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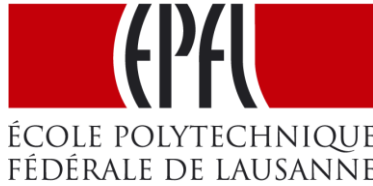SCHOOL OF LIFE SCIENCES

Master's project in Bioengineering and Biotechnology

Effect of carbonated and still beverages containing same dose of caffeine and green tea catechins on energy expenditure

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Abstract

BACKGROUND: Caffeine has been shown to increase energy expenditure, being a good candidate ingredient for thermogenic beverages. CO_2 is commonly used by the pharmaceutical industry to increase the absorption of drugs. Whether CO_2 addition to a caffeinated beverage could increase caffeine absorption and its thermogenic effects is unknown. OBJECTIVE: Determine if effervescence could increase the effects of caffeine on energy metabolism. DESIGN: 12 healthy Caucasian followed a randomized, crossover, single-blind, controlled pilot study. Subjects ingested at two different occasions either a still or a carbonated beverage containing same dose of caffeine (100 mg) and green tea catechins. MEASUREMENTS: Pre- (15 min) and post-treatment ingestion (120 min) energy expenditure (EE) was measured by indirect calorimetry using a canopy system. Substrate oxidation and heart rate were measured during the same time-periods. Caffeine kinetics was evaluated in saliva over 120 min. RESULTS: Caffeine AUC and C_{max} were increased over 120 min (10.1 µg.min/mL, p = 0.040 and 0.19 µg/mL, p = 0.031, respectively) with the carbonated compared to the still treatment. No difference between treatments was observed for change in EE. Both treatments increased EE by 6 kcal compared to baseline (p < 0.040). Over 120 min, still treatment enhanced fat and decreased carbohydrate oxidation by 17.2 kcal (p = 0.004) and 13.3 kcal (p = 0.012), respectively, compared to baseline. CONCLUSION: Caffeine absorption was increased with the carbonated, compared to the still treatment. However, this was not accompanied by an increase in EE compared to still treatment. Still treatment shifted substrate oxidation towards fat, reducing the contribution of carbohydrate as a body fuel. The significant increase in EE induced by both treatments confirms the thermogenic properties of the beverage formulation, independently from its CO_2 content.

Keywords: carbonation, caffeine, energy expenditure, substrate oxidation, indirect calorimetry

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Complementary activities at the Nestlé Research Center

Indirect calorimetry was not new at NRC, since the first instruments were implemented there in 1988. But the recent acquisition of 3 new setups (MAX-II device provided by AEI technologies, Naperville, IL, USA) required their installation, the check of their good functioning, as well as the conformation to NRC quality requirements.

My internship began just when the devices arrived at NRC. Therefore, I was involved into all these processes. Indeed, after a first literature review to understand the technique, I was trained to use the devices by two experts in the field, the scientist Geneviève Major, PhD and the senior scientist Kevin Acheson, PhD. In order to comply with quality requirements, and after a training period, I contributed to the development, writing and implementation of a Standard Operating Procedure (SOP) on the indirect calorimetry devices. This document was published internally, and is now a reference on the use of the indirect calorimetry setups at NRC.

Once the equipment was in agreement with quality standards, I was the main operator in charge of measuring energy expenditure of 72 volunteers in a double-blind, randomized, controlled, parallel-design study of two groups. I performed that work part time from October 2009 to May 2010. The energy expenditure measurements lasted 30-min, and each participant was tested twice, in fasting state. Prior to the beginning of this study, I measured the caloric content of the meals that were given to the subjects to test their *ad libitum* energy intake. This was done by burning pellets of dehydrated mashed food into an oxygen combustion bomb (calorimeter provided by Parr instrument company, IL, USA), and measuring the temperature increase of the water surrounding the bomb (water volume was precisely known).

I was also involved in discussions related to the development the present pilot study. Therefore, I reviewed the literature concerning carbonation, caffeine and carbonation, caffeine and energy expenditure and wrote the study protocol under Dr Major's supervision and in line with the good clinical practices (GCPs). After this step, and in constant collaboration with the Metabolic Unit at the NRC, the protocol was sent to the Ethics Comity of the CHUV for approval.

Once approved, subjects were recruited by the Metabolic Unit at the NRC. I was the main operator in charge of measuring energy expenditure, together with another research assistant. I was therefore blind to the study treatments the participants received. The setting of the rooms where energy expenditure is recorded had to be adapted to the new study, and I found solutions for to adapt the room setup, allowing the participants to be able to ingest treatments without disturbing energy expenditure measurements.

After the end of the recordings, and for each subject, I developed and implemented that concept of a "Dossier santé" which is a small document to communicate to participant their own energy expenditure and heart rate results. Individual results were explained and compared to "normal" values, and sent to each subject.

Conjointly with a mass spectrometry expert, I worked to develop a UPLC (ultra pressure liquid chromatography) method to analyze caffeine content in saliva, and did the analysis of the saliva samples.

It has to be mentioned that achieving the present study in less than 5 months was only possible due to the fact that I was already familiar with the indirect calorimetry setup prior the beginning of the clinical phase, and that every person involved into the study gave its best to work efficiently.

I also contributed to writing an invited review for the Journal of Applied Physiology, Nutrition and Metabolism (National Research Council Canada Press), on the topic of micronutrients and obesity. The review will soon be submitted for publication.

Over all the internship, I worked as technician, research assistant and study coordinator, and took active part to the life of the research group. I attended to weekly group meeting presentations and monthly seminars of the Department of Nutrition and Health. I also did a seminar to present the results of my study to the Energy and Metabolic Health Group. The variety of tasks I performed during my 11 months of internship gave me the opportunity to become more familiar with a clinical research environment and the main activities related to clinical studies.

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°C	Degree Celsius
µL	Microliter
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
bpm	Beat per minute
cAMP	Cyclic adenosine monophosphate
C_{max}	Maximal concentration
COMT	Catechol O-methyltransferase
d	Day
EE	Energy expenditure
EGCG	Epigallocatechin gallate
Eq.	Equation
ESI	Electrospray ionization
eV	Electron Volt
$FeCO_2$	Fraction of expired CO_2
Fig.	Figure
GTE	Green tea extract
h	Hour
kcal	Kilocalorie
Kg	Kilogram
LC	Liquid chromatography
m	Meter
m/z	Mass to charge ratio
mg	Milligram
mL	Milliliter
mm Hg	Mercury milligram

min	Minute
MS	Mass spectrometry
NADH	Reduced nicotinamide adenine dinucleotide
NE	Norepinephrine
NRC	Nestlé Research Center
NPRQ	Non-protein respiratory quotient
$NPVO_2$	Rate of non-protein oxygen consumption
$NPVCO_2$	Rate of non-protein carbon dioxide production
oz	Ounce
p	p-value
PET	Polyethylene terephtalate
PIEE	Post-ingestion energy expenditure
REE	Resting energy expenditure *i.e.* pre-ingestion energy expenditure
rpm	Rounds per minute
RQ	Respiratory quotient
SNS	Sympathetic nervous system
SOP	Standard operating procedure
St. dev.	Standard deviation
T_{max}	Time to reach maximal concentration
UPLC	Ultra pressure liquid chromatography
V	Volts
V_0, V_1, V_2	Visit zero, visit one, visit two
VO_2	Rate of oxygen consumption
vol	Volume
VCO_2	Rate of carbon dioxide production

1   Introduction

The incidence of obesity is dramatically increasing worldwide reflecting a daily positive energy imbalance in a large part of the population [70]. Matching energy intake with energy expenditure favors energy balance which is synonymous with body weight maintenance. Although a macronutrient balanced diet and regular physical activity are the best ways to ensure this match, ingredients that have the potential to increase mitochondrial activity[37,56] and subsequently diet-induced energy expenditure can complement a healthy lifestyle and help energy balance maintenance. Indeed, estimation from population studies revealed that a positive energy balance as low as 50-100 kcal/d could explain the annual 1-2 pound body weight gain (~ 0.5-1 kg) that occurred during the last decade in the American population [34,35]. This corresponds to the modest quantitative range of contribution to total energy expenditure that can be expected from thermogenic ingredients considered safe for food application [68]. Therefore, continuous improvement of existing products formulated to increase energy expenditure in order to try to enhance their effect is on avenue to help healthy-weight conscious people meet their objective.

1.1   Energy expenditure: where does it come from and how to measure it?

Energy expenditure can be divided into three main components: resting EE (REE), thermic effect of food and physical activity EE [32]. REE is energy spent to sustain basic life processes during awaken state, accounting for ~ 60-75 % of total daily energy expenditure [59]. Thermic effect of food is the energy expended for food processing such as mastication, digestion and absorption. Physical activity is the most variable component of EE, since it depends on each individual's engagement in exercise. It accounts for ~ 20-40% of EE [59].

