

Supplementary Information:

A LABORATORY APPROACH FOR CHARACTERIZING CHRONIC FATIGUE: WHAT DOES METABOLOMICS TELL US?

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Concise and informative title: **Metabolomics and chronic fatigue**

Content:

1) Patient procedures and selection:

Female patients who were complaining about fatigue were selected during visits to clinicians. Selection was based mainly on fatigue assessment by the clinicians and the completion of a demographic profile, the revised version of the Piper Fatigue Scale and the Medical Symptoms questionnaire. The questionnaires were part of an information guide about the challenge tests.

2) Informed consent:

Potential participating patients were also required to complete a section on informed consent which in essence contains information about the background and aim of the detoxification and oxidative stress evaluation, risks and possible discomforts, confidentiality, withdrawal, acknowledgement of explanation of the test procedure and finally the declaration of consent.

3) PLS-DA of eight substances selected

The PLS-DA for the low and high fatigue groups when comparing substances before and after the challenge tests using the eight substances mentioned above, show complete separation, as expected

4) Summary table of the clinical diagnosis

Summary table of the clinical diagnosis information of 39 patients in low fatigue group and 31 patients in high fatigue group.

5) Table of a Pearson and a Spearman's correlation test

Table of a Pearson and a Spearman's correlation test between the energy and mental fatigue scores for four of the detoxification metabolites (acetaminophen glucuronide; acetaminophen sulfate; acetaminophen mercapturate and salicyluric acid).

1) Patient procedures and selection:

A. BIOTRANSFORMATION AND OXIDATIVE STRESS STATUS PROFILE

Background information

In today's world of processed food and pollution, toxic substances exist almost everywhere. They are in the food we eat, the water we drink, and the air we breathe. It is estimated that 60 to 80 percent of all cancers are a direct result of these environmental toxins. Environmental toxins may also play a role in the development of neurological disorders such as Alzheimer's and Parkinson's disease, and in mental or behavioural disorders such as depression, anxiety, schizophrenia and attention deficit disorder. Since the nervous system is intricately connected to the immune system, toxins also affect our immune function. Thus, our very ability to think and feel normal can be drastically affected by exposure to toxins.

The liver is a key organ in your body's self-defence system. Inside the liver cells there are sophisticated mechanisms that have evolved over millions of years to break down toxic substances into forms which your body can safely eliminate. The liver uses two mechanisms called Phase I and Phase II biotransformation, designed to convert fat soluble toxins into water soluble substances so that your body can easily excrete these substances via watery fluids such as bile and urine.

In Phase I, your body's enzymes convert a toxin into a substance which can be further metabolised in Phase II. This is achieved by various chemical reactions and during this process, free radicals are produced which can damage the liver cells. Antioxidants (such as vitamin C and E) can reduce the damage caused by free radicals, but if antioxidants are low and toxin exposure high, these toxins become more dangerous and cannot be eliminated as rapidly or as completely. Slower biotransformation results in more toxic substances circulating in the body which are eventually stored for years in fatty body tissue, being released during times of exercise, stress or fasting. During the release of these toxins, symptoms such as head-

aches, poor memory, stomach pain, nausea, fatigue, dizziness and palpitations may occur.

Phase II is also called the conjugation pathway whereby other enzymes in liver cells add another substance to metabolites from Phase I.

This makes the toxin water soluble and less harmful, so it can then be excreted from the body via watery fluids such as bile or urine. For efficient Phase II biotransformation, the liver cells require sulphur-containing amino acids such as taurine and cysteine. The nutrients glycine, glutamine, choline and inositol are also required for efficient Phase II biotransformation. Major Phase II pathways include glutathione conjugation, sulphate conjugation, glycine conjugation and glucuronide conjugation.

A number of conditions affect how well the liver performs its detoxifying duties. Repeated exposure to toxins in food, water and the environment increases the biotransformation burden and if the Phase I and II biotransformation pathways become overloaded, there will be a build-up of toxins in the body.

Biotransformation and oxidative stress status test

One method to assess the liver's detoxifying ability is to examine the overall state of your health. Toxic substances are contributing factors in a wide range of health problems.

