2 (SII)**
1	118.71 (15.74)	112.29 (13.69)
2	67.28 (29.68)	63.85 (47.22)
3	58.14 (24.53)	69.00 (16.96)
4	45.57 (19.39)	46.85 (20.47)
5	74.85 (12.79)	85.28 (27.09)
6	68.57 (21.11)	61.28 (10.67)
7	64.14 (19.38)	59.50 (17.79)
8	61.28 (20.61)	61.85 (29.95)
9	65.85 (31.98)	71.28 (25.61)

Standard deviation indicated in brackets.

1.3 Dietary analyses

Energy and nutrient intakes of athletes were calculated (as described) on three occasions during each trial period:

- the week prior to start of trial period
- week three
- week seven

In this section, the total energy intake (kJ/day), as well as the composition of the athletes, diets (percentage energy from fat, CHO, and protein respectively), are presented. Individual values for each athlete are shown, in order to effectively demonstrate any variation in individual eating patterns. In order to interpret these results, the percentage difference in value from the first analysis of every trial

period, was calculated and is shown. (A percentage increase is indicated with a '+'. A percentage decrease is indicated with a '-').

Dietary intervention did not form part of this research project; athletes were merely instructed to continue with their normal diets, and to record their dietary intake on the prescribed times. From the energy and nutrient calculations (Table 8), it became evident that some athletes, when considering their training programmes, did not take in enough energy; e.g. athlete number 7's daily energy intake during the first dietary recording was a mere 7 410kJ/day; the athlete was subsequently advised to increase his energy intake, and did show a 44.3% increase in daily energy intake during the following recording. From Table 8 it is evident that athletes' daily energy intake, as well as the composition of their diets, did show some variation throughout the research period. The athletes' poor socio-economic circumstances has to be taken into account when interpreting the data; the athletes had to eat what is affordable and available, and not neccessarily what would be beneficial to them.

Table 8: Dietary analyses of individual athletes for trial period one (SI) and trial period two (SII). Mean values per day, and percentage differences are indicated. (E = energy, ▲ = Percentage difference between analyses)

DIETARY ANALYSES

		Total E (kJ/day)			%E from Fat (%/day)			%E from CHO (%/day)			%E from Protein (%/day)		
Athlete	**Period**	**Before**	**Week 3**	**Week 7**	**Before**	**Week 3**	**Week 7**	**Before**	**Week 3**	**Week 7**	**Before**	**Week 3**	**Week 7**
1	**SI**	9148	8135	7726	31.4	29.8	35.2	50.6	55.0	53.6	18.2	14.9	12.0
	▲		*-11.0*	*-15.5*		*- 5.0*	*+12.1*		*+14.1*	*+ 5.8*		*+ 3.3*	*+ 9.2*
	SII	13186	11577	10248	39.0	32.1	34.6	49.5	56.5	52.4	11.9	12.3	13.0
	▲		*-12.2*	*-22.2*		*-17.6*	*-11.2*		*+14.1*	*+ 5.8*		*+ 3.3*	*+ 9.2*
2	**SI**	15038	15643	10845	25.2	35.9	21.3	63.3	45.6	65.9	12.3	19.5	12.8
	▲		*+ 4.0*	*-27.8*		*+42.4*	*-15.4*		*-27.9*	*+ 4.1*		*+58.5*	*+ 4.0*
	SII	13248	13897	11947	23.0	29.7	26.7	64.7	55.3	60.3	13.0	15.7	13.4
	▲		*+ 4.8*	*- 9.8*		*+29.1*	*+16.0*		*-14.5*	*- 6.8*		*+20.7*	*+ 3.0*
3	**SI**	8176	8241	10379	32.1	33.1	36	52.6	51.3	45.0	15.6	16.8	19.0
	▲		*+ 0.7*	*+26.9*		*+31.3*	*+12.1*		*- 2.4*	*-14.4*		*+ 7.6*	*+26.6*
	SII	9082	9349	10156	31.9	30.2	36.0	48.5	52.4	46.7	20.2	18.1	18.0
	▲		*+ 2.9*	*+11.8*		*- 5.3*	*+12.8*		*+ 8.0*	*- 3.7*		*-10.3*	*-10.8*
4	**SI**	11056	10135	11287	33.5	17.9	18.7	56.8	70.7	68.7	10.7	12.9	14.5
	▲		*- 8.3*	*+ 2.0*		*-46.5*	*-44.1*		*+24.4*	*+20.9*		*+20.5*	*+35.5*
	SII	12980	9393	11265	26.4	20.0	24.9	64.3	68.8	63.2	10.7	13.0	11.9
	▲		*-27.6*	*-13.2*		*-24.2*	*- 5.6*		*+ 6.9*	*- 1.7*		*+21.4*	*+11.2*

		Total E (kJ/day)			%E from Fat			%E from CHO			%E from Protein		
Athlete	Period	Before	Week 3	Week 7	Before	Week 3	Week 7	Before	Week3	Week 7	Before	Week 3	Week 7
5	SI	13175	12680	13210	38.0	34.3	33.0	47.1	50.0	52.0	15.2	15.7	14.0
	▲		*- 3.7*	*+ 0.2*		*- 9.7*	*-13.1*		*+ 6.1*	*+10.4*		*+ 3.2*	*- 5.9*
	SII	10795	11527	12230	32.4	38.0	33.7	48.9	48.1	50.7	18.3	13.7	15.6
	▲		*+ 6.7*	*+13.2*		*+17.2*	*+ 4.0*		*- 1.6*	*+ 3.6*		*-25.1*	*-14.7*
6	SI	23535	21758	18970	42.6	29.0	29.0	40.4	56.7	52.1	16.4	14.4	18.6
	▲		*- 7.5*	*-19.3*		*-31.9*	*-31.9*		*+40.3*	*+28.9*		*-12.1*	*+13.4*
	SII	15813	12346	12617	33.2	26.4	29.5	50.9	53.0	55.3	16.6	20.6	17.2
	▲		*-21.9*	*-20.0*		*-20.4*	*-11.1*		*+ 4.1*	*+ 8.6*		*+24.0*	*+ 3.6*
7	SI	7410	10697	6484	20.0	25.9	28.0	55.7	53.0	40.0	23.2	20.5	31.2
	▲		*+44.3*	*-12.4*		*+28.2*	*+38.1*		*- 4.8*	*-28.1*		*-11.6*	*+34.4*
	SII	15198	13359	12797	18.5	20.7	21.9	67.6	58.1	62.5	13.5	18.4	15.6
	▲		*-12.1*	*-15.7*		*+11.8*	*+18.3*		*-14.0*	*- 7.5*		*+36.2*	*+15.5*
8	SI	11873	18332	21179	46.2	30.9	32.2	33.5	54.9	53.1	20.3	14.6	15.7
	▲		*+54.4*	*+78.3*		*-33.1*	*-30.3*		*+63.8*	*+58.5*		*-28.0*	*-22.6*
	SII	14251	10528	16980	30.2	27.3	25.9	52.2	60.2	62.7	16.2	13.8	12.0
	▲		*-26.1*	*+19.1*		*- 9.6*	*-14.2*		*+15.3*	*+20.1*		*-14.8*	*-25.9*
9	SI	37168	19748	22200	35.8	31.1	29.5	54.6	60.2	59.1	9.9	9.2	12.2
	▲		*-46.8*	*-40.2*		*-13.1*	*-17.5*		*+10.2*	*+ 8.2*		*- 7.0*	*+23.2*
	SII	14163	14408	13854	34.7	26.0	24.1	48.0	59.8	58.0	18.5	15.4	18.7
	▲		*+ 1.7*	*- 2.1*		*-.25.0*	*-30.5*		*+24.5*	*+20.8*		*-16.7*	*+ 1.0*

2. Blood analyses

2.1 Plasma analyses - Technikon Dax system

In Table 9 plasma analyses - Technikon DAX System SM4-1141L93 - are shown as determined on four occasions (B1, 6W1, B2 and 6W2). Mean values are indicated. A statistically significant difference was noted between B1 and 6W1 on the following values: corrected calcium and full blood count ($p<0.5$), and plasma albumin ($p<0.01$). Plasma albumin, glucose, alanine aminotransferase ($p<0.05$), and creatine ($p<0.01$) showed a significant difference between B2 and 6W2. When comparing the first and second baseline evaluations (B1, B2), plasma potassium, carbon dioxide, albumin, full blood counts ($p<0.05$), and magnesium ($p<0.01$) showed a significant difference.

Table 9: Plasma analyses - Technikon DAX System SM4-1141L93

(B1=Baseline 1, 6W1=after six weeks supplementation-S1, B2=Baseline 2, 6W2=six weeks after supplementation-SII).

PLASMA ANALYSES - TECHNIKON DAX SYSTEM

	Sodium (mmol/l)	Potassium (mmol/l)	Magnesium (mmol/l)	Corrected calcium (mmol/l)	Total calcium (mmol/l)	Carbon dioxide (mmol/l)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)	Full blood (10^{12}/l)
Normal	**137-144**	**3.60-4.70**	**0.70-0.95**	**2.20-2.55**	**2.20-2.55**	**23.0-29.0**	**66.0-79.0**	**39.0-50.0**		**4.40-5.90**
B1	139.30	4.16	0.81	2.40	2.40	24.87	79.60	45.83	33.76	4.92
	(0.95)	(0.34)	(0.03)	(0.08)	(0.04)	(1.81)	(6.45)	(2.04)	(5.31)	(0.27)
6W1	139.02	4.38	0.85	* 2.31	2.38	24.03	82.71	** 49.42	33.28	* 5.16
	(1.57)	(0.32)	(0.05)	(0.09)	(0.09)	(1.52)	(5.57)	(3.02)	(3.82)	(0.35)
B2	139.31	# 4.84	## 0.90	2.37	2.46	# 22.17	80.15	49.73	29.56	# 5.13
	(3.25)	(0.58)	(0.04)	(0.17)	(0.18)	(3.33)	(5.03)	(2.69)	(5.23)	(0.31)
6W2	138.96	4.86	0.89	2.40	2.45	24.32	80.21	* 47.41	32.80	5.26
	(1.30)	(0.52)	(0.04)	(0.05)	(0.05)	(0.75)	(4.35)	(1.96)	(3.91)	(0.30)

*Statistical difference is indicated in brackets. * Indicates any statistical significant difference between B1 and 6W1, and B2 and 6W2 respectively (*p<0.05, **p<0.01). # Indicates any significant difference between B1 and B2 (#p<0.05), ##p<0.01).*

PLASMA ANALYSES (continue)

	Creatinine (µmol/l)	Urea nitrogen (mmol/l)	Uric acid (mmol/l)	Glucose (mmol/l)	LDH (IE/l)	ALP (IE/l)	ALT (IE/l)	AST (IE/l)	GGT (IE/l)
Normal	**81-114**	**3.1-7.8**	**0.31-0.47**	**3.9-5.9**	**90-180**	**38-102**	**6.0-32**	**9.0-34**	**8.0-32**
B1	88.25	3.94	0.27	3.81	222.93	69.55	28.60	37.56	41.23
	(10.84)	(0.67)	(0.05)	(1.15)	(56.30)	(13.80)	(11.80)	(17.61)	(21.62)
6W1	85.15	4.10	0.26	3.83	213.42	73.42	28.00	39.31	43.12
	(6.06)	(0.81)	(0.03)	(0.23)	(50.54)	(10.58)	(15.75)	(20.69)	(21.77)
B2	87.82	4.76	0.29	3.91	183.06	70.55	24.43	29.31	44.05
	(11.96)	(1.06)	(0.06	(0.30)	(35.79)	(11.33)	(14.93)	(5.82)	(33.06)
6W2	** 98.02	4.28	0.25	* 4.26	* 220.96	73.75	27.87	37.80	51.73
	(12.25)	(0.53)	(0.05)	(0.53)	(33.01)	(7.11)	(13.57)	(24.64)	(32.92)

*Standard deviation is indicated in brackets. Lactate dehidrogenase (LDH), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma-glutamyltransferase (AST). * Indicates any statistical significant difference between B1 and 6W1, and B2 and 6W2 respectively (*p<0.05; **p<0.01). # Indicates any significant difference between B1 and B2 (#p<0.05; ##p<0.01).*

2.2 Serum lipid profiles

Table 10: Serum lipid profiles of athletes: Individual as well as mean values are indicated. (B1=baseline1, 6W1=after six week supplementation-SI; B2=baseline2, 6W2=after six weeks supplementation-SII).

	SERUM LIPID PROFILES (mmol/l)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**MEAN**
TOTAL- C N=2.80-4.80										
B1	3.50	4.85	2.08	3.18	4.08	3.91	4.21	4.51	3.36	3.74 (.82)
6W1	*3.94*	*4.94*	*2.06*	*3.29*	*4.61*	*4.09*	*4.55*	*4.94*	*3.75*	*4.11*(.85)*
B2	4.16	4.90	2.70	2.78	4.29	4.20	4.48	5.19	4.34	4.01 (.92)
6W2	*2.96*	*5.30*	*2.36*	*3.27*	*4.74*	*3.73*	*4.06*	*4.57*	*3.52*	*3.72 (.86)*
LDL-C N=1.65-2.90										
B1	1.90	3.11	0.90	0.59	1.42	1.87	2.46	1.87	1.66	1.75 (.75)
6W1	*2.28*	*2.95*	*0.69*	*0.70*	*4.53*	*1.70*	*3.12*	*3.09*	*2.39*	*2.02**(.66)*
B2	2.32	2.89	1.06	0.93	4.76	2.16	2.65	2.19	2.22	2.05 (.95)
6W2	*1.60*	*3.62*	*1.21*	*1.38*	*1.66*	*1.90*	*2.66*	*2.56*	*2.44*	*2.11 (.77)*
HDL-C N=0.70-1.30										
B1	1.17	0.88	0.80	1.56	1.88	1.58	1.60	2.09	1.24	1.40 (.43)
6W1	*0.92*	*1.16*	*0.74*	*1.60*	*1.90*	*1.61*	*1.14*	*1.61*	*1.02*	*1.55 (.30)*
B2	1.31	1.39	1.26	1.48	1.81	1.67	1.60	2.15	1.51	1.30 (.39)
6W2	0.70	1.17	0.88	1.23	1.54	1.24	1.25	1.48	0.80	1.15 (.29)
TRIGS-C N=0.40-2.10										
B1	0.58	1.27	0.59	0.90	0.75	1.49	1.19	0.95	0.85	0.95 (.31)
6W1	*0.47*	*1.04*	*0.58*	*0.66*	*0.59*	*0.84*	*0.83*	*1.06*	*1.53*	*0.84 (.32)*
B2	0.61	0.87	0.56	0.58	0.55	0.70	1.41	1.11	0.93	0.81 (.29)
6W2	*0.86*	*0.80*	*0.53*	*0.91*	*0.51*	*0.92*	*1.06*	*1.23*	*0.82*	*0.83 (.22)*

*Total-C=Total cholesterol, LDL-C=Low density lipoprotein, HDL-C=High density lipoprotein, Trigs=Triglycerides. N = Normal range. Standard deviation of mean values are indicated in brackets. * p<0.05; **p<0.01.*

Table 10 indicates individual as well as mean serum lipid profile values (mmol/l) as measured on four occasions - baseline 1 (B1), after six weeks of SI supplementation (6W1), baseline 2 (B2) after a five week washout period, and

after six weeks of SII supplementation. Mean values indicate a statistically significant difference in total cholesterol ($p<0.05$), and low density lipoprotein ($p<0.01$) after six weeks of SI supplementation. No significant differences did however, occur during the SII supplementation period.