In animal cells, mitochondrion is the organelle responsible for the transformation of most of the metabolic energy [32]. In this compartment, production of ATP occurs through cellular respiration, the main process that consumes O_2 and releases CO_2, in healthy subjects [48]. CO_2 is a by-product of the citric acid cycle that oxidizes acetyl CoA to give rise to high energy NADH. NADH transmits its energy to the electron transport chain, where O_2 is consumed, to build an acidic gradient in the inter membrane space of the mitochondria. This gradient is the driving force to create ATP from ADP. The overall balance involves that production of cellular energy requires consumption of O_2 and production of CO_2 eliminated through respiration [4,48]. Mitochondrion is the only site of utilization of oxygen, since O_2 cannot be stored in the body. Moreover, cellular respiration is the only mechanism of CO_2 release in normal conditions (no acidosis or alkalosis). Thus, in healthy subjects, assumption is made that all O_2 consumed and all CO_2 released by respiration is due to cellular respiration in mitochondria, and reflects entire body EE [48]. A rough overview of cellular energy generation by the mitochondrion is schematized in Fig. 1.
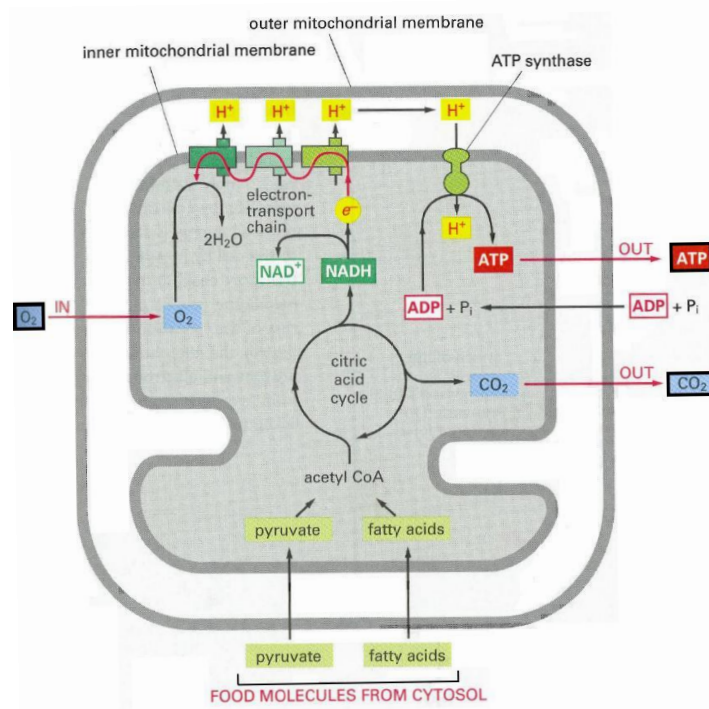

Figure 1 Cellular respiration and energy production: production of ATP requires O_2 consumption and CO_2 release from the mitochondrion. Modified from "Molecular Biology of the Cell" [4]

Evaluation of energy expenditure using indirect calorimetry is realized by measuring respiratory gas exchange (oxygen consumption and carbon dioxide production), which allows to estimate the amount of calories "burned". This technique can be used from mouse to man. Its reliability, relatively low initial and maintenance costs plus the fact that it can describe precisely the type of substrate being oxidized make indirect calorimetry a very good method to measure energy expenditure.

Expiratory gases are collected and their O_2 and CO_2 content are measured. They are compared to the O_2 and CO_2 content of the ambient air that is breathed in by the participant. The difference between ambient and expired O_2 and CO_2 concentrations is assumed to be entirely due to the cellular respiration[48], and to reflect energy expenditure. In practice, indirect calorimetry measurements can be performed when volunteers are in a metabolic chamber, or when they wear a canopy or a mask. The ambient air is pulled into the compartment where subjects breathe, and their respiratory gases are pulled towards the gas analyzer, through a unidirectional valve that prevents gas reflux.

The gold standard equation to determine energy expenditure is the simplified Weir formula[67]:

$$\text{EE (kcal/min)} = (3.941 \times VO_2) + (1.106 \times VCO_2) \qquad \text{Equation 1}$$

Moreover, the type of substrate that is being oxidized (fat or carbohydrate) can be derived from this method, either in observing the ratio of carbon dioxide production to oxygen consumption, or in evaluating the weight of substrate oxidized.

Oxidation of food substrate into the mitochondria consumes O_2 and releases CO_2. Therefore, measurements of expiratory O_2 and CO_2 allow the estimation of substrate oxidation[48]. Indeed, oxidation of glucose ($C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$) requires $6O_2$ and releases

$6CO_2$, whereas oxidation of linoleic acid consumes $5O_2$ and produces $36CO_2$. The method relies on the ratio of carbon dioxide production to oxygen consumption, which gives the respiratory quotient (RQ). It can be assumed to reflect total whole body oxidation of the three principal macronutrients: carbohydrate, fat, and protein. The changes in the RQ reflect the type of substrate that the body is currently oxidizing, see Fig. 2. In normal physiological conditions[39], this parameter can vary between 1 and 0.7. A RQ of 1 indicates that the metabolism rely totally on carbohydrate oxidation, whereas a RQ of 0.7 indicates that fat fuels the energy metabolism[46]. In between values (*i.e.* interval] 0.7 ; 1.0 [ ), indicate that the metabolism relies partly on carbohydrate, partly on fat, and partly on protein.

The gold standard to evaluate substrate oxidation by observing the RQ is the use of equations of Livesey and Elia[46]:

$$RQ \text{ (no unit)} = \frac{VCO_2}{VO_2} \quad \text{Equation 2}$$

As protein oxidation represents a minimal part of whole oxidation in a normal situation (no prolonged starvation, no diabetes, no acidosis)[46], it can be assumed to remain constant. Protein oxidation can be measured through urine nitrogen. Another method is to estimate protein oxidation based on the weight of subjects, using with the following equations [1]:

$$\text{Protein oxidation (mg/min)} = \frac{(0.8 * body.weight) * 1000}{1440} \quad \text{Equation 3}$$

Once protein oxidation has been determined, it is possible to derive the non-protein oxygen consumption ($NPVO_2$) and the non-protein carbon dioxide production ($NPVCO_2$)[46]. These variables reflect O_2 consumption and CO_2 production that does not take into account the portion of gas due to protein oxidation.

$$NPVO_2 \text{ (ml/min)} = VO_2 - protein.oxidation \times 1.01031 \quad \text{Equation 4}$$

$$NPVCO_2 \text{ (ml/min)} = VCO_2 - protein.oxidation \times 0.84361 \quad \text{Equation 5}$$

To calculate the respiratory quotient without taking into account protein oxidation, non-protein respiratory quotient (NPRQ) can be derived from $NPVO_2$ and $NPVCO_2$ (Eq. 4 and Eq. 5).

$$\text{NPRQ (no unit)} = \frac{NPVCO_2}{NPVO_2} \quad \text{Equation 6}$$
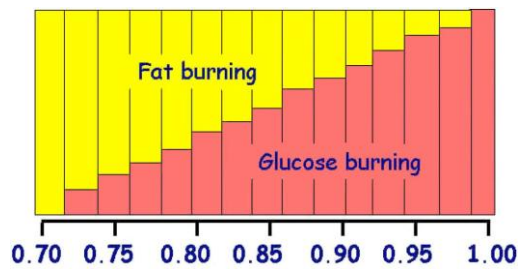
Figure 2 Interpretation of NPRQ evolution

If it is assumed that protein contributes minimally to the three substrate oxidized[46], NPRQ's interpretation is the same than RQ.

From $NPVO_2$ and $NPVCO_2$, it is possible to calculated fat and carbohydrate oxidations[46]:

$$\text{Fat (mg/min)} = \frac{NPVO_2 - NPVCO_2}{2.01494 \times (1 - 0.707)} \quad \text{Equation 7}$$

$$\text{Carbohydrate (mg/min)} = \frac{NPVCO_2 - 0.707 \times NPVO_2}{0.82821 \times (1 - 0.707)} \quad \text{Equation 8}$$

In these equations, energy expenditure is expressed in kcal/min

VO_2, VCO_2, $NPVO_2$ and $NPVCO_2$ are expressed in ml/min

Protein, fat and carbohydrate oxidation are expressed in mg/min

RQ and NPRQ have no unit

Body weight is expressed in kg

Energy expenditure can be modulated by changing physical activity practice or using functional food to modulate the metabolic rate. Among such thermogenic ingredients, caffeine, and to a lower extend tea catechins, have been shown to enhance EE.

1.2 Caffeine and catechins impact on thermogenesis and substrate oxidation

1.2.1 Caffeine

Caffeine belongs to the alkaloid group, and is part of the methylxanthine family, Fig. 3.

Figure 3 Caffeine's developped formula ; 1, 3, 7 tri-methylxanthine, $C_8H_{10}N_4O_2$

After ingestion, caffeine is absorbed in the intestine (80%) and in the stomach (20%)[19]. Although this molecule is hydrophilic, it has sufficient hydrophobic characteristics to cross all biological membranes, and is therefore highly bioavailable[6]. In human, 99% of the ingested caffeine is absorbed within 45 min[13]. Its half-life, in human, is around 4.9h [57,58], but is subjected to important inter-subjects variability[12]. In human, caffeine is primarily metabolized in the liver with the intervention of the cytochrome P450 oxidase enzyme 1A2[18]. Demethylation of caffeine gives rise to paraxanthine (84%), theobromine (12%) and theophilline (4%) [44].

Among the numerous effects of caffeine on the metabolism, its effect on energy metabolism has been widely studied. Acute increase in energy expenditure varying between 3 and 16% increase with doses between 100 and 400 mg caffeine intake has been reported by different authors [2,3,5,8,16,26,36,41]. The thermogenic effect of caffeine is thought to be mainly due to

three mechanisms: adenosine receptor blockage, phosphodiesterase inhibition and ryanodine receptor stimulation. These mechanisms impact either on SNS or directly on muscle stimulation, increasing thermogenesis and lipolysis.

Adenosine receptors blockage (Mecanism 1, Fig. 4)

Adenosine is a neurotransmitter distributed through all the body, which acts on specific cell-surface receptors[47]. Its binding to its receptor leads to inhibition of the release of many neurotransmitters, such as norepinephrine (NE), dopamine, acetylcholine, glutamate and GABA[29]. As shown in Fig. 4 mechanism 1, caffeine can bind to the adenosine receptors. This leads to inhibition of the activity of this receptor, thus increasing the release of the above-cited neurotransmitters, among which NE, enhancing its effects on SNS[24]. More NE in the circulation will further activate β-adrenergic receptors on effector cells, and as a consequence, drive the conversion of ATP into cAMP by adenylate cyclase[48]. Intracellular cAMP signaling produces heat in skeletal muscle and leads to increased lipolysis[10].

Phosphodiesterase (Mechanism 2, Fig. 4).

cAMP, a G-protein second messenger, is degraded to AMP by the widely distributed enzyme phosphodiesterase[48]. Caffeine can inhibit phosphodiesterase (Fig. 4 mechanism 2), *i.e.* increase cAMP lifespan by preventing its degradation[27], leading to increased cAMP levels. Phosphodiesterase inhibition by caffeine has been triggered in vitro with above physiological ranges of caffeine, thus indicating a rather minimal effect of this phenomenon at normal caffeine consumption in human[53].