There are also several different liver tests (such as liver enzyme analysis) which look for clinical evidence of existing liver damage. Unfortunately, by the time these tests register abnormal, liver damage is already present.

A test to assess the liver's biotransformation function can be done. During this test the liver is challenged by common substances such as caffeine,

aspirin and paracetamol in safe doses and samples of blood, urine and saliva are collected. This test is unique because Phase I and II biotransformation pathways as well as the oxidative stress status and antioxidant capacity are assessed. Phase I activity is tested by means of the caffeine clearance test, and in Phase II the production of four conjugates are

determined. The ratio between the activities of the two phases is also calculated to detect imbalances. Blood samples are used to determine the oxidative stress status and antioxidant capacity. This data can then be correlated with the results of the biotransformation capacity test.



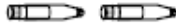




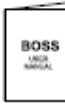

B. TEST PROCEDURE

It is very important that the test procedures are strictly followed. The documentation and all the samples (blood, saliva and urine) should be sent together, before the laboratory can analyse any samples.

About the test kit and procedure

Three compounds, caffeine, paracetamol and aspirin, are taken orally to challenge the liver's Phase I and Phase II biotransformation capacity. Saliva and urine are analysed to determine how well the liver can convert and clear toxins from the body.

The provided kit consists of the following:

	Sample	Amount	Description
A		1	One 1 L urine container
B		1	One small urine container
C + D		2	Two saliva collection tubes (salivettes)
E		1	One 150 mg caffeine tablet
F		2	Two 300 mg aspirin tablets
		2	Two 500 mg paracetamol tablets
G		1	Labels for all containers are supplied and should be completed with the collection time, name and date and applied to the correct containers.
H		1	User manual containing important forms that must be completed and send back.
I		1	Extra plastic bag with document pouch attached to the outside







Pre-test instructions

During this test a normal diet can be assumed except for the following dietary restrictions.

DO NOT consume the following during the test:

- Alcohol, coffee, tea, cocoa, chocolate, cola soft drinks, rooibos tea, health tea;
- Medication containing caffeine, salicylates (aspirin) or paracetamol;
- Stone fruits (eg. sultanas, currants, raisins etc);
- Nuts and seeds, peppermint or liquorice (eg. sweets, lollies, gums, plants/tea);
- Spices (eg. curry, dill, oregano, paprika etc);
- Brassica vegetables like broccoli, cabbage, brussels sprouts, cauliflower and tomato.

INSTRUCTIONS

	Time	Use	Steps to be taken
1	Early morning		Collect an early morning baseline urine sample in the small urine sample container. All urine samples must be frozen as soon as possible.
2	08:00		Take the caffeine tablet before breakfast.
3	10:00		Collect the 1st saliva sample. [Please read detailed instructions on next page]
PLEASE SEE DIRECTIONS ON NEXT PAGE			
4	16:00		Collect the 2nd saliva sample (see above).
5	21:00		Cease eating and drinking (except water). Empty bladder. Take the two aspirin and two paracetamol tablets.
6	Overnight 21:00 - 07:00		Collect all overnight urine until 07:00 the next morning in the 1 L container. All urine samples must be frozen as soon as possible. Please note: bottle has a cap and a seal.
7		Must be done by Pathologists	Collect blood sample in three tubes: two containing EDTA (purple top) and one clotted tube (yellow top). This will be done at the pathology branch when the frozen saliva and urine samples are brought back. Not necessary to fast.
8	Return all samples in the extra plastic bag. Place the blue booklet in the document pouch attached to the plastic bag. Remove strip to seal the document pouch.		

Before saliva collection make sure of the following directions:





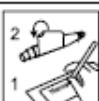

1. Collect saliva at the specific time(s) listed by your physician.
2. Do not eat or drink anything for at least 30 minutes before collecting saliva.
3. Avoid activities that may cause your gums to bleed – do not brush or floss.
4. Remove lipstick and lip balm before collection.

How to collect saliva using the salivettes

Saliva should be collected at the precise time(s) specified by your practitioner. The provided Salivette, is a device made specifically for the purpose of collecting saliva. Please read the following directions carefully before starting your collection.