2.3 Serum organic acid profiles

2.3.1 L-lactate and non-esterified fatty acids

Lactate (mmol/l) and non-esterified fatty acid (NEFA - µmol/l) concentrations of individual athletes are presented in this section. The mentioned values were determined before, directly after, and 30 minutes after each exercise session, on three occasions during each seven week trial period (SI; SII):

- baseline evaluation (B1; B2)
- six week evaluation (6W1; 6W2)
- before and directly after the marathon events (M1; M2)

Data are depicted in the form of lactate and non-esterified fatty acid (NEFA) response patterns. The data were analysed according to

- the extent of the response pattern
- the difference in lactate and NEFA concentrations prior to each exercise session
- the trend in the response pattern.

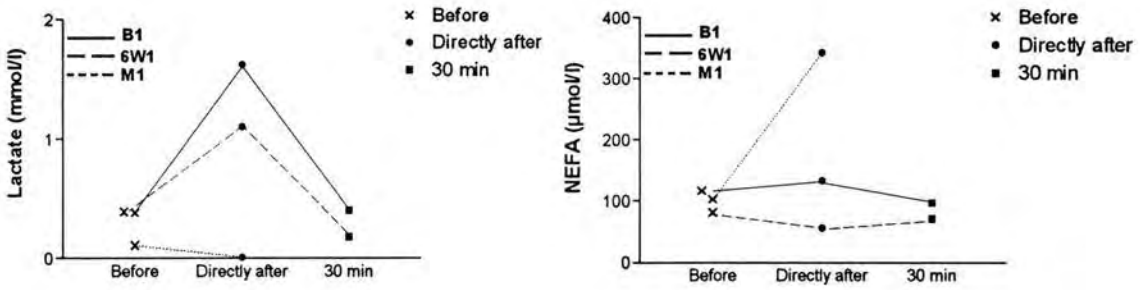
On the basis of the above mentioned parameters, the athletes were divided into three groups. This section highlights the main features of individual athlete's data, with a detailed discussion to follow in Chapter 4. Due to uncontrolable circumstances (e.g. inability of medical doctor to collect an adequate blood sample directly after exercise), some response patterns are unfortunately incomplete.

SERUM ORGANIC ACID PROFILES

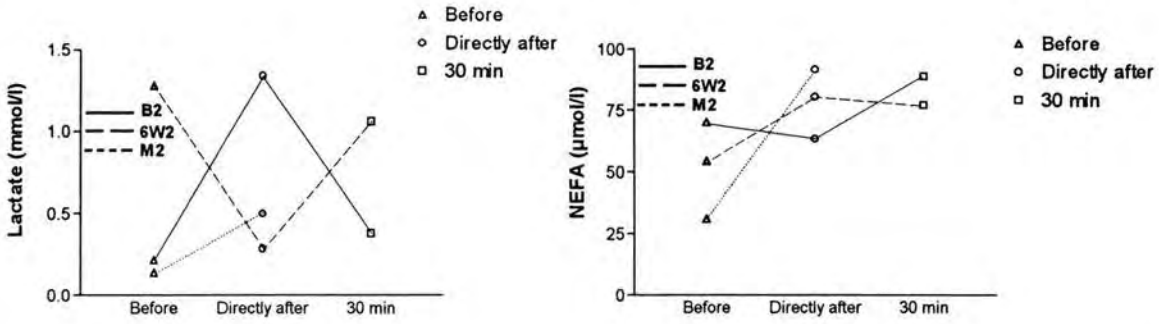
GROUP 1

Athlete 1

Supplement 1



Supplement 2

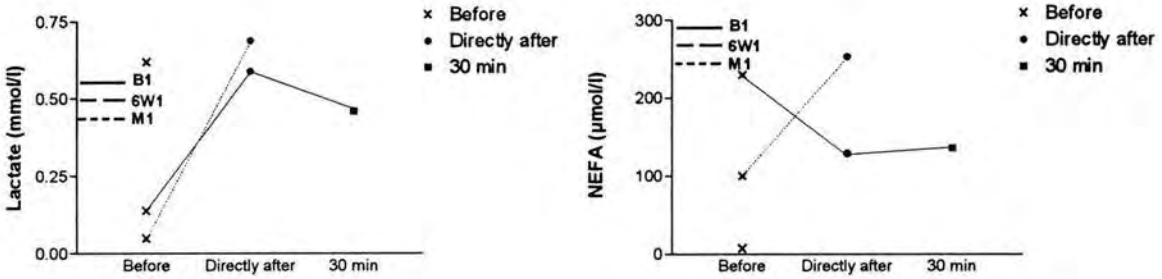


Athletes number 1 and 5 were included in group one on the basis of the following:

- after SI, and SII supplementation (6W1, and 6W2 evaluation), NEFA concentrations were lower than baseline values prior to exercise

Athlete 5

Supplement 1



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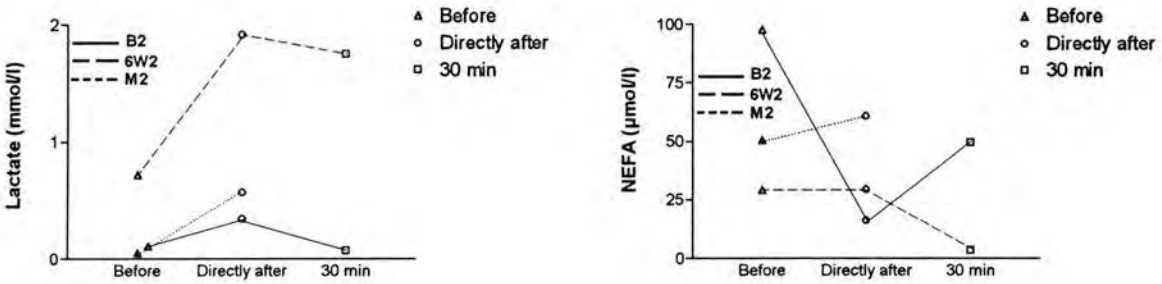


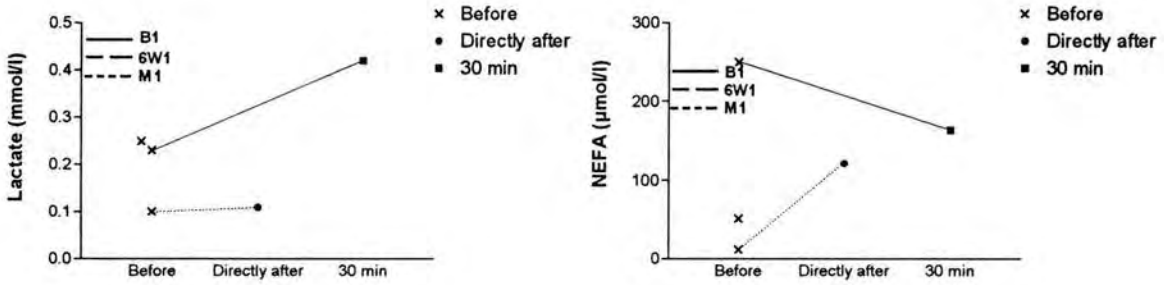
- a clear trend emerged in lactate and NEFA response patterns during the second trial period (SII): prior to exercise, low lactate concentration equals high NEFA concentration, and high lactate concentration equals low NEFA

The response patterns of athlete number 1 during the second trial period displays a fine example of the emerging trend.

GROUP 2

Athlete 7

Supplement 1



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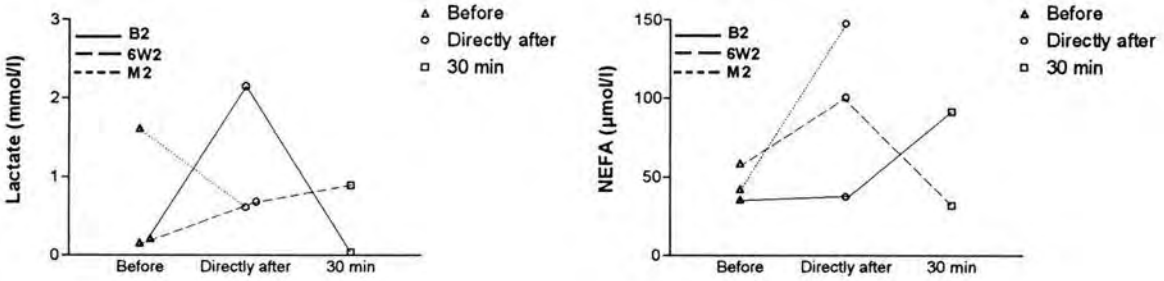
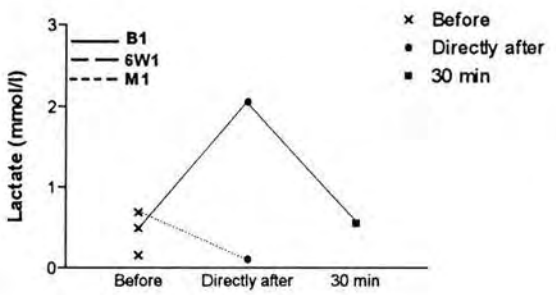


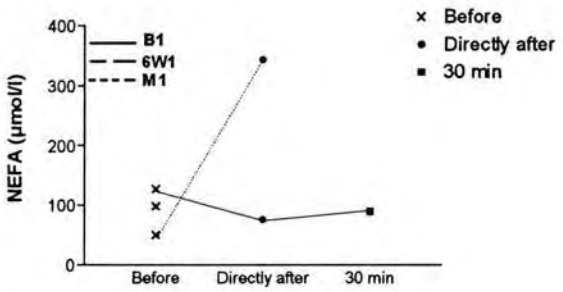
Athletes number 7, 6, 4, 9, 3, and 8 were included in this group based on the following:

- after SI supplementation (6W1), NEFA concentrations prior to exercise, were lower than baseline values

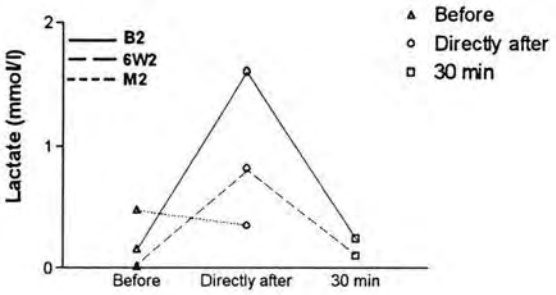
Athlete 3

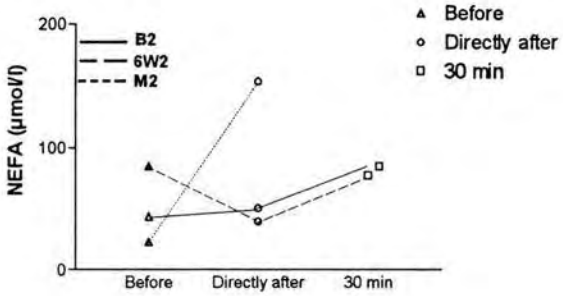
Supplement 1





Supplement 2

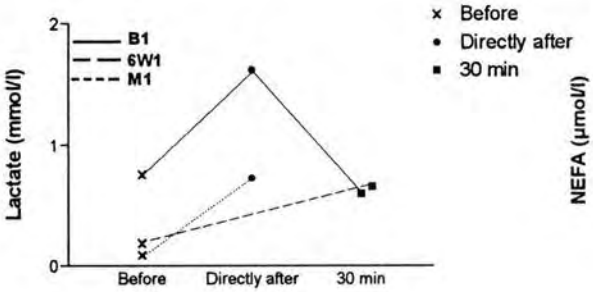


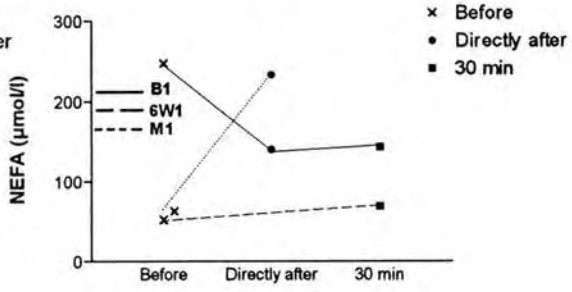


- after SII supplementation (6W2), NEFA concentrations before exercise, were higher than baseline values
- neither supplement one nor two had a dramatic effect on lactate concentrations before exercise, except athlete number 8, where a marked difference was noted in values prior to exercise on supplement two.

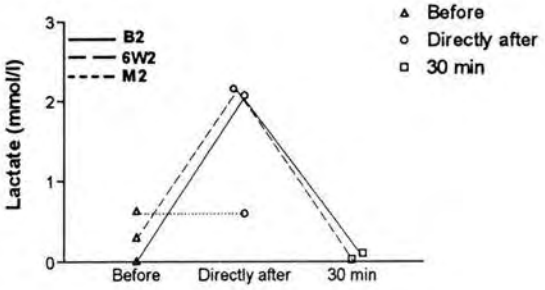
Athlete 4

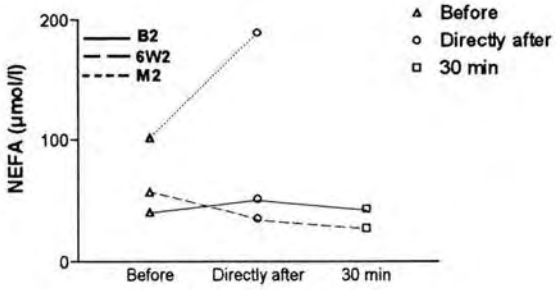
Supplement 1





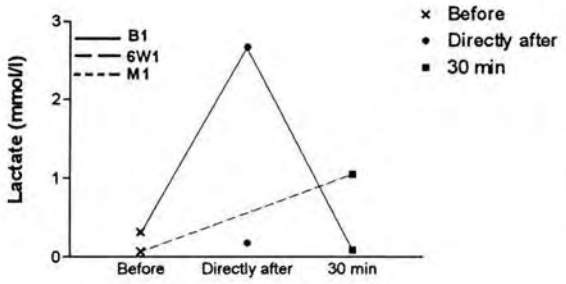
Supplement 2

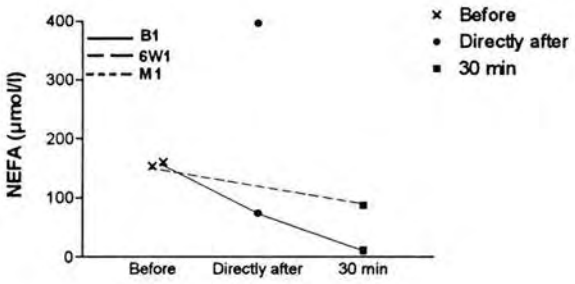




Athlete 6

Supplement 1





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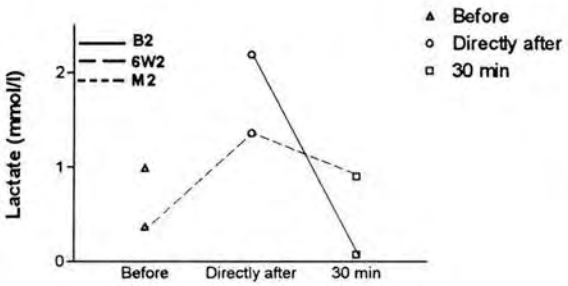
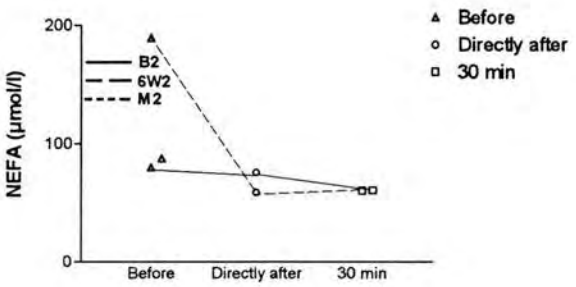
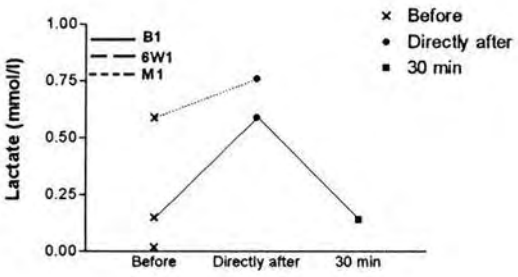
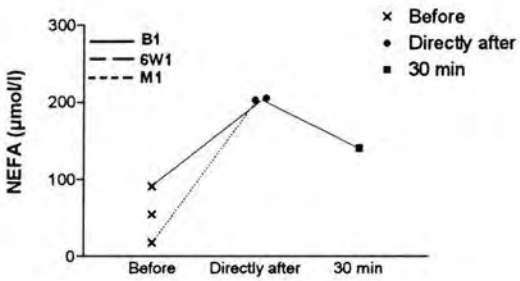