It is to note that both binding of adenosine receptor and inhibition of phosphodiesterase are complementary in leading to increased cAMP levels. These enhance thermogenesis and activate hormone sensitive lipase, which promotes free fatty acids (FFA) in circulation. FFA

can further fuel ATP production by mitochondria, as shown in Fig. 1. Thus, caffeine triggers

fat oxidation more than carbohydrate consumption [2,3,38] and increases thermogenesis.
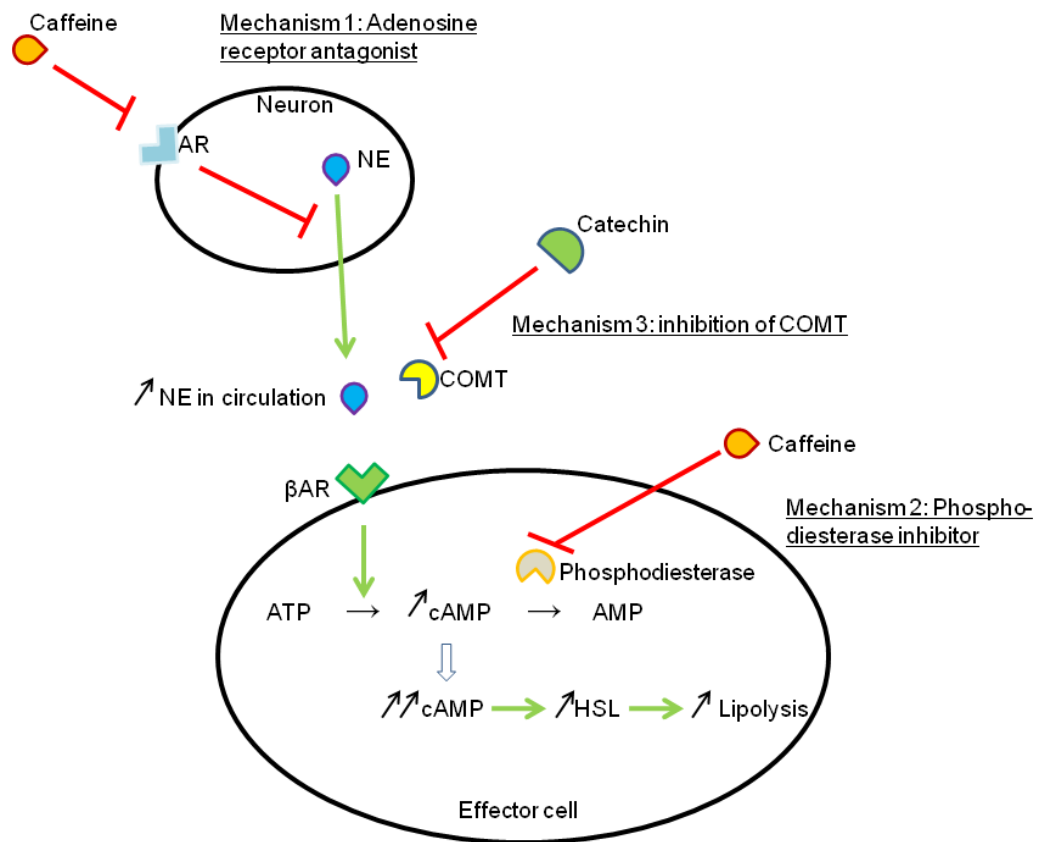
Figure 4 Effects of caffeine and green tea catechins on energy metabolism and substrate oxidation: increase free norepinephrine (activator of SNS), cAMP (mediator of cell thermogenesis) and lipolysis
Abbreviations: AR: Adenosine Receptor; NE: Norepinephrine; βAR: Beta-Adreno Receptor; HSL : Hormone Sensitive Lipase; COMT: Catechol O-MethylTransferase

Release of intracellular calcium

Finally, caffeine can act physiologically and independently of SNS, mainly by stimulating

intracellular calcium release from sarcoplasmic reticulum in skeletal muscle[53]. The ryanodine

receptor, a sarcoplasmic reticulum channel releasing calcium ions into skeletal muscles, can

be activated by caffeine binding. Stimulation of the ryanodine receptor enhances the muscle

contraction, promotes the production of heat, increases ATP turnover and mitochondrial

pyruvate oxidation[21]. By influencing calcium mobilization in skeletal muscle, caffeine may

impact global thermogenesis[2].

The role of resting muscle metabolism in inducing thermogenesis has been shown to be an important determinant of inter-individual variability in energy expenditure[71]. This could indicate that caffeine induced intramyocellar release of calcium is a mechanism impacting importantly on energy expenditure[7].

1.2.2 Catechins (Mechanism 3, Fig. 4)

Catechin is another ingredient being extensively studied for its thermogenic properties. Catechins are part of the flavonoid family, and are found mainly in green tea.
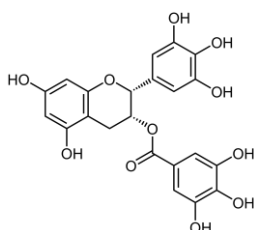
Figure 5 Epigallactocatechin gallate's developped formula ; one of the most potent catechin found in green tea [42]

Catechins can interact in the NE pathway described above (Mechanism 3, Fig. 4). Indeed, catechol O-methyltransferase (COMT) is an enzyme, widely distributed in the body, which degrades several catecholic compounds, among which NE[14]. Green tea catechins, and mainly EGCG, can inhibit this enzyme[69], leading to prolonged half-life of NE, thus extending its activation of β-adrenoreceptors and further cAMP synthesis, as shown in Fig. 4. This leads to enhanced energy expenditure and lipolysis.

Moreover, a synergetic effect between green tea and caffeine is possible. Indeed, both caffeine and catechins act together to enhance the NE pathway (Fig. 4). Caffeine inhibition of cAMP degradation will further increase the cAMP levels, leading to global SNS activation[66,69].

Beside these theoretical mechanisms, the clinical effects of catechins remain unclear. Indeed, 125 mg of catechins triggered no increase in 4h-EE compared to baseline, in the study of Belza *et al* [10]. This might be due to the dose that was too low to induce an effect on EE.

Alternatively, catechins might have to be ingested with caffeine to induce an effect on EE. Indeed, Dulloo *et al* suggested a synergistic effect, after having observed a significant increase in 24h-EE (2.8%) and fat oxidation in 10 human subjects, due to conjoint administration of caffeine and catechins (150 mg and 375 mg respectively) compared to ingestion of 150 mg of caffeine alone[25]. On the other hand, Gregersen *et al* showed that 600 mg catechins plus 150 mg caffeine did not significantly increase 12.5h-EE more than caffeine alone (150 mg)[31]. Rumpler *et al* failed to demonstrate that 270 mg caffeine plus 244 mg EGCG had a greater effect on 24h-EE than 270 mg caffeine alone[64]. Finally, Thielecke *et al* observed that the effect on fat oxidation of 300 mg EGCG and 200 mg caffeine was mainly triggered by caffeine, since they did not observe a difference between this mixture and 200 mg caffeine alone[66]. Moreover, Bérubé-Parent *et al* observed an 8 % increase in 24h-EE with EGCG-caffeine mixtures (per day: caffeine fixed dose of 600 mg plus either 270, 600, 900 or 1200 mg EGCG) compared to placebo; but these doses did not impact fat oxidation and RQ[11]. Thus, even if catechins may have an effect on thermogenesis[25], literature points towards a smaller impact compared to caffeine's effect on energy expenditure and substrate oxidation[10,11,23,31,64,66].

1.3   Carbonation effects

Carbonation and effervescence refer to the dissolution of CO_2 into water or aqueous solution and to the release of CO_2 from these solutions, respectively. There are few evidences suggesting that effervescence increases absorption and bioavailability of drugs. CIMA Laboratories patented in the US the use of effervescence as a penetration enhancer for drugs to improve their solubility and absorption in the gastro-intestinal tract[54]. This could be due to the effect of CO_2 bubbling directly onto the intestinal epithelium which alters the paracellular pathway such as a reduction in transepithelial electrical resistance, as shown in

vitro on a rabbit ileum epithelium[28]. One randomized, double blind, placebo-controlled study compared an acetaminophen-standard to an acetaminophen-effervescent tablet and found that median times to onset of analgesia and meaningful pain relief was significantly shorter with the effervescent table[51], also suggesting faster absorption with the effervescent medium. In accordance with this, Joshi *et al* showed a significantly shorter time to maximum plasma concentration of coenzyme Q10 administered with effervescent and fast-melting formulations compared with a soft-gel or powder-filled formulations, although no difference between the four formulations was observed for maximum plasma concentration and area under the curve[40]. Similarly, the rate of absorption, but not the maximum plasma concentration, was found to be significantly greater for a paracetamol effervescent powder compared to a paracetamol-coated tablet in healthy volunteers[22]. These results raise the question whether carbonation could have similar enhanced-absorption effects on ingredients that are dissolved in beverages for food application.

Schroder *et al* compared in a randomized crossover study the intestinal calcium absorption between a dairy soft-drink, fat-free milk and a calcium-fortified orange juice and found a significantly higher calcium absorption in the first beverage[65]. In another trial implicating 21 volunteers, mean absorption rate of alcohol was found to be significantly faster after consumption of vodka mixed with carbonated water compared to still water, although one third of participants in this study showed no difference or a decrease in absorption rate[60]. Few studies have also compared the absorption rate and kinetics of caffeine in carbonated and non-carbonated solutions. Liguori *et al* showed no difference in peak caffeine absorption and time to peak absorption between 400 mg caffeine administered via 12 oz unsweetened coffee, 24 oz sugar-free cola, or capsules in usual caffeine drinkers[45]. Bonati *et al* compared caffeine kinetics after consumption of 0.22 mg/kg of caffeine as soft-drink (190 ml), 1.54 mg/kg as mocha coffee (50ml), 1.00 mg/kg as aqueous solution (70 ml at 0.1%),

5.00 mg/kg as aqueous solution (70 ml at 0.5 %), and 10.00 mg/kg as aqueous solution (70 ml at 1.0 %) [13]. They found similar kinetics between treatments, although in agreement with another study[49], absorption rate constant was lower after the soft-drink compared to the mocha coffee and aqueous solution[13]. Bonati *et al* suggested that this difference could be due to the lower pH value, the larger volume and the lower concentration of the soft-drink compared to the other solutions. However, in this study, no p value was reported and it is therefore unknown if differences were significant. Moreover, kinetics data were obtained from four volunteers[13].