PLEASE NOTE – SALIVETTES ARE NOT INTENDED FOR USE WITH CHILDREN UNDER THE AGE OF THREE. CHILDREN UNDER THE AGE OF TEN SHOULD HAVE ADULT SUPERVISION.

INSTRUCTIONS - Follow these steps to collect your saliva:

	STEP 1: Remove only the top cap of the tube to expose the round swab. Do not remove the insert (smaller internal container) housing the cotton swab.
	STEP 2: Hold the tube to your lips, and tip the tube so that the swab slides into your mouth. Do not touch the cotton swab with your fingers
	STEP 3: Gently roll the swab around in your mouth for 5 minutes or until you can no longer prevent swallowing excess saliva (the cotton swab should be saturated).
	STEP 4: Place the tube to your lips and allow the swab to slide back into the insert (small internal vessel) and firmly close with the top cap/stopper. Do not touch the swab with your fingers. The salivette should now look exactly as it did when you started, with the cap, insert, cotton swab and centrifuge tube.
	STEP 5: On the supplied labels, write your full name, date of birth, date of collection and collection time and attach it to the correct containers.
	STEP 6: Freeze the salivette tubes and take it to the practitioner or laboratory within 3 days.

C. IMPORTANT PAPERWORK TO BE COMPLETED

PATIENT INFORMATION			
All information from p.4 - 9 must be completed including weight, height and referring doctor.			
Tests requested	Detoxification profile:		
Monster info	Blood:	Saliva:	Urine:
Name:			
Surname:			
Date of birth:			
ID:			Sex:
Cell:			Weight:
Tel:			Height:
Postal address:			
Has anyone in the family been tested? If so, provide us with full names and family connection:			
Referring doctor:			
Medical aid information	Name of medical aid:		
	Guarantor I.D. no		
	Medical aid number:		
	Name of main member:		
ENQUIRIES			
Human Metabolomics, North-West University, Potchefstroom Campus. Enquiries: Cecile Cooke , tel 018 299 2042, fax: 087 231 5527, email: cecile.cooke@nwu.ac.za Postal address: Biochemistry, Private bag X6001, Potchefstroom, 2520			

LIFESTYLE QUESTIONNAIRE			
1	Do you use tobacco products?	No	Yes
2	Do you live or work with someone who smokes in your presence?	No	Yes
3	How often do you eat fast foods	Not often	Weekly Daily
4	How many alcoholic drinks do you have per week	< 1	1 – 5 > 5
5	Do you ever use toxic chemicals such as insect sprays or herbicides?	No	Yes
6	Have you taken any prescription medication two or more times in the past year?	No	Yes
7	Do you take any chronic medication? Please specify if yes.	No	Yes
	Please specify:		
8	Do you take birth-control or hormone replacement medication? Please specify if yes.	No	Yes
	Please specify:		
9	Do you suffer from gastrointestinal problems?	No	Yes
10	Are you often tired for no reason? If yes, please complete Piper Fatigue form p. 7.	No	Yes
11	Diagnosed with medical condition? Please specify if yes.	No	Yes
	Please specify:		

MEDICAL SYMPTOMS QUESTIONNAIRE															
			NB!: POINT SCALE: HOW OFTEN AND SEVERELY DO YOU EXPERIENCE THE FOLLOWING SYMPTOMS - IN THE LAST 30 DAYS:								NB!: POINT SCALE: HOW OFTEN AND SEVERELY DO YOU EXPERIENCE THE FOLLOWING SYMPTOMS - IN THE LAST 30 DAYS:				
			Never / Almost never	Occasionally, effect is not severe	Occasionally, effect is severe	Frequently, effect not severe	Frequently, effect is severe				Never / Almost never	Occasionally, effect is not severe	Occasionally, effect is severe	Frequently, effect not severe	Frequently, effect is severe
			0	1	2	3	4				0	1	2	3	4
HEAD	Headaches		0	1	2	3	4	EYES	Watery or itchy eyes		0	1	2	3	4
	Faintness		0	1	2	3	4		Swollen, reddened or sticky eyelids		0	1	2	3	4
	Dizziness		0	1	2	3	4		Bags or dark circles under eyes		0	1	2	3	4
	Insomnia		0	1	2	3	4		Blurred or tunnel vision (not including near- or far-sightedness)		0	1	2	3	4
EARS	Itchy ears		0	1	2	3	4	SKIN	Acne		0	1	2	3	4
	Ear aches, ear infections		0	1	2	3	4		Hives, rashes, dry skin		0	1	2	3	4
	Drainage from ear		0	1	2	3	4		Hair loss		0	1	2	3	4
	Ringling in ears, hearing loss		0	1	2	3	4		Flushing, hot flashes		0	1	2	3	4
									Excessive sweating		0	1	2	3	4