Athlete 8

Supplement 1





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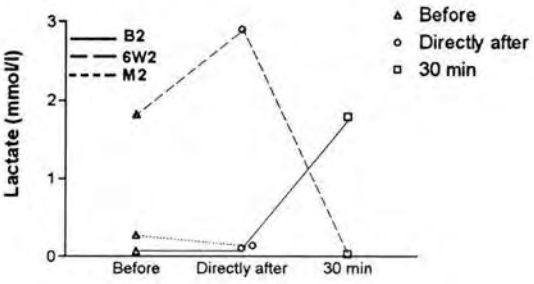
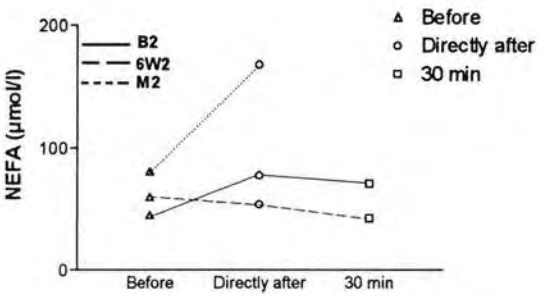
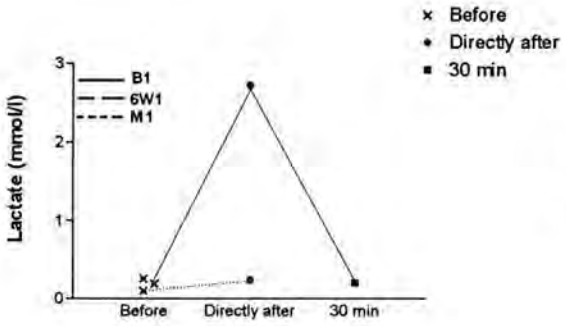
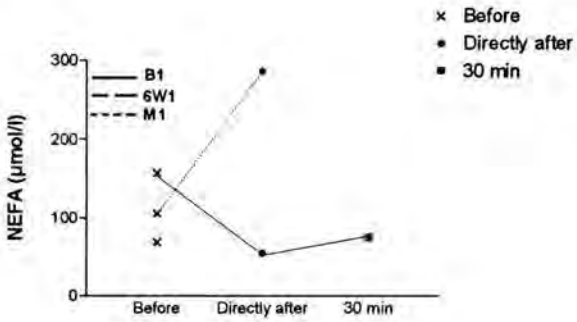




Athlete 9

Supplement 1





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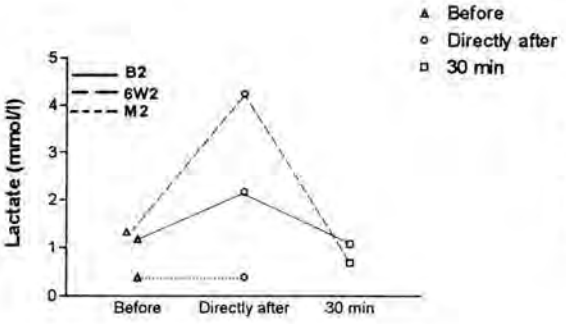
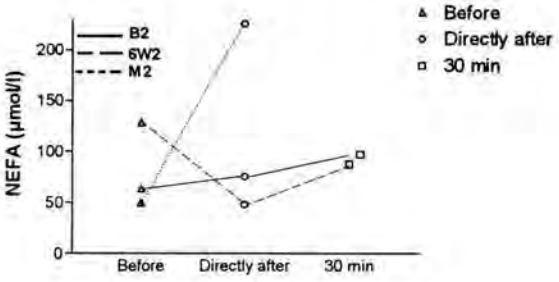
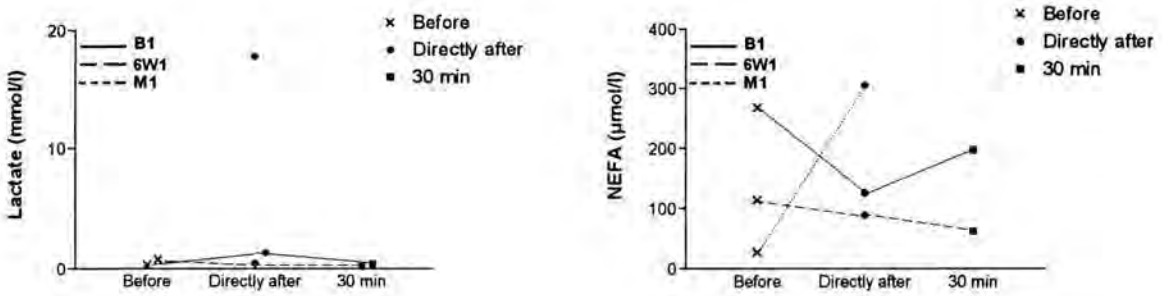




GROUP 3

Athlete 2

Supplement 1



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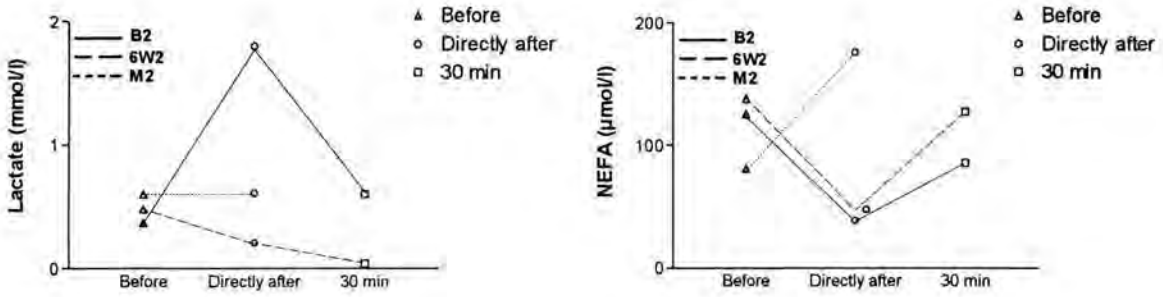


Athlete number 2 was included in group three. This athlete could not be matched in either group one or two:

- a weak lactate response was displayed after SI and SII supplementation
- after SI supplementation, a weak NEFA response occurred
- after SII supplementation, a good NEFA response pattern is displayed.

2.3.2 ß-OH-butyric acid concentrations

Table 11: Mean ß-OH-butyric acid concentrations (µmol/l) of athletes as determined before directly after, and 30 minutes after each VO_2 max. exercise test (B1, 6W1, B2, 6W2).

	ß-OH-BUTYRIC ACID CONCENTRATIONS (µmol/l)		
Evaluation	**Before exercise**	**Directly after exercise**	**30 min after exercise**
Baseline 1 (SI)	19.74	14.82	8.61
	(12.76)	(4.71)	(5.32)
Six week 1 (SI)	6.77	15.68	9.98
	(3.01)	(2.60)	(3.91)
Baseline 2 (SII)	7.09	11.04	7.70
	(6.16)	(5.57)	(7.63)
Six week 2 (SII)	8.49	11.48	10.10
	(5.06)	(4.47)	(5.34)

Standard deviation indicated in brackets.

From Table 11 it is clear that ß-OH-butyric acid concentrations did not differ significantly during the experimental period. The overall trend seemed to be a relative increase in ß-OH-butyric acid concentrations directly after exercise, with a relative decrease in the concentrations 30 minutes after exercise. It is interesting to note that during the second six week evaluation (6W2), the ß-OH-butyric acid concentration stayed high 30 minutes after exercise.

2.4 Plasma carnitine concentrations

Table 12: Mean plasma carnitine concentrations (µmol/l) as determined before, directly after, and 30 minutes after each VO_2 max exercise test (B1, 6W1, B2, 6W2). Mean values are indicated.

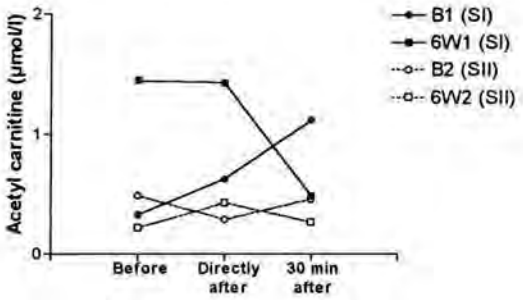
PLASMA CARNITINE CONCENTRATIONS (µmol/l)						
	Free carnitine			Acetyl carnitine		
Evaluation	**Before exercise**	**Directly after exercise**	**30 min after exercise**	**Before exercise**	**Directly after exercise**	**30 min after exercise**
Baseline 1 (SI)	2.48	2.74	2.18	1.10	1.01	0.85
	(0.81)	(0.66)	(0.88)	(0.53)	(0.39)	(0.31)
Six week 1 (SI)	3.14	3.65	2.94	0.75	0.76	0.80
	(0.59)	(1.20)	(1.36)	(0.35)	(0.32)	(0.21)
Baseline 2 (SII)	0.91	# 0.62	# 0.81	# 0.15	# 0.16	# 0.16
	(1.24)	(0.75)	(0.71)	(0.18)	(0.22)	(0.18)
Six week 2(SII)	# 0.94	## 0.80	* # 0.35	# 0.13	## 0.16	# 0.07
	(1.53)	(1.17)	(0.32)	(0.12)	(0.13)	(0.07)

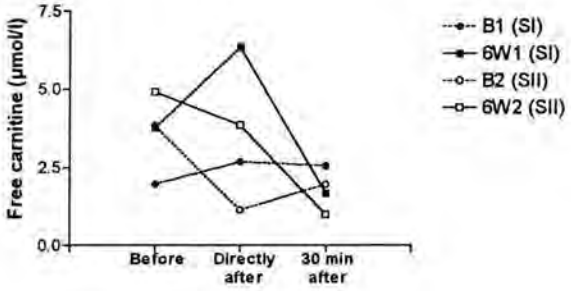
*Standard deviation indicated in brackets. * Indicates any statistical significant difference between B1 and 6W1, and B2 and 6W2 respectively (p<0.05). # Indicates any statistical significant difference between B1 and B2, and 6W1 and 6W2 respectively (#p<0.05; ##p<0.01).*

It is clear that free carnitine concentrations differed significantly ($p<0.05$) when comparing B1 and B2 values directly after, and 30 minutes after exercise, while acetylcarnitine concentrations differed significantly before, directly after, and 30 minutes after exercise ($p<0.05$). When comparing 6W1 and 6W2, it is clear that both free and acetylcarnitine concentrations differed significantly before ($p<0.05$), directly after ($p<0.01$), and 30 minutes after ($p<0.05$) exercise.

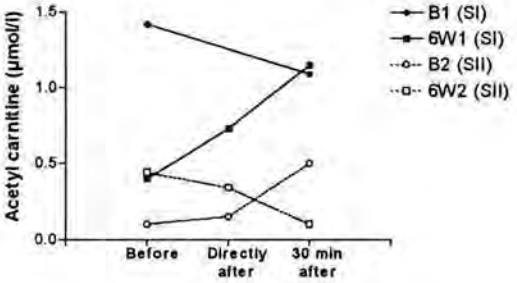
PLASMA CARNITINE CONCENTRATIONS (µmol/l)

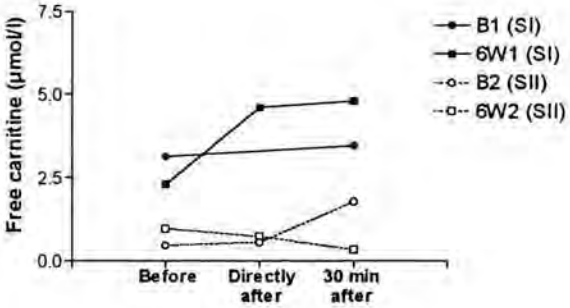
Athlete 1



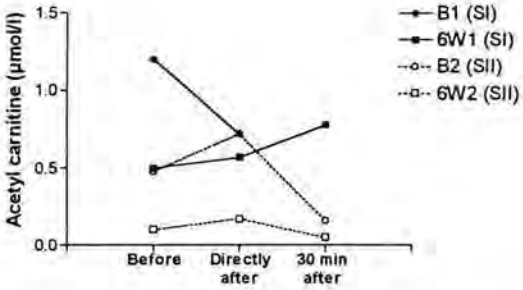


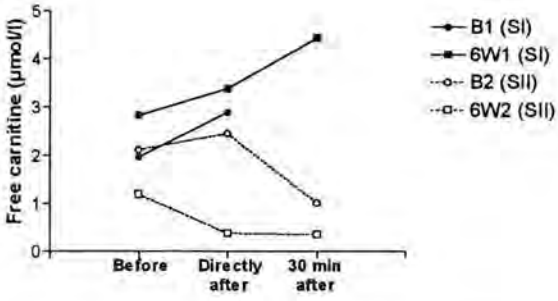
Athlete 2





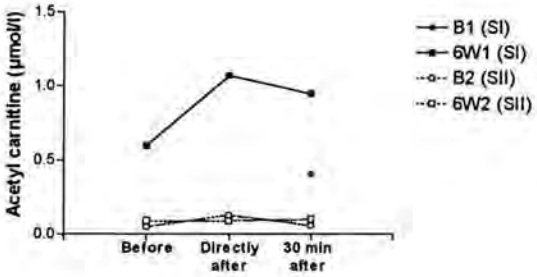
Athlete 3

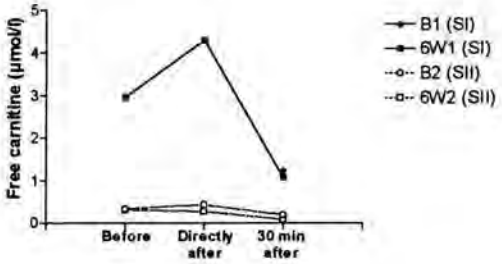




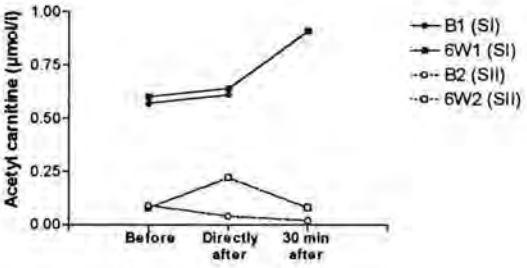
Individual plasma free and acetylcarnitine profiles indicate that in the majority of athletes an increase in acetylcarnitine values are mirrored by an increase in free carnitine values, and vice versa. The significant difference between the first and second trial period's values are clear (B1 and 6W1 versus B2 and 6W2).