1.4   Study treatments

In the scope of energy balance management, it is interesting to evaluate if carbonation can enhance caffeine-induced increase in energy expenditure.

Building on the properties of caffeine and green tea to increase energy expenditure, Nestlé has developed the thermogenic ready-to-drink tea beverage. This tea beverage contains synthetic caffeine (65 mg), green tea extract (899 mg of which 230 mg are catechins, and 35 mg are caffeine) and calcium (195 mg). The beverage exists in two versions containing the same active ingredients: one carbonated and one still. Therefore, this beverage represents a perfect candidate to answer the question whether CO_2 can enhance the effect of caffeine and green tea catechins on energy metabolism.

The acute effects of a mixture of active ingredients in similar proportion to what is found in still beverage has been tested, focusing mainly on energy expenditure, substrate oxidation and heart rate[63]. In this study, three daily doses of this green tea and caffeine beverage was shown to significantly increase 24h-EE by 106 kcal in healthy, normal-weight men and women[63]. If Rudelle *et al* did not observe changes in fat oxidation following ingestion of 300

mg of caffeine and 540 mg catechins, the work of Dulloo *et al* [25] attested of an increase in fat oxidation and of a decrease in carbohydrate utilization after ingestion of 150 mg caffeine and 270 mg EGCG. Heart rate did not change in both studies [25,63].

The following section summarizes the pilot clinical study conducted at the Nestlé Research Center, to compare the acute effect of carbonated compared to still study treatment on energy expenditure, substrate oxidation, heart rate and caffeine absorption.

2   Hypothesis

Since carbon dioxide has been shown to enhance absorption[28,54,65], the hypothesis of this study was that the increase in energy expenditure would be greater following ingestion of the carbonated, compared to the still study treatment, due to enhanced absorption of caffeine and green tea catechins, potentiating their thermogenic effects. This was addressed comparing, between treatments, the change in post-ingestion energy expenditure compared to baseline, over a 135 min measurement period (15 min baseline and 120 min post-ingestion). As caffeine is known to impact substrate oxidation, this parameter was also evaluated in this study.

Similarly, as caffeine is known to influence heart rate, at least at doses as high as 400 mg[8], this variable was also measured. To match the evolution of these parameters with caffeine concentration and to evaluate the impact of carbonation on caffeine absorption, a kinetic of caffeine was derived from its concentration in saliva. It was hypothesized that caffeine would be absorbed faster and in larger proportion in the presence of carbon dioxide compared to the still beverage.

Hypothesis: CO_2 addition into treatment formulation may increase caffeine absorption. This may potentiate caffeine's effects on energy expenditure, substrate oxidation and heart rate.

3   Materials and Methods

For further information, a section "Subjects, Materials and Methods" is attached in Annex A.

3.1   Study design

This study is a single centered, randomized, single-blind, and controlled cross-over clinical

trial of two groups.

3.2   Product description

Characteristics of the study treatments	Carbonated 1 serving = ingested dose	Still 1 serving	Still Ingested dose
Energy (kcal) *	< 10	< 10	< 12
Green tea extract (mg) *	899	899	1364
Total catechins (mg)*	230	230	349
EGCG (mg) *	122	122	182
Caffeine (mg) **	106.5	66	100
Calcium (mg) *	195	195	295
Carbohydrate (mg) *	0	0	0
Fat (mg) *	0	0	0
CO_2 (g) ***	6	0	0
Serving size (mL)	355	330	500

Table 1 Energy content and major ingredients in study treatments
Symbols: * According to recipe ; ** Measured by the Regional Laboratory of Nestlé ; *** Measured by Nestlé's Product and Technology Center Marysville

3.3   Data collection

Parameter	Stabilisation	Pre-ingestion	Ingestion	Post-ingestion				
Time (min)	-20	-5	0 15	30	60	90	//	120
Salivette		S0	S1	S2	S3	S4	S5	
EE Sub. Ox. Heart rate	No measurement	15 min measurement	No measurement	120 min measurement				

Table 2 Phases of measurement, timing and saliva sampling

Visits one and two were identical, with the treatment distributed in a cross-over manner.

4   Results

Tables showing median, mean, standard deviation and range of the measurements are attached in Annex B.

Graphics interpretation: Time 0 min: mean of the 15-min resting period; time 20-120: means over 10 min post-ingestion periods. Graphics (Fig. 6, 8, 9 and 11) represent median of the time intervals, averaged over all the subjects, during the pre- and post-ingestion periods. Fig. 10 represents the caffeine concentration averaged over each subject at each saliva sampling. Fig. 7 represents the subtraction of the median of each post-ingestion time interval and the baseline, averaged over all the subjects, multiplied by the test duration.

4.1   Physical characteristics of the subjects

12 volunteers (5 men and 7 women) were enrolled and all completed the study with no major protocol violation. Therefore, all subjects were included into the intention-to-treat analysis set.

Parameter	Median	Mean	St. dev.	Range
Age (years)	35.0	37.2	8.3	24.0 – 50.0
Weight (kg)	64.1	66.6	8.8	54.0 – 84.0
Height (cm)	173.0	171.8	8.6	160.0 – 190.0
Diastolic blood pressure (mmHg)	80	79	7.0	70 – 90
Systolic blood pressure (mmHg)	120	120	7.1	110 – 130
BMI (kg/m^2)	22.2	22.5	1.7	20.2 – 25.3

Table 3 Demographics and baseline characteristics of participants

All volunteers drank the full treatment volume within 5 min, except one who left 39 mL of the still treatment. Compliance for abstinence from caffeine after the evening meal before V_1 and V_2 was confirmed by measurement of salivary caffeine immediately before products ingestion, which reached 4.8 µmol/L at maximum. This is below the threshold Astrup *et al* used in their study (5µmol/L) [8].

4.2 Primary Outcome: Energy Expenditure
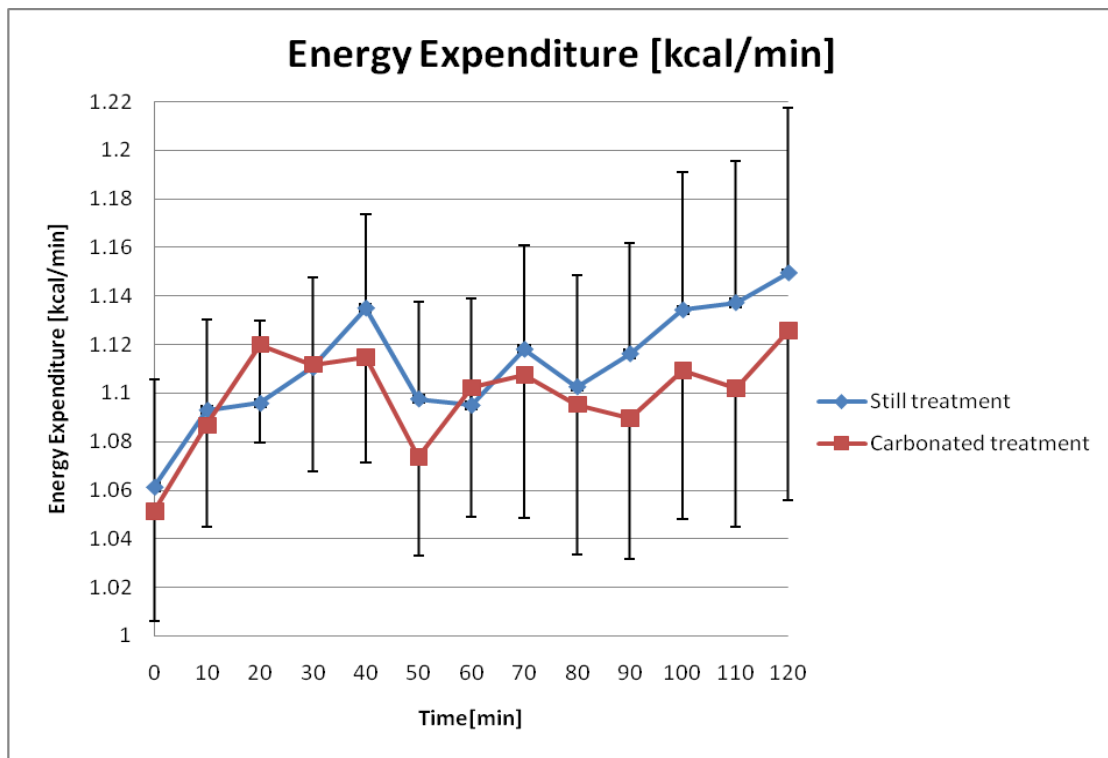
Figure 6 Energy expenditure over time, n = 12
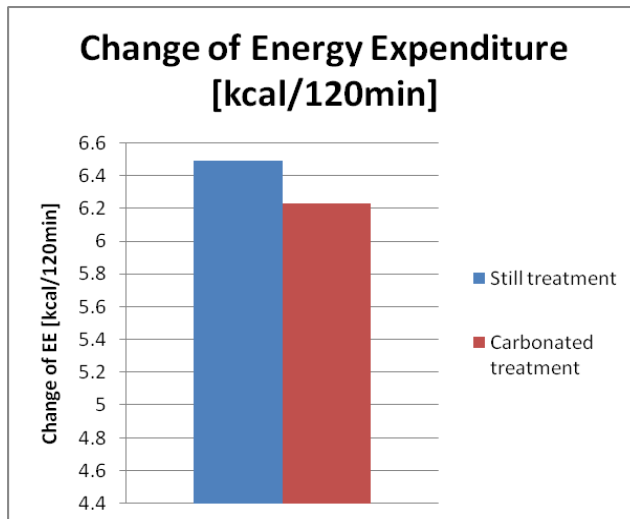
Figure 7 Change in energy expenditure, n = 12

Between both treatments, no significant difference in change in energy expenditure was obtained (p = 0.850). This was reinforced by a supportive analysis (p = 0.908).