		0	1	2	3	4			0	1	2	3	4
NOSE	Stuffy nose	0	1	2	3	4	MOUTH/ THROAT	Chronic coughing	0	1	2	3	4
	Sinus problems	0	1	2	3	4		Gagging, frequent need to clear throat	0	1	2	3	4
	Hay fever	0	1	2	3	4		Sore throat, hoarseness, loss of voice	0	1	2	3	4
	Sneezing attacks	0	1	2	3	4		Swollen or discolored tongue, gums, lips	0	1	2	3	4
	Excessive mucus formation	0	1	2	3	4		Cancer sores	0	1	2	3	4
HEART	Irregular or skipped heartbeat	0	1	2	3	4	LUNGS	Chest congestion	0	1	2	3	4
	Rapid or pounding heartbeat	0	1	2	3	4		Asthma, bronchitis	0	1	2	3	4
	Chest pain	0	1	2	3	4		Shortness of breath	0	1	2	3	4
	Excessive sweating	0	1	2	3	4		Difficulty breathing	0	1	2	3	4
EMO-TIONS	Mood swings	0	1	2	3	4	ENERGY/ ACTI- VITY	Fatigue, sluggishness	0	1	2	3	4
	Depression	0	1	2	3	4		Apathy, lethargy	0	1	2	3	4
	Anger, irritability, aggressiveness	0	1	2	3	4		Hyperactivity	0	1	2	3	4
	Anxiety, fear, nervousness	0	1	2	3	4		Restlessness	0	1	2	3	4
MIND	Difficulty in making decisions	0	1	2	3	4	WEIGHT	Fatigue worsens with exertion (vigorous action or effort), plus post-exertional malaise (feeling ill after exertion)	0	1	2	3	4
	Stuttering or stammering	0	1	2	3	4		Binge eating / drinking	0	1	2	3	4
	Slurred speech	0	1	2	3	4		Craving certain foods	0	1	2	3	4
	Learning disabilities	0	1	2	3	4		Excessive weight	0	1	2	3	4
	Poor memory	0	1	2	3	4	Compulsive eating	0	1	2	3	4	
	Confusion, poor comprehension	0	1	2	3	4	Water retention	0	1	2	3	4	
	Poor concentration	0	1	2	3	4	Underweight	0	1	2	3	4	
Poor physical coordination	0	1	2	3	4	JOINT/ MUSCLE	Pain or aches in joints	0	1	2	3	4	
DIGES-TIVE TRACT	Nausea, vomiting	0	1	2	3		4	Arthritis	0	1	2	3	4
	Diarrhea	0	1	2	3		4	Stiffness or limitation of movement	0	1	2	3	4
	Constipation	0	1	2	3		4	Pain or aches in muscles	0	1	2	3	4
	Bloated feeling	0	1	2	3		4	Feeling of weakness or tiredness	0	1	2	3	4
	Belching, passing excessive gas	0	1	2	3		4	Migratory arthralgia - Pain in different joints at different times as if pain is travelling	0	1	2	3	4
	Heartburn	0	1	2	3		4	OTHER	Frequent illness	0	1	2	3
	Intestinal / stomach pain	0	1	2	3	4	Frequent or urgent urination		0	1	2	3	4
OTHER	Genital itch or discharge	0	1	2	3	4	Genital itch or discharge		0	1	2	3	4
	Painfull lymph nodes (cervical, axillary, inguinal, or supraclavicular)	0	1	2	3	4	Painfull lymph nodes (cervical, axillary, inguinal, or supraclavicular)		0	1	2	3	4