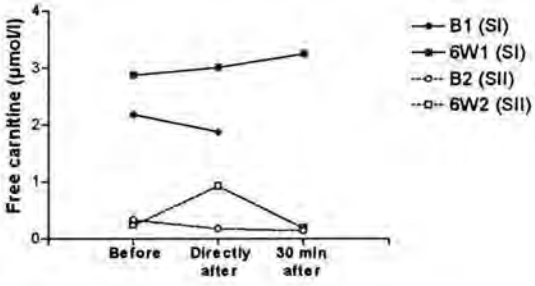
Athlete 4



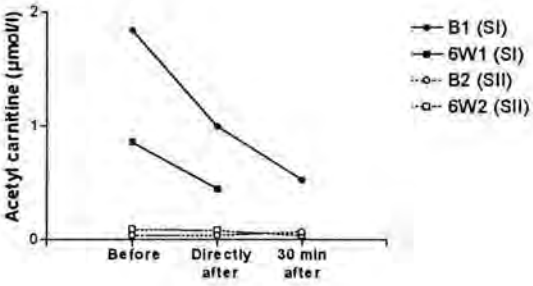


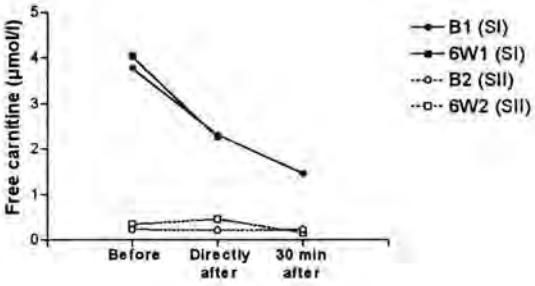
Athlete 5



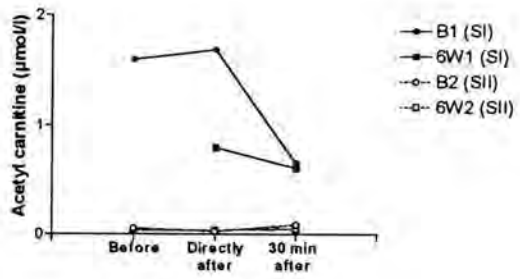


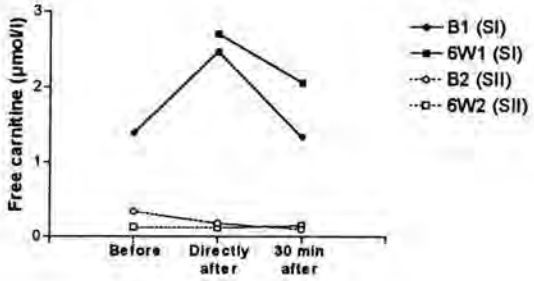
Athlete 6



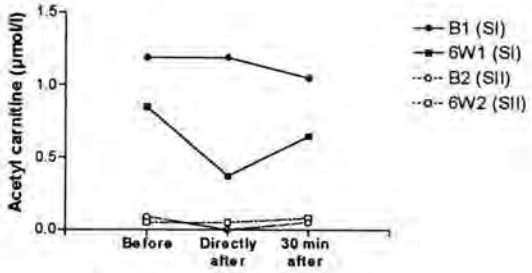


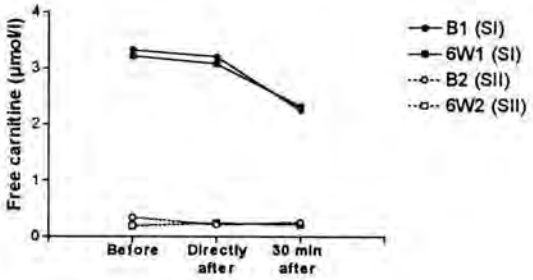
Athlete 7



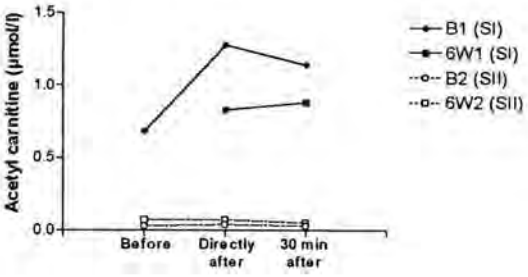


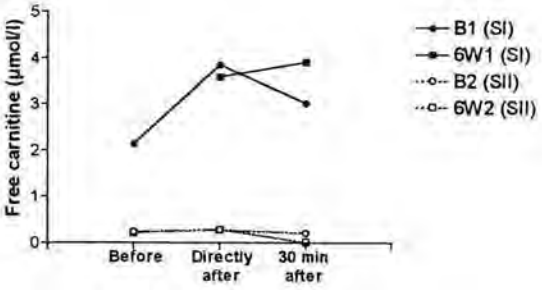
Athlete 8





Athlete 9





3. Performance

3.1 Marathon results

Table 13: Marathon results (hour:minutes.seconds) of marathon 1 and marathon 2. The amount of fluid (ml), in the form of SI and SII respectively, consumed by each athlete during each event is indicated in brackets.

MARATHON RESULTS (h:min.sec)			
Athlete	**Personal best**	**Marathon 1 (SI)**	**Marathon 2 (SII)**
1	3:01.20	3:16.38 (1125ml)	3:44.45 (750ml)
2	2:59.40	3:31.12 (1125ml)	3:29.14 (1375ml)
3	2:36.24	3:05.30 (1125ml)	3:04.58 (1125ml)
4	3:07.56	3:38.14 (1125ml)	4:02.40 (1125ml)
5	2:35.29	2:52.50 (1125ml)	2:57.10 (1125ml)
6	3:45.36	3:53.27 (1125ml)	
7	2:50.04	2:49.37 (1125ml)	3:26.12 (1375ml)
8	3:15.24	3:10.34 (1125ml)	3:15.21 (1125ml)
9	3:10.42	3:14.12 (1000ml)	3:43.02 (1125ml)

In Table 13 each athlete's personal best marathon time during the previous six months is shown, as well as their race times recorded during marathon 1 and 2. The amount of fluid (ml), in the form of SI and SII respectively, ingested by each athlete during the respective events is also indicated.

Marathon 1: The marathon was rated as very difficult - the first 20km comprised of mainly level and downhill running, while the rest of the route was mainly uphill. The temperature at the start of the race was ±9°C, and 16-20°C at the end of the race. The race was run in a light wind. Athlete number 9 experienced gastrointestinal discomfort, and had to stop twice during the race to relieve himself; he did however still completed the race successfully. Athlete number 4 had a difficult run because of blisters on both his feet, due to running with new shoes the previous weekend; he also completed the race without stopping.

Marathon 2: This marathon was rated as easy as it stretched over a relatively flat course. The race was however run in severely adverse weather conditions. At the start of the race the temperature was ±8°C. It remained cold throughout the race, with a 20-30 knot wind further lowering the temperature. Athlete number 6 was not able to complete the race; he experienced severe knee pain and stopped near the 21km mark. Athlete number 4 clocked a very slow time; he was forced to walk the last third of the race since he experienced severe cramps in his thigh muscles. He ascribed the symptoms to the cold weather. Athlete number 7 had a severe bout of flu two weeks prior to the race. His symptoms improved considerably the week prior to the event, and after obtaining medical advice he was declared sufficiently recovered to run. He had a relaxed run, clocking a much slower time than the previous event. Athlete number 1 experienced severe stomach cramps and diarrhoea shortly after starting the race. He subsequently stopped ingesting more supplement, and completed the race in severe discomfort.

3.2 VO_2 max exercise test results

Parameters monitored during the VO_2 max exercise tests included VO_2 max, peak treadmill running speed, respiratory exchange ratio, VCO_2, VO_2, and heart rate. Each athlete performed four VO_2 max tests:

- B1 - baseline one
- 6W1-after six weeks MCT + CHO supplementation
- B2 - baseline two
- 6W2 - after six weeks MCT + CHO + L-carnitine supplementation

In this section, each parameter is presented in the form of two figures - Figure ..a, and Figure ..b. The 'a' figure depicts a comparison between the first and second baseline evaluation, and serves to highlight any variations that might have occurred during the five week washout period, between the two trial periods.

The 'b' figure depicts the following:

- the difference in parameter value between the first baseline and six week evaluation (B1-6W1)
- the difference in parameter value between the second baseline and six week evaluation (B2-6W2)
- variations between the first and second trial periods (SI-SII).

3.2.1 VO_2 max (ml O_2/min/kg)

VO_2 max refers to the maximal capacity of an individual to take up, transport, and utilize oxygen [67]. VO_2 max measures the total capacity of skeketal muscle mitochondria, active during exercise, to utilize oxygen [68].

Figure 8a depicts a relative consistency in VO_2 max between the first and second baseline evaluations. From Figure 8b, an overall decrease in VO_2 max, ranging from 2.2 - 11.9%, is evident during the first trial period (SI) except for athlete number 9, who showed no change in VO_2 max during this period. During the second trial period (SII), athlete number 7 showed an increase in VO_2 max. The remaining eight athletes again showed a decrease in VO_2 max, ranging from 4.3 - 8.5%.

3.2.2 Peak treadmill running speed

The peak treadmill running speed achieved by an athlete during the VO_2 max exercise test, is regarded as the best laboratory test whereby to predict a marathon or ultra marathon athlete's performance, over any distance between 10 and 90 kilometers [69].

Four athletes showed no change in peak treadmill running speed, while three athletes showed a decrease of 1-2km/h, and two athletes an increase of 1-2km/h (Figure 9a).

From Figure 9b, it is evident that two athletes showed no change in peak treadmill running speed during the first trial period (SI), between the baseline an six week evaluation. Three athletes showed a decrease of 1-2km/h, while four athletes' peak treadmill running speed improved by 1km/h during this period. During the second trial period (SII), four athletes achieved the same peak treadmill running speed. Four athletes increased their speed by 1km/h, and one athlete showed a decrease of 2km/h.

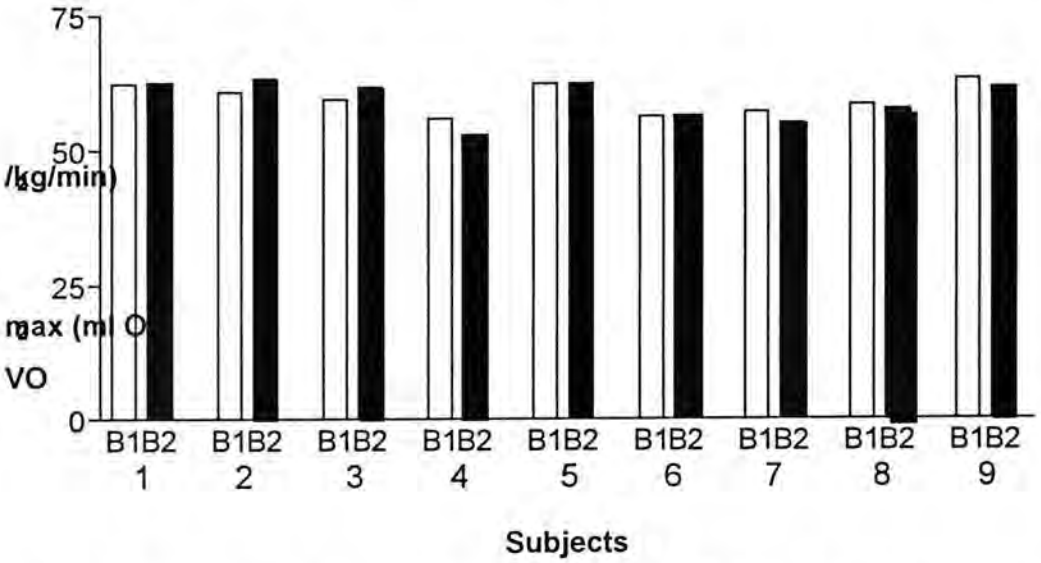


Figure 8a: VO_2 max of individual athletes (n = 1-9) indicating baseline 1 (B1) and baseline (B2) values

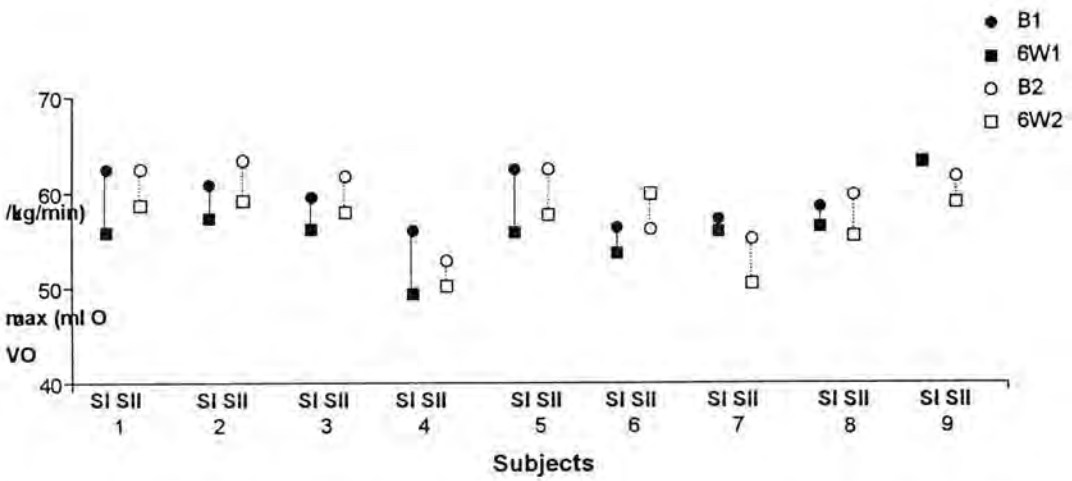


Figure 8b: VO_2 max of individual athletes(n = 1-9) comparing trial period 1 (SI: B1 and 6W1) and trial period 2 (SII: B2and 6W2)

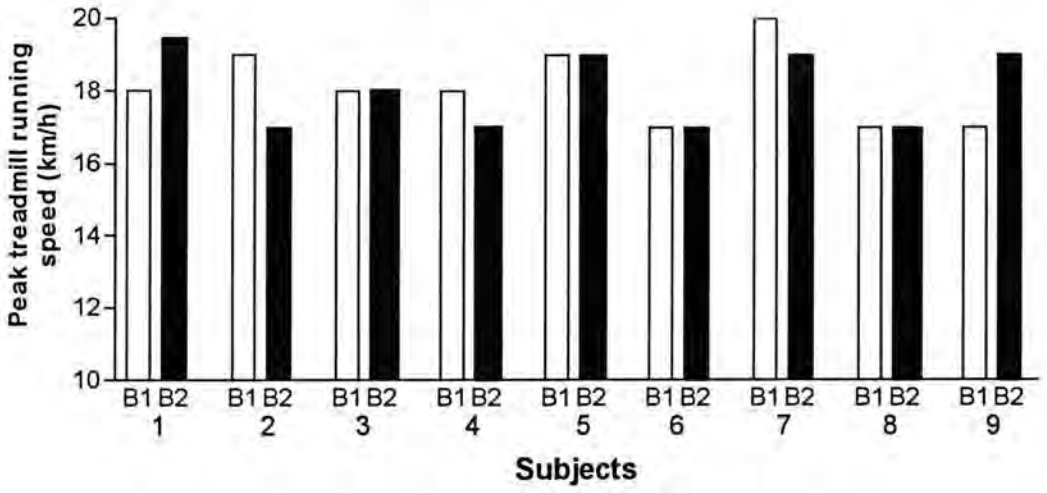


Figure 9a: Peak treadmill running speed of individual athletes (n = 1-9) indicating baseline 1(B1) and baseline 2(B2) values

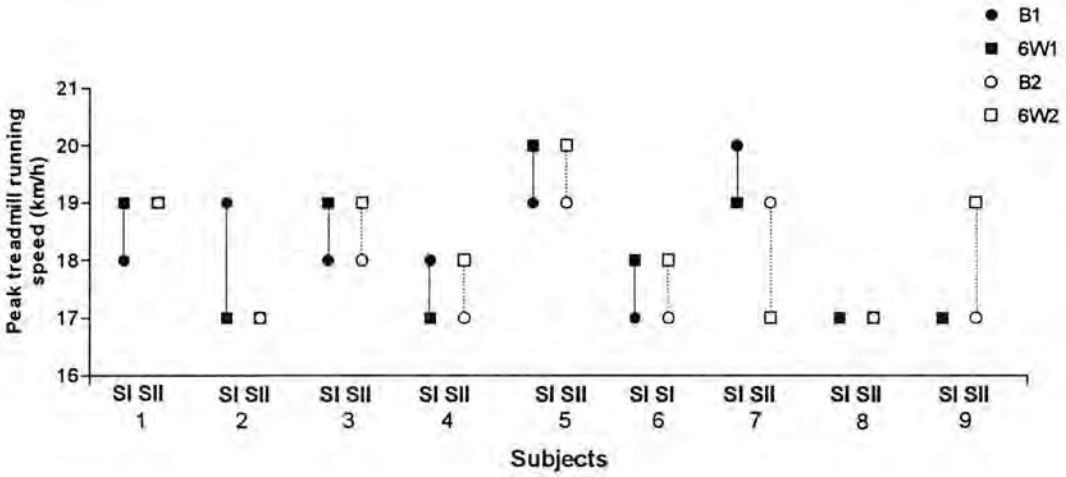


Figure 9b: Peak treadmill running speed of individual athletes (n = 1-9) comparing trial period 1 (SI: B1 and 6W1) and trial period 2 (SII: B2 and 6W2)

3.2.3 Respiratory exchange ratio

The respiratory exchange ratio indicates the volume of carbon dioxide liberated, divided by the volume of oxygen consumed, and is used as an indicator of substrate utilization during exercise at a constant workload [70].