Both treatments triggered an increase of 0.05 kcal/min from baseline (p = 0.038 for still and p = 0.020 for carbonated treatment), *i.e.* 6 kcal/120min.

4.3   Secondary Outcomes

4.3.1   Substrate Oxidation

All the observations on fat and carbohydrate oxidations were reinforced by measurement and statistical treatment of RQ and NPRQ (see in Annex B).

Compared to the still treatment, the carbonated treatment induced significantly lower fat and higher carbohydrate oxidations (10.2 mg/min, $p = 0.002$, and 21.8 mg/min, $p = 0.005$, respectively). Carbonated treatment did not impact significantly substrate oxidation, compared to baseline (fat oxidation: $p = 0.262$, carbohydrate oxidation: $p = 0.935$). Still treatment significantly increased fat oxidation by 15.2 mg/min ($p = 0.004$), and decreased carbohydrate oxidation by 26.5 mg/min ($p = 0.012$), from baseline.
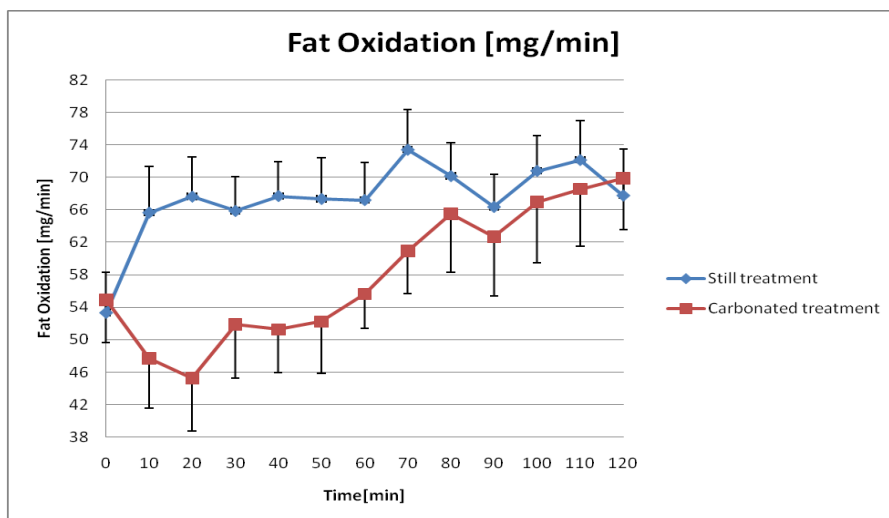
Figure 8 Fat oxidation, n = 12
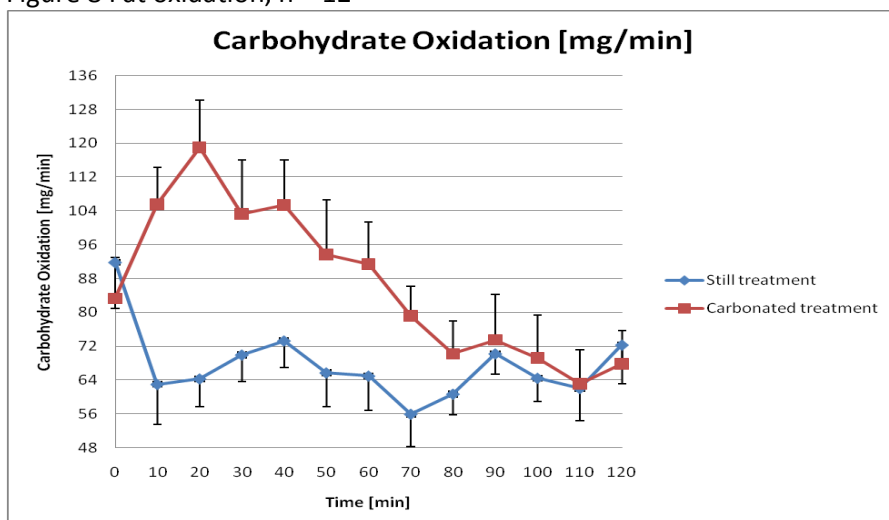
Figure 9 Carbohydrate oxidation, n = 12

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4.3.2 Caffeine Kinetics
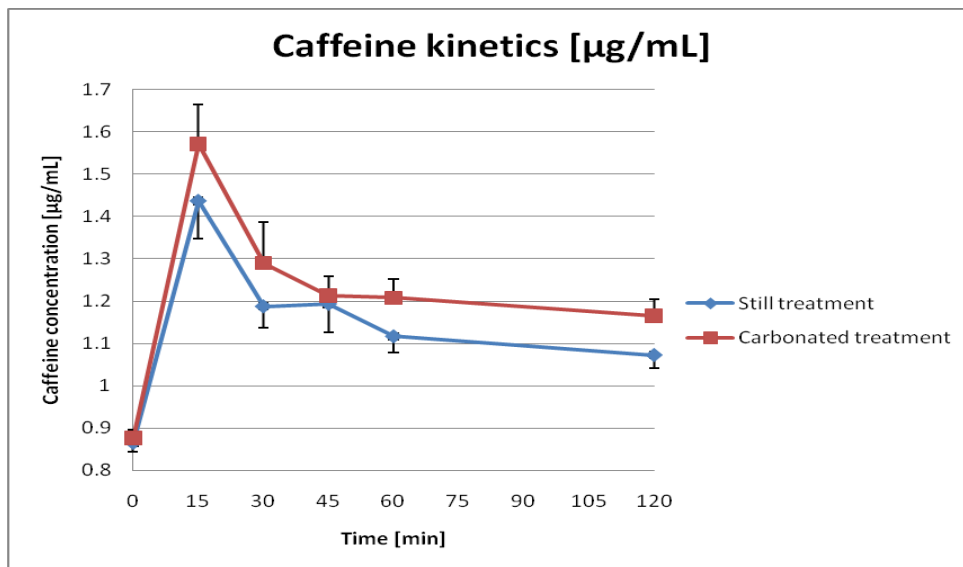
Figure 10 Salivary caffeine kinetics, n = 12

Compared to the still treatment, the carbonated treatment induced higher caffeine concentration AUC and C_{max} (increases by 10.1 µg.min/ml, p = 0.040 and 0.19 µg/ml, p = 0.031, respectively). T_{max} was not different between treatments (p = 0.693).

4.3.3 Heart Rate
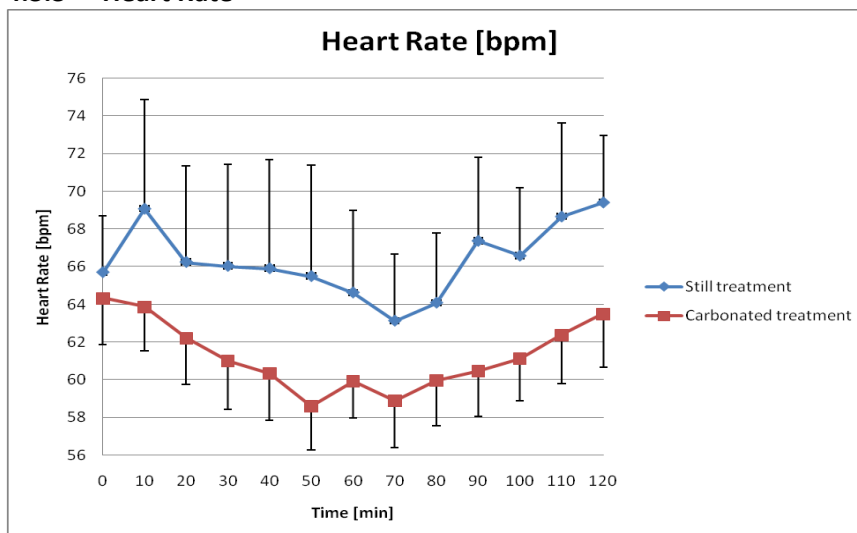
Figure 11 Heart rate, n = 12

Still and carbonated treatments induced a similar effect on heart rate (p = 0.130). Carbonated treatment showed a significant decrease of 3.67 bpm from baseline (p = 0.030), whereas no significant effect was observed for the still treatment (p = 0.958).

5   Discussion

The present study was conducted to investigate the hypothesis that caffeine-induced increase in energy expenditure would be greater following ingestion of a carbonated beverage, compared to the same product in a still version. This hypothesis was based on the assumption that carbonation could increase caffeine absorption, and thus enhance the effects of this ingredient on metabolic rate.

5.1   Caffeine Kinetics

Comparing both products, results revealed significant increase in caffeine absorption (AUC, and C_{max} enhanced) with the carbonated product. This is in line with our initial assumption, and supported by literature [54,22,28,43,65].

Carbonation increased caffeine AUC by 10.1 µg.min/mL over 120 min. As caffeine is known to be completely absorbed in human[13,19], the difference in AUC measured in this study does not reflect a difference in total quantity of absorbed caffeine. However, it may reflect a different pattern of absorption on a 120 minutes basis due to CO_2 addition in the beverage.

An increase in C_{max} may attest of higher efficiency of caffeine to cross gastro-intestinal mucosa due to carbonation, as suggested by Eichman and Robinson[28].

Time to reach maximal concentration did not differ significantly between products (p = 0.693). This is against the current available literature, where time to reach maximal concentration is reported to be shorter with carbonated compared to still treatment formulation. Indeed, with 18 volunteers, Di *et al* showed a decrease in T_{max} with effervescent paracetamol compared to tablets (20.4 min compared to 38.4 min respectively)[22]. Moreover, Joshi *et al* observed that effervescent formulation of a novel coenzyme Q10 triggered faster T_{max} than non-effervescent formulations (2.0 h and 3.7 h respectively)[40]. Finally, Bonati *et al*

observed reduced T_{max} after ingestion of 0.22 mg/kg of caffeine as soft-drink compared to sin-gas formulation 5.00 mg/kg as aqueous (38 min and 47 min respectively) [13].