PIPER FATIGUE SCALE												
Please answer the questions below using the scale measurement in the next column		Scale measurement	Please mark appropriate scale									
			1	2	3	4	5	6	7	8	9	10
1	How long have you been feeling fatigue? (Check one response only): 1 - not feeling fatigue, 2 - minutes, 3 - hours, 4- days, 5 - weeks, 6 months, 7 - other (please describe.....)		1	2	3	4	5	6	7	8	9	10
2	To what degree is the fatigue you are feeling now causing you distress?	1 No distress 10 A great deal	1	2	3	4	5	6	7	8	9	10
3	To what degree is the fatigue you are feeling now interfering with your ability to complete your work or school activities?	1 Non 10 A great deal	1	2	3	4	5	6	7	8	9	10
4	To what degree is the fatigue you are feeling now interfering with your ability to socialise with your friends?	1 Non 10 A great deal	1	2	3	4	5	6	7	8	9	10
5	To what degree is the fatigue you are feeling now interfering with your ability to engage in sexual activity?	1 - Non 10 A great deal	1	2	3	4	5	6	7	8	9	10
6	Overall, how much is the fatigue which you are now experiencing interfering with your ability to engage in the kind of activities you enjoy doing?	1 Non 10 A great deal	1	2	3	4	5	6	7	8	9	10
7	How would you describe the degree of intensity or severity of the fatigue which you are experiencing now?	1 Mild 10 Severe	1	2	3	4	5	6	7	8	9	10
8	To what degree would you describe the fatigue you are experiencing now as being	1 Pleasant 10 Unpleasant	1	2	3	4	5	6	7	8	9	10
9	To what degree would you describe the fatigue you are experiencing now as being	1 Agreeable 10 Disagreeable	1	2	3	4	5	6	7	8	9	10
10	To what degree would you describe the fatigue you are experiencing now as being	1 Protective 10 Destructive	1	2	3	4	5	6	7	8	9	10
11	To what degree would you describe the fatigue you are experiencing now as being	1 Positive 10 Negative	1	2	3	4	5	6	7	8	9	10
12	To what degree would you describe the fatigue you are experiencing now as being	1 Normal 10 Abnormal	1	2	3	4	5	6	7	8	9	10
13	To what degree are you now feeling	1 Strong 10 Weak	1	2	3	4	5	6	7	8	9	10
14	To what degree are you now feeling	1 Awake 10 Sleepy	1	2	3	4	5	6	7	8	9	10
15	To what degree are you now feeling	1 Lively 10 Listless	1	2	3	4	5	6	7	8	9	10
16	To what degree are you now feeling	1 Refreshed 10 Tired	1	2	3	4	5	6	7	8	9	10
17	To what degree are you now feeling	1 Energetic 10 Unenergetic	1	2	3	4	5	6	7	8	9	10
18	To what degree are you now feeling	1 Patient 10 Impatient	1	2	3	4	5	6	7	8	9	10

PIPER FATIGUE SCALE												
Please answer the questions below using the scale measurement in the next colom		Scale measurement	Please mark appropriate scale									
			1	2	3	4	5	6	7	8	9	10
19	To what degree are you now feeling	1 Relaxed 10 Stressed	1	2	3	4	5	6	7	8	9	10
20	To what degree are you now feeling	1 Exhilarated 10 Depressed	1	2	3	4	5	6	7	8	9	10
21	To what degree are you now feeling	1 Able to concentrate 10 Unable to concentrate	1	2	3	4	5	6	7	8	9	10
22	To what degree are you now feeling	1 Able to remember 10 Unable to remember	1	2	3	4	5	6	7	8	9	10
23	To what degree are you now feeling	1 Able to think clearly 10 Unable to think clearly	1	2	3	4	5	6	7	8	9	10
24	Overall, what do you believe is most directly contributing to or causing your fatigue?											
25	Overall, the best thing you have found to relieve your fatigue is:											
26	Is there anything else you would like to add that would describe your fatigue better to us?											
27	Are you experiencing any other symptoms right now?											