Mean respiratory exchange ratios (RER) at running speed 14-20km/h, are presented in Figures 10a, and b. Figure 10a depicts a significant difference in RER between baseline values at 16km/h ($p<0.05$). RER values were <1 during the first baseline evaluation at 14, 16, 17, 19, and 20km/h. During the second baseline evaluation, values were <1 at 14km/h. The number of athletes able to reach a running speed of 18km/h and higher, is clearly indicated.

Figure 10b shows that during trial period one (SI), there was a significant increase in RER at running speed 14-19km/h ($p<0.01$). During the second trial period (SII), the only significant change in RER (increase) occurred at 14km/h. During the baseline evaluation (B2), no athlete was able to maintain a running speed of 20km/h, while after six weeks of SII supplementation, one ahtlete was able to do so.

3.2.4 VCO_2 (ml CO_2/min)

The mean VCO_2 of athletes at running speed 14-20km/h are presented in Figures 11a and b. The first and second baseline values are shown in Figure 11a, and it is evident that there were no significant difference between these values. Figure 11b indicates that during trial period one (SI), no significant changes occurred in mean VCO_2 during the baseline and six week evaluations. During the second trial period (SII), a significant decrease in VCO_2 ($p<0.01$) was evident at 17km/h. It is again clear that only one athlete was able to reach 20km/h after the second trial period (SII supplementation).

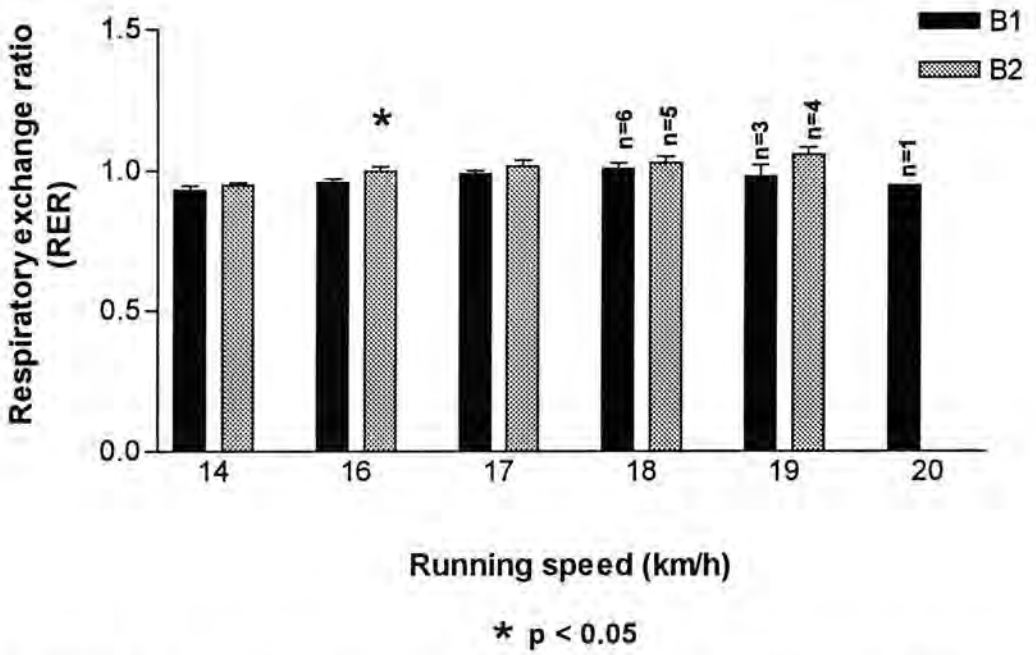


Figure 10a: Mean respiratory exchange ratios of athletes at running speed 14-20 km/h indicating baseline 1 (B1) and baseline 2 (B2) values. Standard deviations are indicated.

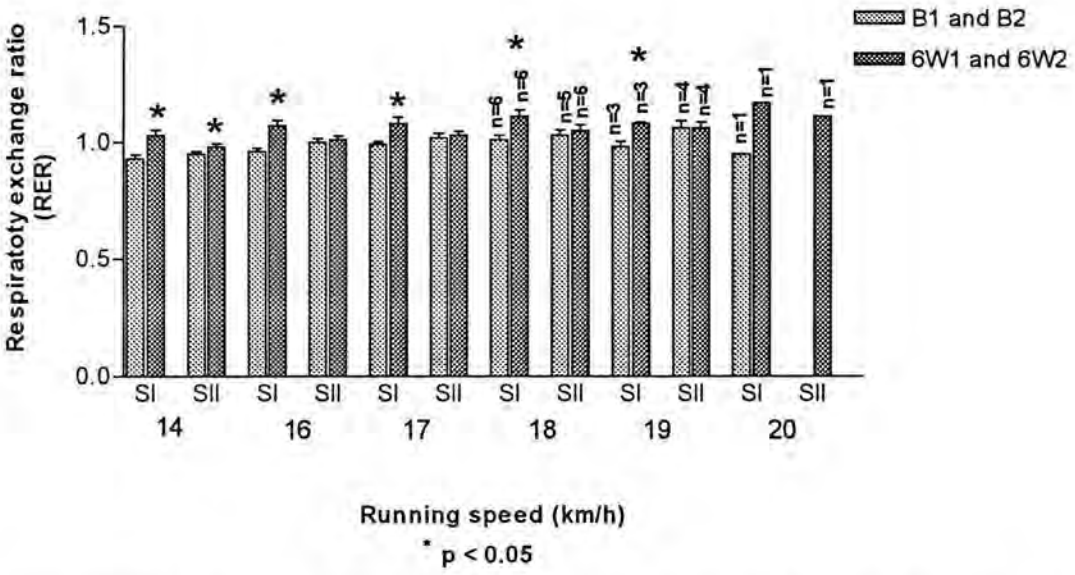


Figure 10b: Mean respiratory exchange ratios of athletes at running speed 14-20 km/h comparing trial perios 1 (SI: B1 and 6W1) and trial period 2 (SII: B2 and 6W2). Standard deviations are indicated

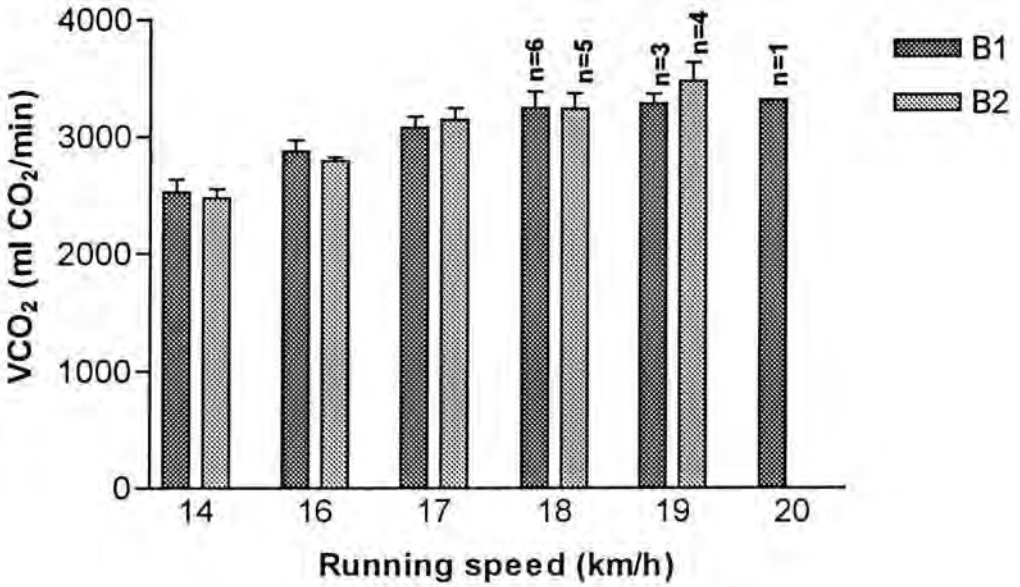


Figure 11a: Mean VCO_2(ml CO_2/min) of athletes at running speed 14-20 km/h indicating baseline 1 (B1) and baseline 2 (B2) values. Standard deviations are indicated

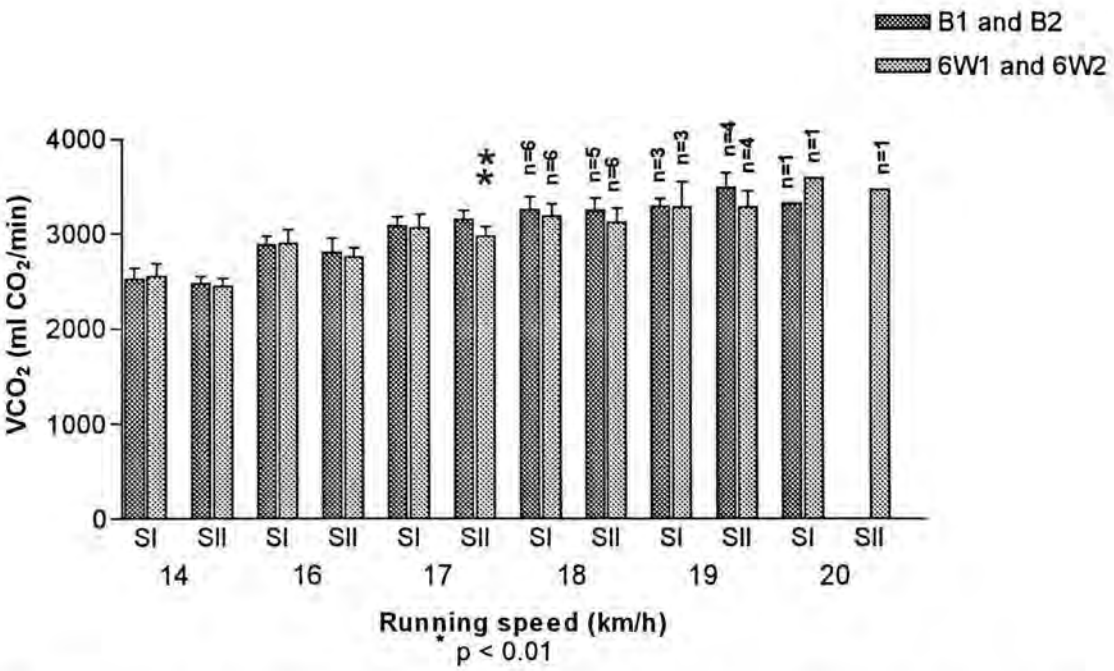


Figure 11b: Mean VCO_2(ml CO_2/min) of athletes at running speed 14-20 km/h comparing trial periods 1 (SI: B1 and 6W1) and trial period 2 (SII: B2 and 6W2). Standard deviations are indicated

3.2.5 VO_2 (ml O_2/min)

Figure 12a shows that there was no significant difference in mean VO_2 values, at running speed 14-20 km/h, during the first and second baseline evaluations. During trial period one (SI), a decrease in VO_2 occurred between the baseline and six week evaluation, being significant at 17km/h ($p<0.05$). At 18km/h an increase occurred (Figure 12b). During the second trial period (SII), a decrease in VO_2 was once again evident between baseline and six week evaluation, except at 18km/h. The decrease being significant at 14, 16 and 17km/h. The number of athletes able to reach a running speed of 18km and higher, is again noted.

3.2.6 Heart rate (beats/min)

From Figure 13a it is clear that a significant decrease occurred in the mean heart rate of athletes ($p<0.05$), at 14, 16, and 17km/h, between the first and second baseline evaluation. At 18, and 19km/h, an increase occurred, though not significant. During the first trial period (SI), a decrease in the mean heart rate is evident between the baseline and six week evaluations, at 14, 16, and 17km/h, though not significant (Figure 13b). The second trial period (SII) showed no significant difference in the mean heart rate between the baseline and six week evaluations; a slight increase occurred at 18 and 19km/h.