Looking deeper into T_{max} distribution (see table 6B in Annex B and Fig. 12), discrepancies can be observed. Indeed, mean time to reach maximal concentration is much longer than its median. Out of 12 participants, 9 and 8 of them triggered a T_{max} at T = 15 min in following ingestion of still and carbonated treatments, respectively, see Fig. 12. However, the mean is 2.5 min shorter with carbonated (25 min) than with still treatment (27.5 min). Although it is not significant, the trend of lower T_{max} due to carbonation is observed here, as described in literature [13,22,40]. It has to be mentioned that it is difficult to determine whether it reflects physiological values, or whether it was affected by the 15 min interval between each saliva sampling and the last measurement at 120 min with still study treatment, which would have impacted on the mean. Anyways, more accurate T_{max} values would have been obtained if saliva sampling had been more frequent, leading to a normalization of T_{max} distribution.

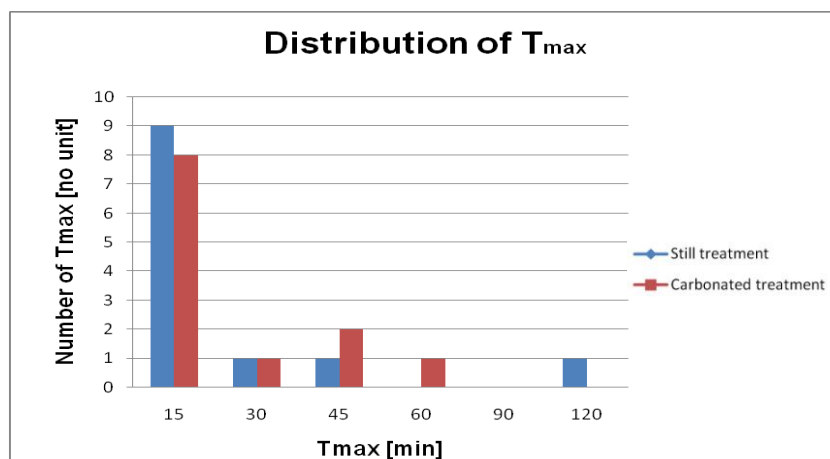Figure 12 Distribution of T_{max} for both treatments, n = 12

Salivary caffeine concentration has been shown to reflect plasma caffeine concentration[6,33,52,72]. Caffeine concentration can be monitored in saliva independently of the salivary pH or the flow[33]. The gold standard for this method is to rinse the mouth to remove the caffeine that could remain and which would false real plasmatic concentration[30].

In the present study, it was uneasy to have the subjects rinse their mouth after beverage ingestion, because the presence of the canopy over their head prevented them to spit. Since energy expenditure was the first outcome of this study, it was decided not to remove the canopy during the test, including during treatment ingestion, in order to avoid losing respiratory gas immediately after treatments ingestion. For this reason, treatments were ingested using a straw and intervals between saliva samplings were relatively long (15 min). As a consequence participants could not rinse their mouth following treatment ingestion. Therefore, caffeine kinetics may have been affected by non-physiological caffeine remaining in the saliva due to the drinking of the caffeinated test products, enhancing artificially AUC and C_{max}. However, if this is the case, it is most likely that only the first saliva sampling would have been affected, since 100 mg caffeine was diluted in 355 mL or 500 mL.

The hypothesis of an artefact at the first measurement is reinforced by the fact that median T_{max} are reached in 15 min with both treatments. Indeed, in the literature, caffeine T_{max} is very widely distributed (from 15 to 120 min), but strong evidence is that time to reach maximal concentration is about 40 min [6,8,13,49,58]. The fact that the still treatment presents a lower than expected T_{max} indicates that the highest concentration present at the first post-ingestion measurement is not due to carbonation, but more likely to residual caffeine in saliva, remaining from beverage ingestion.

This artefact may have influenced the absolute values AUC, C_{max} and T_{max}, but not the differences between the two treatments for AUC and C_{max}. Therefore, the observation that caffeine was better absorbed due to the presence of carbonation on a 120 min basis remains relevant.

5.2   Energy Expenditure

5.2.1   Comparing Treatments

The carbonation-induced increase in absorption of caffeine does not appear to have been sufficient to significantly affect energy expenditure in our study. This is against our initial hypothesis, which was that the change in energy expenditure between carbonated and still beverage versions would be different. Therefore, we could conclude that carbonation does not represent a solution to enhance the thermogenic functionality of caffeine. However this conclusion should be interpreted with caution, since the present study did not investigate the effect of pure caffeine into water but the effect of a mix of active ingredients. Moreover, the ingested volumes were not identical. As discuss below, these are factors that might have influenced our results.

Increased Volume

Study beverage is formulated to contain 100 mg caffeine. However, analysis of the caffeine content revealed that the batch of still treatment contained less caffeine (66 mg). As a consequence, the volume was adjusted so that both study beverages could contain 100 mg caffeine, which is a dose shown to affect the resting metabolic rate by 3-4 % over 2.5h [26]. Therefore, 500 mL of the still beverage were necessary to insure this caffeine content, whereas 355 mL of the carbonated drink were sufficient to deliver 100 mg caffeine.

Water Induced Thermogenesis

Based on the results of Boschmann *et al*, it could be argued that the greater volume ingested with the still beverage lead to an increase in energy expenditure [15]. In this study involving 14 healthy subjects, drinking 500 mL of water led to an increase of energy expenditure of 30 %, 60 min after beverage ingestion (p < 0.001). Despite the significant effect of water observed in this study, this article was challenged by a review[17], which concluded, based on several other papers, that water did not enhance energy expenditure, by itself. Indeed, in a randomized crossover design where 8 human subjects ingested 7.5 mL/kg (about 518 mL) of

distilled water or 0.9 % saline solution, Brown *et al*, could not observe any effect on energy expenditure[17]. As this team does not support an effect of water volume *per se* on energy expenditure, the decision to match caffeine content rather than total volume ingested was taken in the present study.

Temperature

In the above described study, Boschmann *et al* suggested that energy expenditure was increased of 12 % only to fuel the warming of 500 mL water from 22° to 37°C [15]. However, Brown *et al* reported an increase in EE of only 4.5 % due to ingestion of ~ 7.5 mL/kg distilled water at 3°C [17]. Considering this number, ingestion of a still treatment volume surplus of 145 mL at 3°C would lead to an increase of 1.3 % in EE, which could have been sufficient to hide an effect of carbonation on caffeine-induced effect on energy expenditure.

Catechin Content

Another aspect to consider is the fact that the increase of volume of the still treatment have led to an increase in GTE and its catechins, as well as calcium. The concentrations of these elements have not been measured, and it is therefore difficult to evaluate precisely their absolute quantity in 500 mL. According to recipe, the addition of 145 mL of still beverage increased absolute intake of catechin by 119 mg, bringing total ingested catechins amount to 349 mg in still compared to 230 mg in carbonated treatment. In the present study, the contribution of catechins cannot be distinguished from that of caffeine, due to the fact that no control was done with either caffeine or catechins alone.

Dulloo *et al* found that daily ingestion of 375 mg catechins and 150 mg caffeine increased significantly 24h-EE by 2.8 % compared to 150 mg caffeine alone[25]. Such an effect of catechins on energy expenditure, beyond the effect of caffeine, has not been observed in several other studies [10,11,23,31,64,66]. Indeed, Gregersen *et al* did not observe higher EE due to ingestion of caffeine (150 mg/d) and catechins (range from 493.8 to 684.0 mg/d) compared to same dose of caffeine alone.

Based on the above mentioned doses of catechins needed to significantly enhance EE it is likely that the increase in catechin content ingested with the extra volume of the still treatment (evaluated to 119 mg catechins) could not influence significantly EE.

In summary, based on available literature, we can conclude that ingestion of the additional volume *per se* (145 ml) and of the additional quantity of catechins (119 mg), could not affect significantly energy expenditure. However, it is possible that the thermogenic cost of heating a surplus of beverage have influence the effect of the still treatment on energy expenditure.

5.2.2 Treatment Effect

This study could complement the research of Rudelle *et al* in investigating the effect of one single serving of beverage on energy expenditure. Indeed, these authors addressed the effect of three servings of a similar beverage (daily dose: 2100 mg GTE, 300 mg caffeine and 633 mg calcium), and observed diurnal EE increased by 4.7 kcal/h compared to placebo (p = 0.005) [63]. This is comparable to the 6 kcal/h (compared to baseline) noticed in the present work which was achieved with a single beverage serving.

Ingestion of either treatment in the present study showed an increase in EE of about 4.6 %. This is in the same range that the diurnal-EE observed in the study of Dulloo (increase of 4.5 % due to ingestion of 150 mg caffeine and 375 mg catechins, compared to placebo) [25].

The effect observed on 120-min EE is greater than what was reported by Dulloo and Rudelle during the diurnal phase of the tests. This may be attributable to the fact that we focus on a 120 min effect –when the effect is suspected to be the highest–, whereas they measured EE for 24h and 23h, respectively. Indeed, caffeine maximal concentration in blood is reached in approximately 40 min [6,8,13,49], and that there is a correlation between plasma caffeine and thermogenic response [8]. To cover the global effect of treatments on EE, it would have been

interesting to extend the measurement until energy expenditure return to baseline values. However, this would have probably required more than three hours, since neither Acheson *et al*, Astrup *et al* nor Dulloo *et al* could return to baseline after 3h measurements following administration of caffeine in a dose of 8 mg/kg body weight [3], or of 100 mg [8,26].

Based on results from the present study and from the available literature, it seems reasonable to attest that carbonation by itself failed to enhanced caffeine and catechins-induced increase in energy expenditure. It can also be concluded that one serving of beverage significantly increased 120 min-EE by 6 kcal.

5.3 Substrate oxidation

The still treatment induced significant changes from baseline, towards increase in fat and a decrease in carbohydrate (17.2 kcal and 13.3 kcal, respectively, over 120 min) oxidation, which is in line with literature [2,3]. Indeed, Acheson *et al* reported an increase in change in fat oxidation following 8 mg/kg caffeine ingestion compared to placebo (fat oxidation was increased by 33 mg/min with caffeine, whereas it was only 16 mg/min with placebo, compared to respective baselines) [3]. Moreover, in a study by Acheson *et al*, ingestion of caffeine (10 mg/kg) triggered higher lipid oxidation than baseline [2].