2) Informed consent

INFORMED CONSENT	
Biotransformation and oxidative stress status evaluation	
1	Background and aim of the detoxification and oxidative stress evaluation
<p>Detoxification of exogenous and endogenous toxins is a natural and critical biological process consisting of several biochemical pathways. The effectiveness of these pathways varies between individuals and can significantly affect the health of an individual. Similarly, the steady state between the formation of free radicals and the antioxidant defence system of the human body to cope with these free radicals, which may elevate under certain conditions mostly as a result of endogenous factors, also varies between individuals and affects health and ageing. When the antioxidant defence cannot keep the free radicals at a steady state, oxidative stress increases and incurs damage to the body. These two processes, detoxification and oxidative stress, can be measured in biological material using several tests and are useful to estimate the effectiveness of these health-related processes. It may also help decide if lifestyle habits, such as nutrition, of an individual should be adapted to support these processes. The combination of tests that is done on the material provided aims to evaluate these two processes.</p>	
2	Procedures
2.1 Evaluation of detoxification	
2.1.1	Two saliva samples will be taken two and eight hours after caffeine loading respectively. These samples will be used to assess Phase I of the detoxification profile.
2.1.2	One 10 hour urine sample will be taken after the aspirin and paracetamol loading. The urine sample will be used to assess Phase II of the detoxification profile.

Continue on next page ...

<p>2.2 Oxidative stress evaluation 2.2.1 Two blood samples will be taken simultaneously to a total of 15 ml blood. 2.2.2 The blood samples will be processed for an array of tests that measures markers of free radical damage and antioxidant status.</p> <p>2.3 Molecular genetic testing 2.3.1 DNA may be isolated from blood samples for research purposes only. This will help identify genetic and epigenetic variations that play a role in biotransformation, oxidative stress and energy metabolism deficiencies.</p>	
3	Risks and possible discomfort for the patient / individual
The usual risk and possible discomfort experienced when a blood sample is obtained.	
4	Declaration of confidentiality
Information provided will be treated as highly confidential. Only individuals of the research group and the referring practitioner will have access to information. Data published in a scientific journal will include no information that could identify a patient or his/her family.	
5	Withdrawal clause
I understand that I may request withdrawal of the evaluation at any time. My participation or that of my child is therefore on a voluntary basis until I request otherwise.	
6	The above-mentioned project was thoroughly explained and the following additional information pointed out to me
<p>6.1 That the blood, saliva and urine samples may be used for all relevant tests to evaluate detoxification function and oxidative stress status and that no compensation for this material will be provided.</p> <p>6.2 That the biological material provided will reside only at the Centre for Human Metabonomics, North-West University, Potchefstroom, until I request (in writing) the destruction of this material at any time.</p> <p>6.3 That the withdrawal clause was explained to me and that I understand its implications.</p> <p>6.4 If you have any further enquiries please refer to p.4 for our contact details.</p>	
7	Declaration of consent
<p>I, _____ (print full name and surname) hereby consent to:</p> <p>7.1 participation in the above-mentioned evaluation of detoxification and oxidative stress. I also consent that the following samples may be used for these purposes and to storage thereof until such time as it is needed for further relevant tests: (a) 15 ml venous blood sample which was taken from me as specified, (b) the urine sample which was taken as specified, and (c) the saliva samples which were taken as specified OR</p> <p>7.2 participation of my child, _____ (print full name and surname) in the above-mentioned evaluation of detoxification and oxidative stress. I also consent that the following samples may be used for these purposes and to storage thereof until such time as it is needed for further relevant tests: (a) 15 ml venous blood sample which was taken from the child named above as specified, (b) the urine sample which was taken as specified, and (c) the saliva samples which were taken as specified</p> <p>I understand that I or my child are participating in the evaluation. I hereby acknowledge that I understand and are fully aware of the content of the forms and by signing this form I give the necessary permission.</p> <p>Signed at _____ (place) on _____ (date).</p> <p>Signature _____ (A parent/guardian must give consent in the case of minors.)</p> <p>Signature of responsible individual: _____ Individual who explained the project and informed consent form to the participant)</p> <p>Witness 1: _____ Witness 2: _____</p>	

3) PLS-DA of eight substances selected

The PLS-DA for the low and high fatigue groups when comparing substances before and after the challenge tests using the eight substances mentioned above, show complete separation, as expected (Fig. S1).