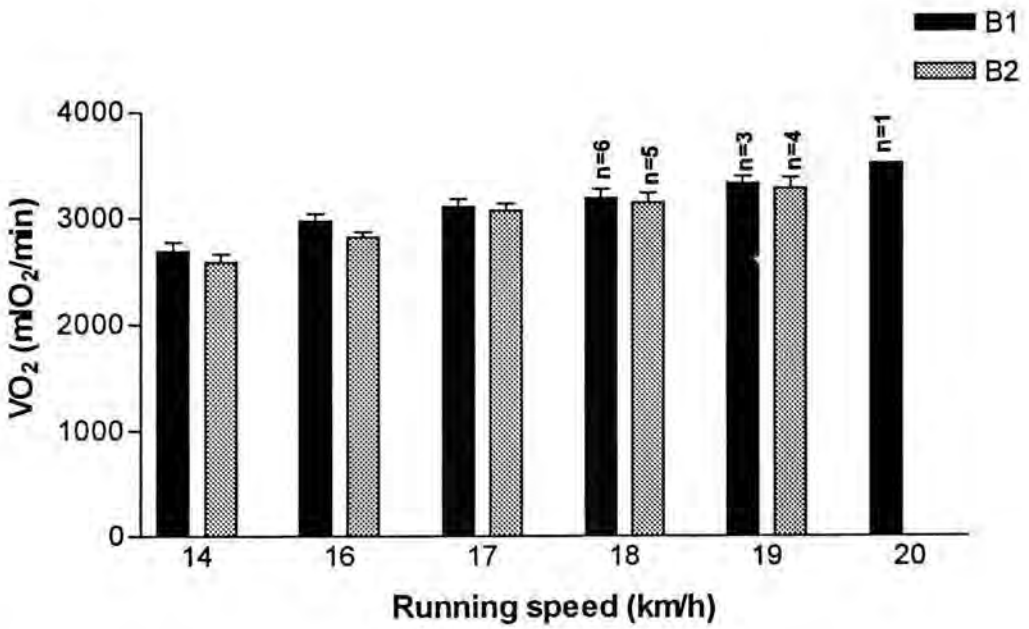


Figure 12a: Mean VO_2 (mlO_2/min) of athletes at running speed 14-20 km/h indicating baseline 1 (B1) and baseline 2 (B2) values. Standard deviations are indicated

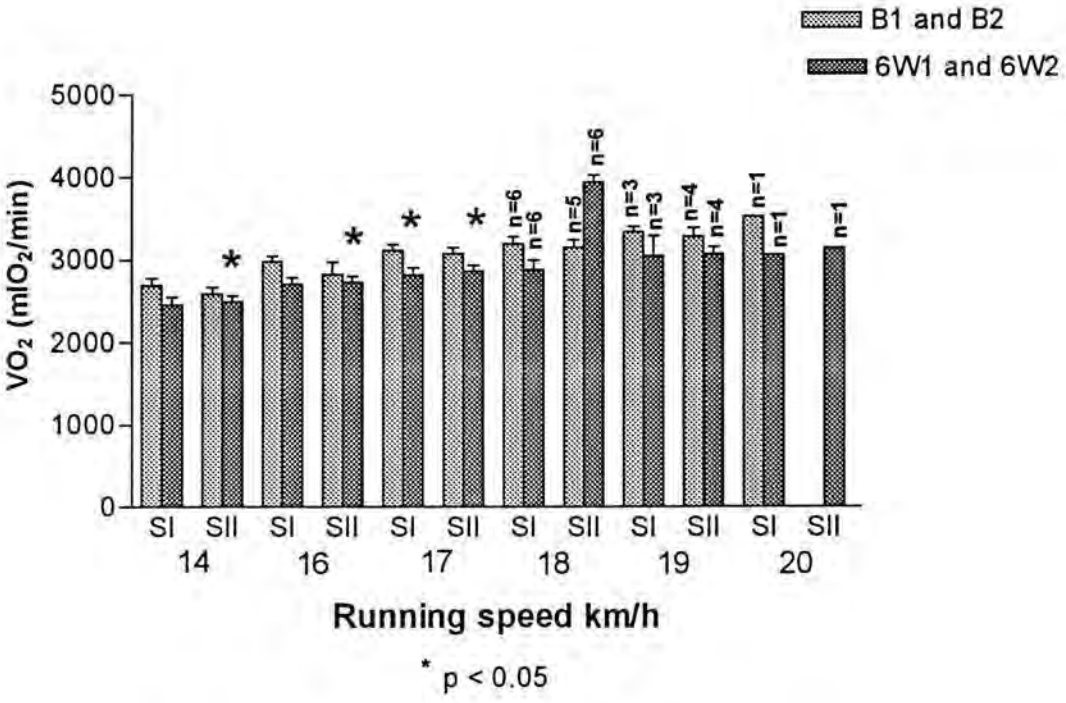


Figure 12b: Mean VO_2(mlO_2/min) of athletes at running speed 14-20 km/h comparing trial perios 1 (SI: B1 and 6W1) and trial period 2 (SII: B2 and 6W2). Standard deviations are indicated

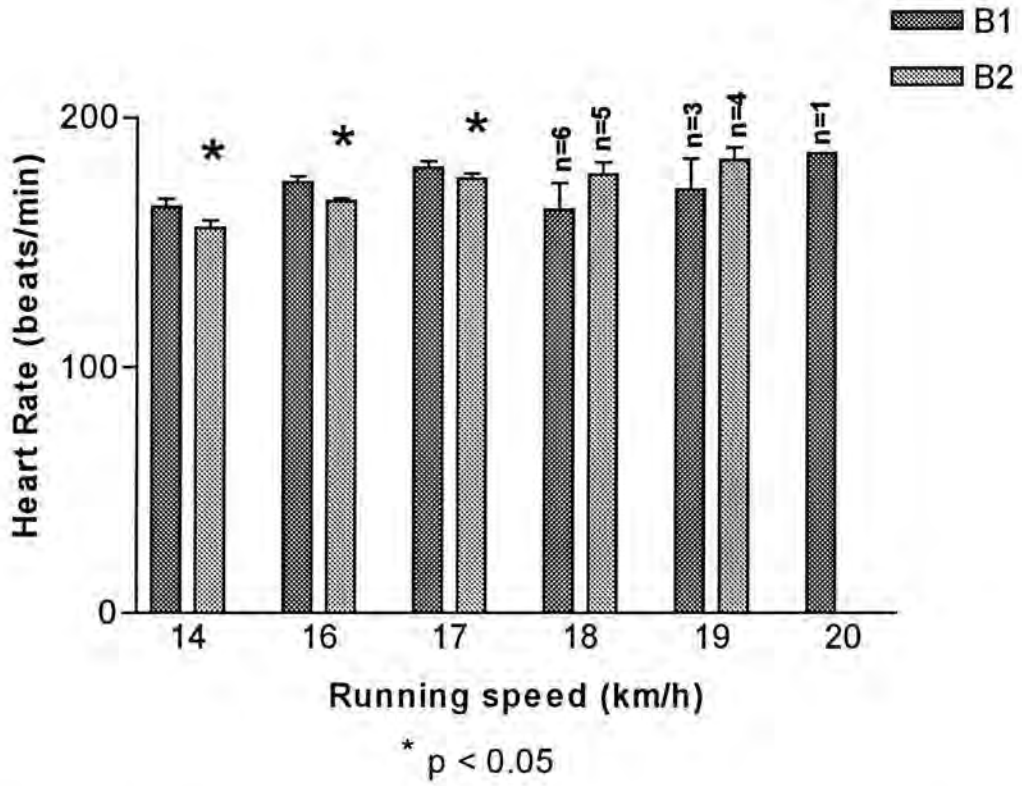


Figure 13a: Mean heart rate of athletes at running speed 14-20 km/h indicating baseline 1 (B1) and baseline 2 (B2) values. Standard deviations are indicated

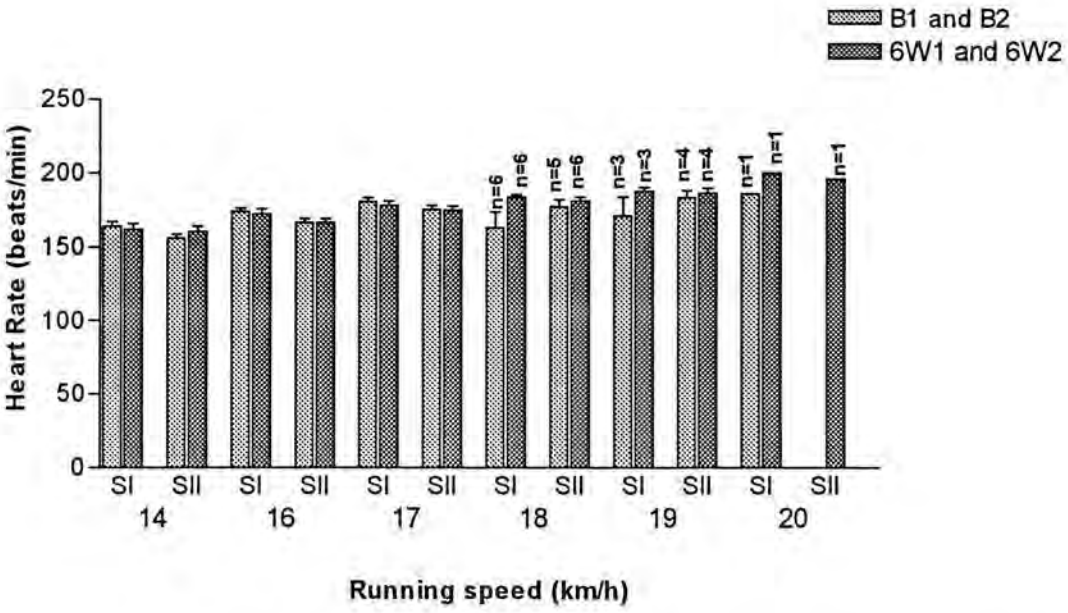


Figure 13b: Mean heart rate of athletes at running speed 14-20 km/h comparing trial perios 1 (SI: B1 and 6W1) and trial period 2 (SII: B2 and 6W2). Standard deviations are indicated

CHAPTER 4: DISCUSSION

In this study, the effects of medium-chain triglyceride, carbohydrate and L-carnitine supplementation on the performance and metabolic parameters of male marathon athletes were investigated. The divine individuality of the human physiology came to the fore, emphasizing the need to always delve deeper when unravelling results; e.g. a mere glance at a group average might lead one astray, masking meaningful individual metabolic and physiological response patterns. This discussion is thus presented, bearing the above in mind.

1. Dietary analyses

From a health perspective, the advocating of a moderate to high fat diet, even for the endurance athlete, has been criticized for its association with the development of obesity and cardiovascular disease [73]. During this study, athlete's diets were merely monitored, and not modified in any way. Fat, in the form of a liquid MCT supplement (containing carbohydrate and L-carnitine) was added to the athlete's diets over an extended period of time.

Diet should certainly be considered a major contributor to any athlete's performance. During this study, the energy and nutrient intake of athletes' basic diets was monitored on three occasion during each trial period (Chapter 3, section 1.3, Table 8). Careful evaluation (Table 8) revealed inconsistent eating patterns in some athletes, while others showed a relatively consistent contribution from fat, carbohydrate, and protein to the total daily energy intake.

It has to be mentioned, once again, that these athletes came from mostly underprivileged socio-economic circumstances. Food choices are therefore limited, and they are basically forced to eat what is available. The total daily energy intake of some athletes (e.g. athlete number 7, during the first dietary recording) were clearly insufficient to meet their energy requirements. Consider the fact that these athletes averaged between 45-119km/week during training

(Chapter 3, section 1.2, Table 7). It would therefore not be unreasonable to regard the athletes as relatively nutrient deficient. Appropriate advice, e.g. to increase daily energy intake, was given throughout the study when dietary analyses produced disconcerting results.

Inadequate carbohydrate intake can increase lipolysis, and initiate the metabolic response to starvation, even when energy intake is sufficient to meet resting energy requirements [74]. It is advocated that carbohydrates should make up at least 55-60% of the athlete's diet [75]. Table 8 shows that only one athlete's (athlete number 4) diet contained adequate amounts of carbohydrate throughout the experimental period. It is also quite clear that the athletes did not follow any specific carbo-loading regime prior to an important race, e.g. the standard marathon included at the end of each seven week trial period.

Fat should preferably constitute 25-30% of the energy of the athlete's diet. From Table 8 it is evident that six athlete's (numbers 1,3,5,6,8, and 9) fat intake was within these recommendations. However, some analyses revealed fat intakes as high as 46% among the mentioned six athletes. Body fat percentages (Chapter 3, section 1.1, Table 6), and serum lipid profiles (Chapter 3, section 2.2, Table 10) were seemingly not affected by these high levels of fat intake, and were well within normal limits.

Protein intake, in some cases, constituted less than 15% of the athletes' diets. This could be considered too low, and may lead to endogenous protein catabolism in order to meet the body's protein requirements as measured in creatinine, urea, etc.

2. Blood analyses

2.1 Nutritional status parameters

As mentioned, fat, in the form of a MCT supplement, was added to the athletes' diets over an extended period of time. This intervention indicated the need to monitor basic metabolic and nutritional status parameters (Chapter 3, section 2.1, Table 9). These remained well within normal limits throughout the study, however, with some exceptions.

Plasma potassium and magnesium concentrations, as well as full blood counts were significantly lower ($p<0.05$) when comparing the second baseline evaluation with the first (bearing in mind that a five week washout period followed the first trial period, during which no intervention occurred). The added vitamins and minerals, included in the MCT+CHO supplement (Chapter 2, section 5.1, Table 1), might have contributed to the above mentioned variation from normal. The unreliability of measuring magnesium concentrations in blood [47], was highlighted as a possible cause in that a significant increase ($p<0.01$) occurred in plasma magnesium concentrations between the first- and second baseline evaluations, whereas supplement two (SII) contained extra magnesium - SI: 62.4mg/40g dose; SII: 1586.6mg/45g dose (Chapter 2, section 5, Table 2 and 5 respectively). No significant difference however occurred in plasma magnesium concentrations during the second trial period, despite the increased magnesium content of the supplement; on the contrary, plasma magnesium values decreased after SII supplementation. For a more reliable result, magnesium should be measured in the red blood cell.

The reason for the significant decrease ($p<0.05$) which occurred in plasma carbon dioxide concentrations, when comparing the second baseline evaluation's results to the first's, is unclear. L-carnitine supplementation has been reported to lower carbon dioxide production [22], but in this study, carnitine supplementation only commenced after the second baseline evaluation.

Plasma albumin concentrations showed significant decreases during the first and second trial period (SI and SII), and could possibly be linked to the "carrier protein" function of albumin: blood free fatty acids bind to albumin, and are then carried throughout the body via the circulation [73]. Fat infusion (triglyceride emulsion), has been reported to increase the blood fatty acid concentration [28]. The effect of MCT supplementation could therefore have been involved in the variations occurring in plasma albumin levels; a rise in plasma free fatty acids (as a result of ingesting MCTs), would mean more fatty acids are available to bind to plasma albumin, thereby lowering the plasma albumin concentration.

The presence of creatinine in blood (as well as urea and uric acid), is an indication of the transport of nitrogenous waste products, resulting from the breakdown of nitrogen-containing substances in food and tissues [39]. Lactate dehydrogenase (LDH) could be linked to microtrauma occurring in skeletal muscle during intensive exercise [39,40]. The significant increase in the plasma concentration of the above mentioned parameters during the second trial period could, therefore, speculatively be linked merely to the effect of intense physical exercise.

In view of the contentious issue of cholesterol, serum lipid profiles were carefully monitored during this trial (Chapter 3, section 2.2, Table 10). MCTs purportedly have a slight cholesterol lowering effect. This effect has been accounted for by a decrease in the intestinal absorption of cholesterol, and a slowing of it's synthesis from acetyl-CoA in the liver [36]. Results obtained from this study differ however from the above reported effect. A significant increase occurred in mean serum total and LDL-cholesterol levels (Table 10) during the first trial period (SI), with LDL-cholesterol also showing an increase during the second trial period (SII), though not statistically significant. No significant changes occurred in HDL and triglyceride levels. Interestingly, total cholesterol levels showed a significant increase ($p<0.05$) between the first and second baseline evaluations.

In examining individual serum lipid profiles (Table 10) at the end of the entire 19 week experimental period, some interesting observations were made. A clear tendency towards a rise in LDL cholesterol levels emerged, with eight out of nine athletes showing an overall rise in LDL. Seven out of nine athletes showed an overall decrease in HDL levels, and six out of nine athletes an overall rise in total cholesterol levels. Although these tendencies were not statistically significant, it appeared to be noteworthy. One positive trend was the overall decrease in serum triglyceride levels.

A mere glance at these figures may create some cause for concern. Further investigation revealed, however, that the difference in lipid values between the first and second baseline evaluations, were of the same magnitude as the differences between the first six week and baseline, and second six week and baseline evaluations. Therefore, should any significance be attached to the reported increases occurring during the supplementation periods? Bear in mind, that a five week wash-out period followed the first trial period; therefore, theoretically, the first and second baseline values should have been more or less consistent. They differed however, without any intervention occurring. Do these observations not merely display the dynamic nature (normal fluctuations over time) of cholesterol levels, well within the realms of normal individual physiological parameters, affected by factors such as stress, and the level of lipid oxidation?

2.2 Serum organic acid profiles: non-esterified fatty acid (NEFA), and L-lactate concentrations

In an attempt to identify any possible beneficial effect that either MCT supplementation, or MCT and L-carnitine supplementation (both including carbohydrate), might have had on free fatty acid metabolism, dynamic metabolic evaluations were performed during this study. Serum non-esterified fatty acid (NEFA), and serum lactate profiles were studied. The latter being labile parameters; under fasting conditions these parameters were determined prior to, directly after, and 30 minutes after intensive physical exercise, in order to

determine or identify if an individual relies predominantly on fat oxidation, or carbohydrate oxidation, or both, to produce energy during exercise. The profiles were also used in an attempt to identify a possible shift towards fat (free fatty acid) oxidation, as a result of MCT and L-carnitine supplementation.