If carbonation would enhance the effects of caffeine on substrate oxidation, an increase in fat and a decrease in carbohydrate oxidation would be expected with CO_2 added recipe compared to sin-gas treatment, as described above [2,3,38]. In contrast with the above mentioned studies, our results presented an inverse pattern. This indicates that either the higher volume of the still product has impacted substrate utilization, or that carbonation itself has an effect on substrate oxidation.

Increased content of active ingredients in still treatment may have triggered a higher impact on substrate oxidation. Catechins may trigger substrate oxidation towards fat rather than carbohydrate oxidation. Indeed, Dulloo *et al* reported lower RQ due to ingestion of 375 mg catechins and 150 mg caffeine, compared to same dose of caffeine alone [25]. However, some studies also failed to demonstrate this effect of catechins on substrate oxidation [9-11,31,63,66]. Indeed, Gregersen *et al* could observe no significant effect of GTE (150 mg caffeine and 493.8 - 684.0 mg catechins) on RQ over 12.5h compared to placebo [31]. Moreover, Thielecke *et al* did not observe a difference in fat oxidation between EGCG-caffeine mixtures (per day: caffeine fixed dose of 600 mg plus either 270, 600, 900 or 1200 mg EGCG) and 200 mg caffeine alone [66]. Thus, the increase in catechin content in still compared to carbonated treatment could be an explanation of the inverted substrate oxidation pattern observed. However, this hypothesis is less plausible when one considers the small increase in catechins ingested and the conflicting literature.

Another more plausible explanation in the context of this study is that drinking carbonated beverage may have lead to non-physiological CO_2 appearance in exhaled gas, invalidating CO_2 concentration measurements. Higher $NPVCO_2$ leads to greater carbohydrate and lower fat calculated oxidations. Thus, the pattern observed following carbonated treatment ingestion could be explained by the treatment's CO_2 exhaled by the participants in the canopy, between two sips during treatment ingestion or afterwards due to gastric reflux. Similarly, this non-physiological CO_2 may have also affected carbonated treatment's-induced energy expenditure and also explain why compared to baseline, carbonated treatment failed to significantly affect substrate oxidation oxidations but showed a strong trend towards increased carbohydrate and lower fat oxidations compared to pre-ingestion values.

To evaluate the impact of the non-physiological presence of CO_2, Fig. 13 and Fig. 14 show the evolutions of O_2 consumption and CO_2 production rates. Comparing these two figures, it appears that CO_2 production at least during the first 40 min, reflects non-physiological values because this increase is not followed by O_2 consumption.
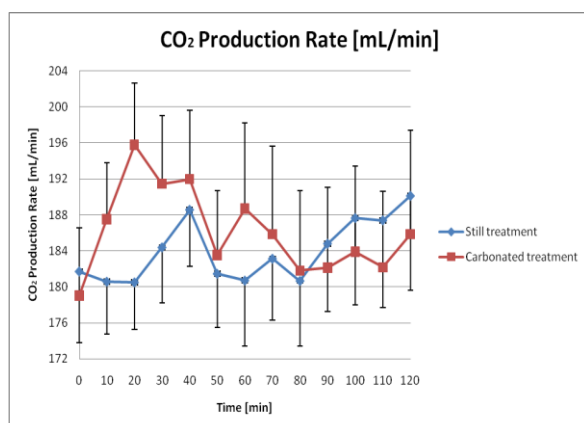
Figure 13 Rate of CO_2 production over time, for both treatments, n = 12
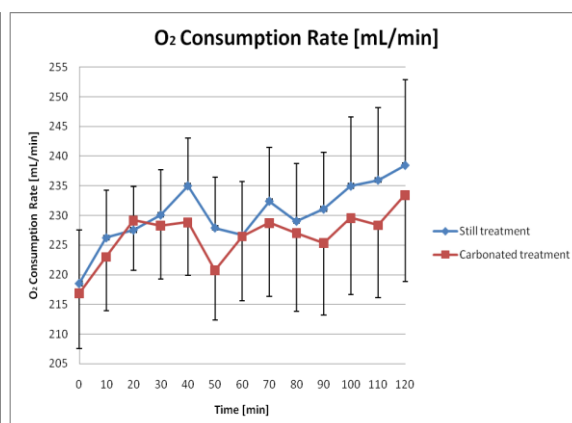
Figure 14 Rate of O_2 consumption over time, for both treatments, n = 12

Although it could have been an interesting information, it was not possible to subtract beverage CO_2 content to VCO_2, due to the fact that it is not known how much CO_2 was rejected during breathing, and how much stayed into the gastro-intestinal tract since gastric partial O_2 and CO_2 pressures were not monitored.

Therefore, changes in substrate oxidation observed in this study could be influenced by the non-physiological CO_2. Indeed, VCO_2 is multiplied by a factor 1.106 in EE equation (Eq. 1), such that an increase in VCO_2 would influence energy expenditure in a lower extend than $NPVO_2$ which is has a factor of 1.69 for fat oxidation (Eq. 7) and 4.12 for carbohydrate oxidation (Eq. 8). This could explain why carbohydrate oxidation is increased of 21.8 mg/min and fat oxidation decreased of 10.2 mg/min with still compared to carbonated treatment, whereas EE does not seem to be widely affected.

The increased carbohydrate oxidation and decreased fat oxidation triggered by carbonated treatment compared to still (by 21.8 mg/min and 10.2 mg/min, respectively) correspond respectively to 10.93 kcal and 11.55 kcal over the 120 minutes of measurements, according to conversion factors of Livesey and Elia [46]. The resulting difference of energy is then 0.62 kcal, which is negligible on a 120 minutes test. Therefore, even if substrate oxidation was impacted differently by both beverages, this failed to differently influence energy expenditure.

Regarding this artefact, carbonated treatment may not have triggered physiological substrate oxidation values and as a consequence, comparisons between beverages may not be accurate. However, ingestion of still treatment triggered real changes in substrate oxidation, which do reflect physiological variations, and indicate greater fat and lower carbohydrate oxidation.

5.4   Heart Rate

The non-significant effect of still treatment on heart rate compared to baseline, and the non-significant differences comparing both products, were expected, based on the important doses of caffeine that need to be ingested to influence this parameter. Indeed, 400 mg of caffeine failed to increase significantly heart rate, from baseline in the study of Astrup *et al* [8]. Moreover, 300 mg caffeine did not affect significantly heart rate, compared to placebo in the study of Rudelle *et al* [63]. Acheson *et al* could not observe an effect of 10 mg/kg caffeine on heart rate, compared to baseline [2]. It was therefore expected that a caffeine intake of 100 mg would not modify heart rate significantly.

Both products present the same non-significant trend towards an initial decrease of heart rate after test beverage ingestion, and a further regain. This pattern was already reported in

literature [8,61]: Astrup *et al* noted that after 100, 200 or 400 mg caffeine intake, heart rate dropped by 3-4 bpm from 30 to 90 min post-ingestion, and raised afterwards (from 90 to 180 min post-ingestion). Anticipation, which is known to increase importantly resting heart rate [50], could explain this pattern. Indeed, subjects may be anxious before and just after treatment ingestion, relaxed at mid-test, and willing the test to end towards the end of measurements.

Carbonated treatment decreased significantly heart rate by 3.67 bpm, compared to baseline ($p = 0.030$). This is against the hypothesis that increased caffeine absorption increase heart rate. Thus, to the best of our knowledge, this result remains unexplained.

6 Conclusion

The main goal of this study conducted as part of the Master thesis, was to assess the difference between the effects of two products, one carbonated and one still, containing similar amounts of caffeine and green tea catechins, on energy expenditure and substrate oxidation.

The results of the present study suggest an increased caffeine absorption pattern over 2h due to CO_2 addition in beverage formulation, which is in line with literature. Despite the increased caffeine absorption, energy expenditure was not increased by carbonation. This is probably mainly attributable to the fact that for a similar dose of caffeine, the small increase in caffeine absorption is not sufficient to enhance its effect on energy expenditure. Ingestion of one serving of either treatment leads to a 6 kcal increase in energy expenditure over 120 min. Still treatment consumption shifted substrate oxidation towards fat burning and decreased the contribution of carbohydrate as a body fuel, as expected. Finally, ingestion of carbonated treatment decreased heart rate by 3.67 bpm.

The results of this study will need to be reproduced to confirm our conclusions, since it was impossible to discard from the analysis the potential CO_2 coming from the carbonated beverage, that may have interfered with gas exchange measurements during the ingestion of the carbonated treatment. It is also possible that absorption of the other active ingredient of the treatments (catechins and calcium) was affected by carbonation, or that their contents were increased together with the increase in still ingested volume.

A new design would be required to strengthen the present results. For instance, a carbonated water control could be added, to subtract the non-physiological CO_2 from indirect calorimetry measurements. Another solution to solve the problem related to CO_2 measurement would be to test subjects with direct calorimetry. However, that would not allow insights on substrate oxidation and was not possible in the context of the present study since no time was available to identify an external lab where direct calorimetry measurements could have been preformed. Another improvement would be to measure caffeine concentration in plasma, rather than in saliva as it was done in the present study due to the absence of available nurse. This would avoid the artifact in salivary caffeine concentration coming from the impossibility to rinse the mouth during indirect calorimetry using a canopy. Finally, it is clear that the content of each active ingredient of next study's treatments would be analyzed prior to the beginning of the study, to avoid any volume confounding factor. Although all the solutions listed above were explored and discussed during study planning, they could not be implemented due to limited resources and time for this project.

A full understanding of the role of CO_2 addition on substrate absorption would require to run cellular and tissular scale studies. Indeed, histology, pharmacology, gastroenterology and fluid dynamics could help develop a more holistic comprehension of CO_2's roles on different ingredients absorption.

This study confirmed the effect of still beverage on energy expenditure and substrate oxidation, reinforcing its position as a thermogenic beverage. It confirmed that CO_2 enhance the absorption of some ingredients, caffeine in the present context. But to draw firm conclusions on the other results, a new design would be required.