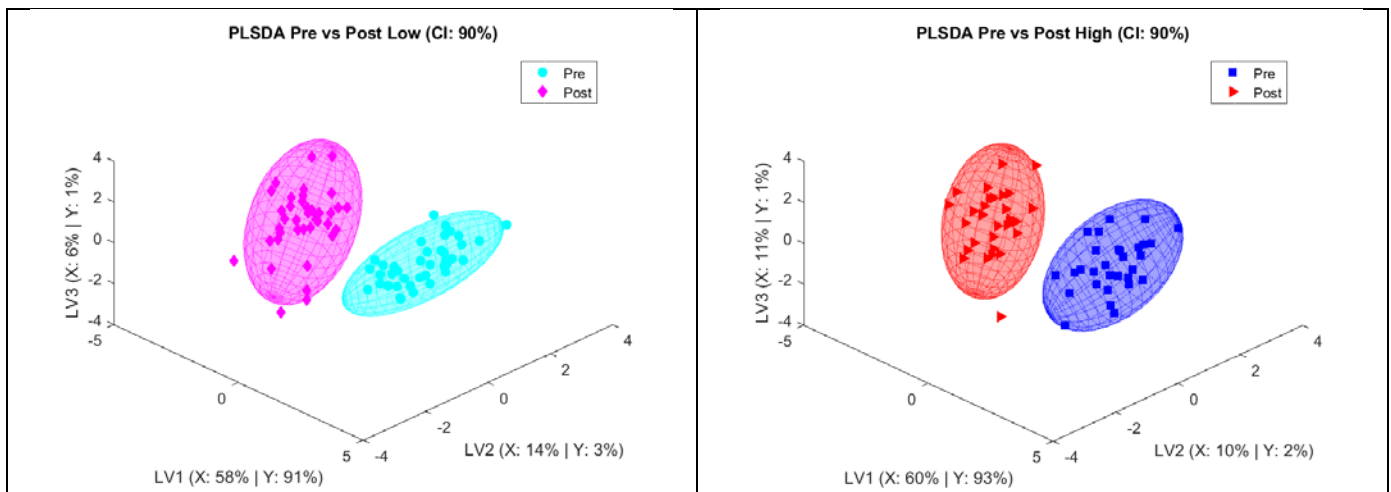


Figure S1 - Group separation between experimental groups through multivariate analysis based on eight substances, identified as important from the equidistant binning data. PLS-DA, indicating the separation between the low (a) and high (b) fatigue cases before and after the hepatic challenges.

Neither PLS-DA models validated well with respect to predictive accuracy. Therefore, the findings should only be interpreted within the context of the current study and should not be generalized.

Table S1: Summary table of the clinical diagnosis information of 39 patients in low fatigue group and 31 patients in high fatigue group.

		Mean	SD	ES
Energy	High	8.90	0.77	9.49
	Low	1.61	0.75	
Mental	High	8.44	0.90	7.52
	Low	1.66	0.71	

Table S2: A Pearson and a Spearman’s correlation test between the energy and mental fatigue scores for four of the detoxification metabolites (acetaminophen glucuronide; acetaminophen sulphate; acetaminophen mercapturate and salicyluric acid). The outcome is shown in the table below:

Measure	Pearson correlation coefficient		Spearman’s rho correlation coefficient	
	PFS Energy	PFS Mental	PFS Energy	PFS Mental
Acetaminophen glucuronide	0.112**	0.060	0.090*	0.035
Acetaminophen sulphate	-0.015	0.007	-0.033	-0.012
Acetaminophen mercapturate	0.085*	0.017	0.034	0.019
Salicyluric acid	0.053	0.023	0.015	0.004

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).