When examining NEFA and lactate profiles, it should again be stressed that response patterns, and not the serum levels per se, should, and have been regarded as meaningful. As discussed in Chapter 3, section 2.3.1, athletes were divided into three groups on the basis of the extent of the response patterns, the magnitude of the difference in lactate and NEFA concentrations prior to each exercise session, and the trends in the response patterns.

The degradation of fatty acids is a much more lucrative manner in which to produce ATP than glucose oxidation (the breakdown of fatty acids still do depend on a backround level of carbohydrate catabolism, to provide oxaloacetic acid to combine with acetyl-CoA in the citric acid cycle). A greater total yield of ATP is possible if muscle could oxidize fatty acids sufficiently during intense exercise, than is possible when relying predominantly on carbohydrate. Consider the fact that a six-carbon glucose molecule yields 38 ATP via glycolysis and the citric acid cycle, whereas an 18-carbon fatty acid (stearic acid) yields 147 ATP via ß-oxidation and the citric acid cycle. Thus, a 1.3-fold greater yield of ATP/carbon molecule, or a 3.9-fold greater yield of ATP from fat [73,40]. It should however also be mentioned that the oxygen requirement for glucose oxidation is 77% less than the oxygen required to oxidize stearic acid.

Fatty acids are supplied by both exogenous (adipose tissue), and endogenous (intramuscular) lipid reserves [26]. Adequate intramuscular triglycerides have been regarded as being critical for supplying free fatty acids to the working muscle [76]. Therefore, just as depleted muscle glycogen levels may lead to impaired performance, low muscle triglyceride stores may have the same effect [26].

Studies have demonstrated that in endurance trained athletes, basal lipid kinetics are shifted toward increased mobilization and oxidation of fat [74,77]. A high rate of triglyceride-fatty acid (TG-FA) substrate cycling in endurance trained athletes during the resting state, has been suggested as a possible reason for the shift towards lipid mobilization. Romijn *et al.* [74] found two to four-fold higher basal rates of triglyceride breakdown, fatty acid release into plasma, fatty acid oxidation, and TG-FA cycling in trained, compared to untrained athletes. They found the concentrations of the lipolysis regulating hormones, i.e. catecholamines and insulin, to be the same in both trained and untrained athletes, suggesting that increased fat oxidation was not solely due to hormonal stimulation.

The circulating FFA concentration curve at the commencement of, during, and after exercise, could be described as the end result of FFA flux into the circulation, and efflux into, and utilization by various tissues [1]; thus the appearance of FFA in the plasma represents the net result of FFA release from the adipose tissue, and FFA uptake by the active muscle.

In this study, athletes in group one (number 1 and 5), and group two (number 3,4,6,7,8, and 9) had lower fasting NEFA concentrations prior to exercise, after six weeks of SI supplementation (6W1), when compared to the baseline evaluation (B1). This would therefore imply that plasma FFA had either been utilized by muscle, or were re-esterified. A high-carbohydrate, low-fat diet has been shown to inhibit the optimal refilling of the muscle TG pool; this would require an increased uptake of plasma FFA in order to maintain the intramuscular FFA pool [26]. Dietary analyses revealed, however, that athletes generally consumed enough fat. Therefore, it could be argued that the drop in NEFA concentrations after MCT and CHO supplementation, were due to increased fat oxidation. This is also reflected in a decrease in mean serum triglyceride levels after SI supplementation (Chapter 3, section 2.2, Table 10). Considering the important role of carnitine in fatty acid transport (Chapter 1, section 3.5), it could subsequently be stated that athletes had enough endogenous carnitine to be able to transport mobilized long-chain fatty acids into the mitochondria. This assumption is reflected in mean plasma

carnitine concentrations being within normal range during the first trial period (Chapter 3, section 3.2.4, Table 12).

In contrast to group one, athletes in group two displayed higher NEFA concentrations prior to exercise, after SII supplementation (6W2), when compared to the baseline evaluation (B2). Athletes in group one displayed the same trend after SII supplementation, as they did after SI supplementation (a decrease in NEFA concentration after supplementation). This notion could be explained after again studying plasma carnitine values (Chapter 3, section 3.2.4, Table 12). From Table 12 it is clear that some external factor must have been responsible for a statistically significant decrease in plasma carnitine concentrations after the five week washout period (B2). Bearing in mind that carnitine supplementation only commenced after the second baseline evaluation. Table 12 also shows that not withstanding carnitine supplementation, plasma carnitine levels still decreased during the second trial period.

A factor worthy of consideration could have been the fact that the first trial period stretched over the late summer, early autumn period, while the second trial period was in mid winter. The athletes had to rise early to report to the laboratory on time in very cold conditions, with neither the comforts of adequate warm clothing, nor being able to ingest a hot beverage, as exercise tests were performed under fasting conditions. The exposure to low temperatures gave rise to an increase in lipid oxidation, subsequently leading to a decrease in carnitine levels. These sentiments have been echoed by Bording *et al.* [78], who verified that several physiological (or pathological) situations, such as cold exposure or high fat feeding, have been reported to influence carnitine metabolism *in vivo*. Situations associated with a high degree of fat oxidation, generally act to lower muscle carnitine concentrations. The rise in mean serum total- and LDL-cholesterol levels, as well as the mentioned decrease in mean serum triglycerides, now seem to fit more clearly into the total picture, with an increase in lipid oxidation being reflected in higher serum cholesterol levels.

The consequence of the significant decrease in plasma carnitine levels at the start, and during the second trial period, was an endogenous carnitine deficiency in the athletes. However, the athletes in group one were still able to mobilize and utilize FFA; reflected in the decrease in NEFA prior to exercise after SII supplementation. Thus, it could be accepted that these two athletes benefited from the carnitine supplementation, and that they were predominantly "fat burners", relying mainly on fatty acids to produce energy during exercise. These two athletes consistently performed the best in terms of peak treadmill running speed (Chapter 3, section 3.2.2, Fig. 9b), and high VO_2 max values (Chapter 3, section 3.2.2, Fig. 8b).

A clear trend emerging from their response patterns (group one) during the second trial period, was that prior to exercise, low lactate concentrations equaled high NEFA concentrations, and high lactate equaled low NEFA concentrations. This trend is in agreement with reports that L-carnitine supplementation decreases plasma lactate accumulation during exercise [19].

Carnitine supplementation during the second trial period was, however, not adequate to compromise for the endogenous carnitine deficiency in the second group of athletes. This was reflected in higher NEFA concentration prior to exercise after SII supplementation; fatty acids mobilized, could not be utilized efficiently, and therefore plasma FFA concentrations increased. The abundance of plasma FFA were most probably re-esterified, and could be the reason for the sharp decrease in NEFA concentration in most of these athletes, directly after exercise. Group two's athletes were therefore considered as a "grey group"; neither predominantly "fat burners", nor predominantly "carbohydrate burners". The magnitude of lactate and NEFA response patterns, could consistently be described as average to significant in these athletes.

2.3 Plasma carnitine concentrations

The effect of exercise on plasma carnitine levels has been widely studied; results to this effect have been reported in Chapter one, section 3.6. To recapitulate, exercise generally seems to cause a rise in plasma acylcarnitine levels [57,59,61], coinciding with a decrease in plasma free carnitine. The increase in acylcarnitine is accompanied by an increase in plasma ß-OH-butyric acid concentrations [78], thereby supporting the enhancing effect of carnitine on lipid oxidation, in that increased lipid oxidation gives rise to an increase in ketone body production. This phenomenon seems to occur with or without carnitine supplementation [78], and is generally ascribed to a wasting of excess muscle acylcarnitines to the plasma after exercise [59,79]. The influence of carnitine supplementation on physical exercise, has also been reported on (Chapter 1, section 3.7).

Plasma carnitine levels are believed to represent the balance between the synthesis, tissue uptake, and excretion of carnitine via the kidneys. Free carnitine represents the unbound carnitine fraction, whereas acylcarnitine represents the esterified carnitine fraction, be it long- or short-chain acylcarnitines. Friolet *et al.* [80] reported that acetylcarnitine represents the most abundant acylcarnitine compounds in the skeletal muscle carnitine pool, both under resting conditions, and after exhaustive exercise. They reported a three- to fivefold increase in the skeletal muscle acylcarnitine content during exercise above the lactate threshold, with a corresponding decrease in free carnitine. They subsequently suggested that predominantly free carnitine is used for the formation of short-chain acylcarnitines, and that acetylcarnitine formation accounted for 50% of the exercise associated increase in the skeletal muscle short-chain acylcarnitine content under normoxic conditions.

During this study, plasma free and acetylcarnitine levels were determined on four occasions (B1, 6W1, B2, 6W2), prior to, directly after, and 30 minutes after exercise. In examining mean carnitine values (Chapter 3, section 2.4, Table 12), most conflicting results came to the fore; an overall increase occurred in free

carnitine levels after SI supplementation (containing no carnitine), with a decrease in acetylcarnitine. These changes were not significant, but extremely noteworthy, and in sharp contrast to previously mentioned findings. During the second trial period (SII), much lower baseline free and acetylcarnitine levels, remained more or less unchanged, not withstanding the fact that L-carnitine was supplemented during this period. No statistically significant differences occurred in plasma ß-OH-butyric acid concentrations during either of the trial periods (Chapter 3, section 2.3.2, Table 11). Significant changes in both free, and acetylcarnitine levels occurred between the first and second baseline evaluations without carnitine supplementation, as well as between the first and second six week evaluation (significant decrease in free and acetylcarnitine concentration in spite of carnitine supplementation).

The question now arises as to whether there is any functional significance in the above mentioned conflicting changes in free and acetylcarnitine. Roger *et al.* [81] pondered over the same question while studying changes in muscle free and acetylcarnitine in the thoroughbred horse during physical exercise. In agreement with the generally reported trend (i.e. an increase in acetylcarnitine and a decrease in free carnitine) [78,80], he also found an increase in acetylcarnitine, mirrored by an equal decrease in free carnitine. He questioned if these changes did not simply reflect the obligatory action of the carnitine acetylcarnitine translocase enzyme, in maintaining thermodynamic equilibrium when confronted with a greatly increased rate of acetyl CoA formation. The the changes in acetyl= carnitine may therefore merely represent a side-reaction of an enzyme primarily involved in the metabolism of short-chain acyl groups. The alternative is that these changes do reflect some advantage to the cells.

The confusing picture (concerning free and acetylcarnitine levels) became considerably clearer after studying individual free and acetylcarnitine profiles (Chapter 3, section 2.4.1). These profiles portray a trend in the free and acetylcarnitine response patterns: during both trial periods (SI and SII), the acetyl carnitine response pattern was mirrored in the free carnitine response pattern, in

that a rise in free carnitine was accompanied by a rise in acetylcarnitine, and vice versa. This trend was consistent in most of the athletes, with athletes numbers 4, 5, 6, 8, and 9 being the clearest examples. Athletes number 1, 2, and 3 showed some minor variations.

The question thus arises as to how we should interpret the mentioned observations in this study. It seems to point towards a suggestion that before, during, and after exercise, response patterns should be regarded as more meaningful than plasma carnitine values per se. Considering the fact that these athletes could be regarded as normal, healthy individuals, should the trend in plasma carnitine response patterns not be regarded as a norm? Therefore, should any apparent deviation of significant magnitude emerge, it could warrant further investigation into the athletes' metabolic status, and might point to some weakness or defect which could adversely affect the athlete's performance, or even his health.

A second obvious trend, evident in the plasma carnitine profiles, needs to be highlighted. The significant difference in the baseline plasma free and acetyl= carnitine levels is quite clear. This occurrence seemed peculiar, considering the fact that a five week wash-out period followed the first trial period, and that carnitine supplementation was only incorporated into the experiment after the second baseline evaluation.

Two questions come to mind. Firstly, could the measuring of plasma carnitine levels be regarded as a true and reliable indicator of an individuals carnitine status? Famularo and De Simone [82] stated that L-carnitine is found in high concentrations in leukocytes, including peripheral blood mononuclear cells, and that L-carnitine and its congeners play a regulating role in the immune response. They found decreased serum levels of carnitine in most AIDS patients, with a small minority presenting with normal of even high carnitine levels. However, consistent in these groups were low intracellular (i.e. peripheral blood

mononuclear cells) carnitine levels. They consequently regarded serum carnitine measurements as a fallacious index of an individual's true carnitine status.

The second question, as to what could possibly have caused the obvious significant drop in plasma free and acetylcarnitine levels between the two trial periods, has already been discussed in section 2.1 (i.e. the effect of cold exposure during the winter had an overriding effect on carnitine supplementation).

3. Performance

During this study, progressive treadmill exercise tests during each trial period were performed at the start, and after six weeks of supplementation. During the treadmill tests, performance parameters were monitored and analysed (i.e. VO_2 max, peak treadmill running speed, respiratory exchange ratios, VCO_2, VO_2 and heart rate). In an attempt to validate the measured parameters, a field test (standard marathon) was included at the end of each supplementation period.

3.1 Marathon results

As mentioned, the marathon events were an attempt to validate performance results obtained during controlled laboratory tests. Under these field conditions, it could also be ascertained if the MCT supplements could be tolerated during a marathon event.

Concerning the marathon results, it could not be unequivocally stated that the athletes' performance improved on either the MCT + CHO, or the MCT + CHO + L-carnitine supplement. One must however consider various uncontrollable factors e.g. weather conditions, nutritional status of the athlete on the day, injuries etc. The marathons might therefore be described as "subjective" tests. Athletes reported on feeling stronger during the marathons each time after ingesting a sachet of either supplement.

What the marathon events did however serve to highlight, was that both supplements could easily be tolerated by the majority of athletes over a relatively short period of time. Athletes, on average, consumed 1125ml of supplement in a three to four hour period. This amounted to 4.5 x 20g dose of SI, and 4.5 x 22.5g dose of SII, equalling approximately 13.36g of MCT per marathon. The amount of 13.36g within three to four hours, falls well within the advocated maximum amount of ~30g MCT in three hours, as proposed in previous studies [12].

3.2 VO_2 max

Scientists in Germany and Sweden [83] have been determining the VO_2 max of athletes for more than 70 years. They use VO_2 max to predict the endurance capacity of athletes and their studies indicate that the highest VO_2 max values (expressed relative to body weight) are measured in the best endurance athletes. Modern studies did however show that VO_2 max per se, is neither an indisputable indicator of fitness, nor an effective yardstick to predict performance [69]. There is however an intricate connection between aerobic capacity, expressed as VO_2 max, and the ability of an individual to endure physical activity over an extended period of time (i.e. endurance exercise). VO_2 max does reflect the total capacity of skeletal muscle mitochondria, active during exercise, to utilize oxygen [69]. Considering the fact that the mitochondria are the target of carnitine activity, and the role carnitine plays in providing lipid substrates to the mitochondria, the individual's capacity to resynthesize adenosine 5'-triphosphate during mitochondrial oxidative phosphorilation, is reflected in VO_2 max. Optimal, or above normal carnitine levels, in conjunction with higher enzyme activity levels, should, theoretically, potentiate oxidative skeletal muscle mitochondrial oxidation [84].

The latter should therefore be reflected in higher VO_2 max values. Carnitine has indeed been reported to increase VO_2 max [79,62]. The opposite has however also been recorded [23]; despite an improvement in performance, VO_2 max values decreased. During the first trial period in this study (SI), a decrease in VO_2 max

ranging from 2.2-11.9% was recorded. During the second trial period (SII-containing L-carnitine), eight athletes again showed a decrease in VO_2 max, ranging from 4.3-8.5% (Chapter 3, section 3.2.2, Fig. 8b). The reason for this notion is still unclear. The previously mentioned issue, regarding the use of VO_2 max as performance predictor, is thus highlighted [69].

3.3 Peak treadmill running speed

Peak treadmill running speed, reached during the VO_2 max test, has been described as the most effective laboratory test to predict an athlete's performance [70,85]. In this study, four athletes showed an increase in peak treadmill running speed after SI supplementation, whereas five athletes performed better after SII supplementation (Chapter 3, section 3.2.2, Fig 9b); it would probably have been six, had athlete number 7 not been recovering from a bout of flu. After SI supplementation, two athletes performed consistently, while after SII supplementation, three athletes equaled their performance. It could therefore be stated that there was a trend towards improving performance after SII supplementation (containing L-carnitine). Carnitine supplementation has been reported to improve power output/workload during exercise [23]. Wyss *et al.* [20], however, reported no change in power output after carnitine supplementation. Their supplementation period only stretched over seven days prior to the commencing of exercise testing.

3.4 Respiratory exchange ratio (RER)

Carnitine purportedly enhances lipid metabolism [19,23] by means of supplying the mitochondria with oxidative substrate (i.e. long-chain fatty acids), in a more efficient manner. This phenomenon has been reflected, or verified, with decreased respiratory exchange ratios during exercise when supplementing carnitine [19,20,23,73]. According to Martinez and Haymes [72], changes in RER values during exercise, are indicative of a shift in the relative contribution of carbohydrate and fat to the total energy metabolism.

Results to the contrary have, however, also been reported. After administering carnitine intravenously at the start of a bicycle ergometer exercise test, no changes in RER were recorded by Brass *et al.* [44] during exercise. They concluded that carnitine supplementation had no effect on muscle carnitine metabolism. They proposed that the efficacy of carnitine supplementation in modifying exercise performance, must either be due to a nonmuscle site of action, or altered muscle carnitine content caused by long term carnitine administration. They also considered altered muscle carnitine handling in pathophysiologic states. Vukovich *et al.* [86] supported the above, after finding no change in RER during exercise after carnitine supplementation.

In this study, results of RER values during exercise were slightly confusing (Chapter 3, section 3.2.3, Fig. 11b). After SI supplementation, an overall increase occurred in mean RER values, being statistically significant at 14, 16, 17, 18, and 19km/h. The RER values after supplementation were consistently >1.00, indicating predominantly carbohydrate oxidation during exercise. It has already been explained (section 2.2) that according to NEFA profiles, athletes in group one and two, were able to utilize fatty acids during exercise after SI supplementation. However, only two athletes were considered as predominantly "fat burners", while the rest of the athletes were considered a "grey group". Results of RER supports this categorization; although group two's athletes were able to utilize some fatty acids, RER values seemed to indicate that they benefited more from the carbohydrate included in the supplement, than from the MCTs.

After SII supplementation, a significant increase occurred in mean RER values only at 14km/h (Chapter 3, section 3.2.3, Fig. 11b). The rest of the results indicated a relative consistency in RER before (B2) and after (6W2) carnitine supplementation. RER values after SII supplementation, were also much closer to one, thereby indicating a tendency towards lipid metabolism, and supporting the notion of carnitine's enhancing effect on lipid metabolism.

NEFA profiles (Chapter 3, section 2.2) have indicated that group two's athletes did not benefit from the carnitine supplementation as much as the "fat burners" in group one. This was ascribed to the endogenous carnitine deficiency, as a result of cold exposure. RER results however, seem to indicate that carnitine supplementation did have an effect, however slight, on lipid metabolism.

3.5 VCO_2

In this study, results on VCO_2 (chapter 3, section 3.2.4, Fig. 11b) supported the deductions made from RER results. The respiratory exchange ratio reflects the relative amount of carbohydrate and lipid being oxidized, as well as carbon dioxide produced to buffer lactic acid production. Martin *et al.* [87] declared that endurance trained athletes generated nearly 50% more energy from fat oxidation during submaximal exercise, when compared to untrained athletes. In these trained athletes, they observed lower RER as well as carbon dioxide levels during exercise. Therefore, higher rates of fat oxidation, coincides with lower levels of carbon dioxide production.

After SI supplementation, VCO_2 levels slightly increased at 14 and 16km/h, with a more noticeable increase at 20km/h, remained constant at 17 and 19km/h, and slightly decreased at 18km/h. The remark made in section 3.4, that most of the athletes still relied predominantly on carbohydrate metabolism after SI supplementation, is thus supported.

Carnitine's purported role in lipid metabolism is verified, in that VCO_2 levels showed an overall decrease after SII supplementation, being statistically significant at 17km/h ($p<0.01$). The decrease was small, but again fits in with the noted tendency towards lower RER values, as well as RER values being closer to one after carnitine supplementation. It is important to again point out that NEFA profiles, as well as plasma carnitine profiles, indicated an endogenous carnitine deficiency in the majority of athletes. This probably explains the minor changes

observed in RER and VCO_2 values. The tendency observed should however, not be disregarded.

3.6 VO_2 and heart rate

In a previous study [23], L-carnitine supplementation (2g/day for six weeks) caused a significant decrease in VO_2 values during exercise, concurrent with lower heart rates. Athletes did however perform better after carnitine supplementation. It was deduced that after L-carnitine supplementation, athletes were able to exercise more economically; i.e. exercise at the same intensity as prior to carnitine supplementation, requiring less oxygen, and inducing less cardiovascular stress.

In this study, results on VO_2 seem to mirror previous findings (Chapter 3, section 3.2.5, Fig. 12b). After L-carnitine supplementation (SII), there was a decrease in VO_2 (except at 18km/h), being statistically significant at 14, 16, and 17km/h. These results support lower VO_2 max values. However, the same occurred after SI supplementation; an overall decrease in VO_2, being significant at 17km/h.

Results of heart rate (Chapter3, section 3.2.6, Fig. 13b) did however not mirror results of VO_2. After SII supplementation, heart rates remained relatively constant throughout. The same occurred after SI supplementation, with noticeable increases at 18, 19 and 20km/h (though not significant).

Muoio *et al.* [26] found an increase in VO_2 during exercise in athletes consuming a high fat diet (38% energy from fat), and ascribed the increase in oxygen consumption as being due to the higher oxygen cost of producing ATP from fat. Consider that six molecules of oxygen are required to completely oxidize one molecule of glucose, while 26 molecules of oxygen are required to completely oxidize stearic acid [73]. Considering the above, it was confusing to note that VO_2

values were lower after SII supplementation (containing carnitine), and VCO_2 and RER data indicated that there was a shift towards lipid metabolism.

A possible mechanism for the increase in oxygen consumption observed by Muoio *et al* . [26], has been proposed to be an enhancement of ß-oxidation capacity, as a consequence of enzymatic adaptations necessitated by the high fat diet. The reasons for the disparities observed during this study, remain unclear.

4. Concluding Remarks

The effects two different MCT supplements, SI containing MCT+CHO and SII containing MCT+CHO+L-carnitine, had on the performance and metabolic parameters of male marathon athletes, were investigated in this study.

When taking into account the variability associated with exercise performance in humans, it is extremely difficult to produce conclusive results regarding performance, as it clearly came to the fore during this study. Consider the adverse weather conditions during the second marathon, athletes periodically suffering from niggling injuries throughout the experimental period, and the effect winter colds and flu had on training and performance.

In terms of peak treadmill running speed and VO_2 max, it could not be stated unequivocally that athletes' performances improved after SI supplementation (MCT+CHO). Some athletes were able to improve their peak treadmill running speed, and all the athletes showed a decrease in VO_2 max; the latter could not be regarded as a negative indicator, considering that improved performance, not withstanding a decrease in VO_2 max, had previously been reported [23].

During the second trial period (SII supplementation), a slight improvement in performance was evident. Only one athlete showed a decline in peak treadmill running speed. An overall decrease in VO_2 max was again evident. Previous studies, investigating the effect of L-carnitine supplementation on endurance performance, were discussed in Chapter 1, section 3.7. Carnitine supplementation (2g/day for four weeks) has also been proven to increase respiratory chain enzymes in endurance runners. Furthermore, carnitine supplementation has been shown to improve exercise tolerance in patients with impaired exercise tolerance, e.g. patients with cardiac disease [93]. In contrast, 500mg of L-carnitine per day for four weeks in competitive male runners, did not seem to increase maximal work output during a 69 minute endurance cycle event [88], neither did carnitine

supplementation (4g/day for two weeks) have any significant effect on the time required to complete a simulated 5km run on a treadmill [79].

The slight observed improvement in performance observed during this study after combined MCT+CHO+L-carnitine supplementation (SII), could be ascribed to the effect of L-carnitine supplementation. It should be noted, that the amount of carnitine supplemented was only 400mg/day. This small dose might have been responsible for the slight variation in results. In studies producing more pronounced results, a dose of 2-4g/day was most often used. However, bear in mind that in this study, carnitine was incorporated in a palatable, liquid, MCT and CHO supplement. Thus, the supplement is "usable" to the average man in the street. The question arises, as to whether a dose of 2-4g carnitine/day could be incorporated into a MCT+CHO supplement in the same successful manner? That seems an important, and as yet unanswered question.

A major unforeseen, and definitely unexpected factor emerging during this study, was the severe effect the winter and continuous cold exposure, had on plasma carnitine levels. The latter had an overriding effect on the supplementation. It could be speculated that more conclusive results would have been obtained had this factor not come into play; especially considering the fact that there was definitely a move towards improved performance during the second trial period.

Another issue that must be addressed, is if either/or MCT+CHO, as well as MCT+CHO+L-carnitine supplementation had an altering effect on the body's metabolism, enabling it to predominantly utilize fat as an energy source during exercise; thus, the question of fat adaptation. Studies supporting the notion of fat adaptation have been reported; the combination of fat feeding and training, was found to improve aerobic performance in dogs and horses [90]. This strategy also served to spare glycogen utilization, and reduced lactate accumulation. The fat adaptation in horses appeared to facilitate metabolic regulation in order to achieve power needs; glycolysis decreased during aerobic work, but increased during anaerobic work, with blood lactate changes following accordingly. The

latter verified the results obtained from NEFA and lactate profiles in this study, with low lactate concentration equaling high NEFA, and high lactate concentrations equaling low NEFA.

A seven day high fat (compared to a normal and high carbohydrate) diet, have also been reported to improve the endurance performance in trained male runners. Muoio *et al.* [26] offered evidence that humans and animals adapt to a high fat diet in a similar manner as to the response observed to endurance training, i.e. by increasing skeletal muscle oxidative capacity, therefore, dietary manipulation which facilitates lipid utilization, may lead to an increased power output from fat oxidation.

In this study, NEFA profiles indicated that the majority of athletes were able to mobilize and utilize fatty acids during exercise after SI supplementation (MCT+CHO). However, RER and VCO_2 data suggested that the majority of athletes still relied predominantly on carbohydrate metabolism during exercise. Therefore, no clear answer could be given as to whether fat adaptation did indeed occur during the first trial period. What was however obvious, was that the MCT+CHO supplement had no detrimental effect on any of the athletes.

After adding L-carnitine to the MCT+CHO supplement (SII) during the second trial period, a shift towards lipid metabolism was definitely observed. Consistent with the above, were lower VCO_2 and RER values. NEFA profiles served to identify two "fat burners", relying predominantly on fat metabolism to produce energy during exercise. These two athletes consistently performed the best in the group, and the latter was ascribed to their "fat burning" capacity. It was clear from NEFA profiles that they benefited most from both supplements. It could therefore speculatively be stated, that both the MCT supplements orchestrated adaptive changes in their metabolism, enabling them to utilize fat effectively, thereby improving their performance. Despite an endogenous carnitine deficiency during the second trial period, the carnitine supplementation enabled them to still utilize fat as substrate.

The shift towards lipid metabolism during the second trial period, would most probably have been more pronounced, had it not been for the unexpected effect of the winter on plasma carnitine levels. The majority of athletes did however perform better. Despite NEFA profiles indicating that the majority of athletes did not utilize fatty acids as effectively during the second trial period, VCO_2 and RER results verified a slight shift towards fat metabolism. Because of the relative carnitine deficient state of athletes during the second trial period, either fat adaptation, or carnitine supplementation could probably be held responsible for the observed shift toward lipid metabolism. However, if fat adaptation was responsible, the same observed changes in VCO_2 and RER should have taken place during the first trial period. Therefore, the carnitine supplementation must have been responsible for the shift. But, because of the endogenous carnitine deficiency, it was the identified "fat burners" that benefited most from the carnitine supplementation.

To summarize:

- Two "fat burners" were identified. The MCT supplementation induced adaptive changes in their metabolism, enabling them to utilize fat efficiently, thereby improving their performance. Despite an endogenous carnitine deficiency, they were still able to utilize fat; ascribed to the fat adaptive changes.
- The majority of athletes still relied predominantly on carbohydrate metabolism during exercise, despite the MCT supplementation. However, carnitine supplementation did induce a slight shift towards lipid metabolism. The endogenous carnitine deficiency in athletes did have an effect on their ability to utilize fatty acids (evident from NEFA profiles), despite the carnitine supplementation.

It therefore seemed that fat adapted athletes could utilize fatty acids in a relative carnitine deficient state, when being supplemented with carnitine. Athletes relying predominantly on carbohydrate metabolism, could not utilize fatty acids as efficiently as the "fat burners", in a carnitine deficient state, despite carnitine

supplementation. Carnitine supplementation did however still induce a shift towards lipid metabolism.

It is clear from the above discussion that carnitine induced a slight shift towards lipid metabolism. It could not however be assumed that carnitine was directly involved in the transport of MCFAs. Carnitine may well be involved in improving mitochondrial function, as has been reported in patients with chronic fatigue syndrome [91]. *In vitro* tests have shown depressed mitochondrial respiration with reduced aerobic work capacity, as well as reduced intracellular concentrations of adenosine triphosphate at peak exercise intensities, thereby suggesting mitochondrial abnormalities in patients with chronic fatigue syndrome. Higher carnitine levels in these patients, following carnitine supplementation produced significant correlations with the severity of clinical symptoms (higher carnitine levels = less severe symptoms and improved physical ability). Carnitine therefore seems to improve mitochondrial function, thereby increasing the efficacy of lipid oxidation.

6. Final conclusion and recommendations

A supplement designed to contain medium-chain triglycerides, carbohydrate and L-carnitine, seems to have promising effects on endurance performance. The latter presumably due to the glycogen sparing effect of fat utilization during exercise, as well as the enhancing effect carnitine has on lipid metabolism. Such a supplement could comfortably be tolerated when supplied to athletes during a marathon event.

More pronounced effects might be obtained if the carnitine dose was to be increased, in order to override any existing endogenous carnitine deficiency. Consideration should in future be given to how much of an administered dose of carnitine is actually absorbed, and reaches its target, i.e. the mitochondria. This issue has already been addressed in studies on the effect of oral L-carnitine supplementation on muscle and plasma carnitine concentrations in thoroughbred horses [92]; urinary excretion of carnitine increased after suppplementation, and free carnitine accounted for 60-75% of the total carnitine measured in urine. Attention should therefore also be directed towards investigating urinary excretion of carnitine during supplementation in humans.

In order to get more conclusive results regarding fat adaptation and MCT suppementation, a longer supplementation period is proposed. Carnitine should definitely be included in the supplement; a higher dose should be considered if practically possible. Intermittent tests on serum cholesterol levels should be performed during the extended supplementation period, especially in a population with a genetic tendency towards higher cholesterol levels.