7 Annexes

A: Subjects, Material and Methods

B: Data and Results

C: Dossier santé

8 Reference List

[1] Panel on macronutrients, panel on the definition of dietary fibers, subcommittee on upper reference levels of nutrients, subcommittee on interpretation and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes, food and nutrition board, and institute of medicine of the national academies. dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients) (National Academies Press, 2005).

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Annex A:

Subjects, Materials and Methods

I. Protocole

The protocol and informed consent were approved by an Independent Ethics Committee, the "Commission d'Ethique du Centre Hospitalier Universitaire Vaudois (CHUV)". The trial was conducted according to the relevant legal and operational requirements (Good Clinical Practices, Standard Operating Procedures).

1. Study design

This study is a single center, randomized, single-blind, and controlled, cross-over clinical trial of two groups. It is aimed at testing the acute effect of carbonation into a caffeinated beverage on energy expenditure, substrate oxidation, caffeine absorption and heart rate.

1.1 Inclusion and exclusion criteria

The subjects had to comply with the following main inclusion criteria: age between 20 and 50 years old, healthy, men and women, BMI between 20 and 27 kg/m^2. After having been informed of the study details, subjects had to be freely willing to participate to the study.

The main exclusion criteria were smoking, history of diseases that could affect intestinal absorption or resting metabolic rate, intake of medication that could influence these parameters. Frequent practice of physical activity (> 300 min of moderate or intense exercise per week), pregnancy and intake of more than 5 cups of caffeine containing beverages per day were also judged as exclusion criteria.

1.2 Study population

Healthy Caucasian men and women matching inclusion criteria were recruited from April to May 2010, at the Nestlé Research Center in Lausanne, Switzerland. Among 22 volunteers screened, 12 were enrolled into the trial.

2. Product allocation

Subjects were assigned to each of the 2 products randomly in a cross-over order and with sex as a stratification factor.

2.1 Product description

Test product 1 (Carbonated treatment): is a water based beverage containing natural citrus flavors, artificial sweeteners (aspartame, acesulfame K). One serving is 355 mL steel can. This serving contains the following active ingredients: 899 mg green tea extract (GTFT-X) of which 122 mg are epigallocatechin gallate, 195 mg of calcium, 100 mg caffeine (of which 35 mg is from the green tea extract and 65 mg from added caffeine) and 6g CO_2.

Test product 2 (Still treatment): is exactly the same recipe than product one, but it does **not contain CO_2**, and it is served in a 330 PET bottle.

Characteristics of study treatments	Carbonated 1 serving = ingested dose	Still 1 serving	Still Ingested dose
Energy (kcal) *	< 10	< 10	< 12
Green tea extract (mg) *	899	899	1364
Total catechins (mg)*	230	230	349
EGCG (mg) *	122	122	182
Caffeine (mg) **	106.5	66	100
Calcium (mg) *	195	195	295
Carbohydrate (mg) *	0	0	0
Fat (mg) *	0	0	0
CO_2 (g) ***	6	0	0
Serving size (mL)	355	330	500

Table 1A Energy content and major ingredients in study treatments
Symbols: * According to recipe ; ** Measured by Nestlé's Regional Laboratory;
*** Measured by Nestlé's Product and Technology Center Marysville

2.2 Product distribution

Products were stored at 4°C. Immediately before presenting them to the subjects, test beverages were poured into a glass. Test beverages were drunk in less than 5 min, with a straw put through the canopy.

For quality reason, caffeine content of both beverages was assessed by the Regional Laboratory of Nestlé in Orbe. Unexpectedly, although the sparkling drink contained the indicated dose (100 mg), the still beverage contained only 66 mg. To correct this defect and insure a caffeine intake of 100 mg for each test beverages, a dose of 500 mL of still beverage was administered to the subjects, whereas the dose of carbonated drink remained 355mL. As shown in Table 1A, volunteers ingested the same dose of caffeine, even though the volumes were non identical. Catechin content of both products was not measured, and therefore the potential greater catechin intake due to volume addition is not known.

3. Data collection, management and validation

3.1 Data collection

Participants were recruited internally, amongst NRC employees. The persons who responded to the recruitment add were first asked to fill-in the medical questionnaire. This was followed by a standard medical evaluation performed by the research Medical Doctor at NRC who ensured volunteer fulfilled the eligibility criteria. Decision to enroll or not subjects was taken afterwards. During their first visit, at V_0, subjects received information on the study content and planning and were informed on the tests to be performed. They were shown the indirect calorimetry measurement setup, to allow them to familiarize with the canopy system. At the end of V_0, they were given the informed consent with the instruction to return the form signed at the latest at V_1 in order to be enrolled into the trial.

At the next visit, V_1, the subjects were welcomed at the Metabolic Unit, asked to fulfill the compliance questionnaire, weighted by medical staff. Subjects were then comfortably installed in semi-recumbent position in a reclining bed, wearing a ~ 15L Plexiglas canopy. After a stabilization period of ~ 5 min, resting metabolic rate was measured for 15 minutes, as shown in table 2A.

Volunteers were then asked to ingest the randomly assigned treatment beverage contained in a glass within 5 min with a straw put through the ventilated canopy. During beverage ingestion, energy expenditure measurements were stopped. The energy expenditure measurement (postprandial phase) continued for another 120 min as shown in table 2A.

Heart rate was recorded by using a portable device on the chest of the participants during all the energy expenditure measurements (pre- and post-prandial phases), see table 2A.

Immediately before drinking the test product, and at time 15, 30, 45, 60, and 120 minutes after ingestion of the test beverages, volunteers were asked to chew cottons wool swabs for the harvesting of saliva. This is, schematized in table 2A.

After 120 minutes post-ingestion, measurement were terminated, canopy removed from over participants head, and participants were invited to go to the adjacent dining room where they were offered a breakfast.

Parameter	Stabilisation	Pre-ingestion	Ingestion		Post-ingestion				
Time (min)	-20	-5	0	15	30	60	90	//	120
Salivette		S0		S1	S2	S3	S4		S5
EE Sub. Ox. Heart rate	No measurement	15 min measurement	No measurement		120 min measurement				

Table 2A Phases of measurement, timing and saliva sampling

V_2 was identical to V_1, but the test product was given in a crossover manner.

Over all, the clinical testing lasted 6 weeks.

3.2 Procedures:

In order to eliminate as many confounding factors as possible, the subjects were tested after a 10-h over-night fast. They were asked not to consume food (except water) after 22h00 on the evening before V_1 and V_2. Moreover, consumption of caffeine was not permitted after the evening meal before V_1 and V_2, to allow for clearance of caffeine which has a half-life reported to last from 4.9 ± 1.8 [58] to 10 hours [55,61,62], depending on gender and use of oral contraceptives.

On the test days, volunteers were requested to use their car or public transportation to come to NRC in order to keep morning physical activity level as low as possible.

During the tests, participants were asked not to fall asleep, to move and speak as less as possible, but they could watch television.

Between the visits, they were asked to continue on their usual diet and physical activities.

As menstrual stage has been shown to influence energy expenditure [20], women were tested only in the follicular phase of their cycle.

3.3 Sample size:

Since the literature on the influence of carbonation on the effects of caffeine is very limited, it was not possible, to do any power calculation to evaluate the number of subjects to enroll into the trial. Since this study was considered a pilot trial it was arbitrarily decided to test 15 participants, with an estimated dropout rate of 20 %. That is 12 participants needed to complete the study.

Participants recruited at the Nestlé Research Center are known to be relatively compliant. Moreover, the trial did not have many constraints, which could be challenging for study participants and increase risk of drop outs. Therefore, it was chosen to do the analyses as

per-protocol but not as intention-to-treat. It was planned that subjects withdrawing from the study would not be treated as per protocol.

3.4 Data management and quality aspects

All the data captured by the investigator were directly recorded on the paper case report forms. Data entry was done at NRC into the Clintrial® database with double entry.

Height and weight measurements, as well as saliva sampling were assessed by health professionals, following the appropriate SOPs. A control of eventual caffeine ingestion before the tests was done by measuring the caffeine concentration in saliva prior to beverage ingestion. Research assistants were present during the entity of indirect calorimetry tests, as recommended in the relevant SOP.

Energy expenditure results were provided electronically (Excel files) to data manager after a manual selection of interesting variables. More precisely, minutes of test beverages ingestion and saliva sampling were not considered in the statistical analysis.

The caffeine content of the test beverages was analyzed by the Regional Laboratory, with a certified method.

Caffeine concentration in saliva was dosed in duplicates, under supervision of a metabonomics expert, using a new UPLC-ESI-MS/MS method.

All data were processed by the data management group, and the statistics were performed by a qualified statistician.

II. Techniques and measurements

1. Energy expenditure measurements

The measurements were performed with the MAX-II device provided by AEI technologies, Naperville, IL, USA. The sampling rate of the MAX-II machine is 2 averaged samples per minute. EE measurements were obtained directly from the MAX-II device, which uses the equations of Weir [67], see Eq. 1.

Resting: At the beginning of the measurements, values are unstable due to stabilization of gas concentrations in the mixing chamber and subject habituation to the conditions of the environment. These values were not taken into consideration and the recording of data begun after this 5-10 min stabilization period (see table. 2A), when the RQ and the fraction of expired CO_2 ($FeCO_2$) became stable.

A recording of resting energy expenditure of 15 min was then performed.

After this base line measurement, volunteers were given the test product. The measures during ingestion were discarded.

Postprandial: Post-ingestion energy expenditure of the volunteers was monitored for 120 min.

2. Substrate oxidation:

Carbohydrates and fat utilization as well as RQ and NPRQ were calculated using the VO_2 and VCO_2 values obtained from the MAX-II device during the same timeline than energy expenditure.

As protein oxidation represents a minimal part of whole oxidation in a normal situation (no prolonged starvation, no diabetes, no acidosis) [46], protein oxidation was not measured in the present study, but it was estimated from the weight of the subjects, see Eq.3.

Equations used by statisticians to derive substrate oxidation and respiratory quotients were the equations mentioned in introduction [46] (Eq. 8, Eq. 7, Eq. 2 and Eq.6).

3. Biological samples:

<u>Sample collection:</u>

Saliva samples were collected with Sarstedt Salivette tissues without citric acid provided by SARSTEDT AG & Co, Nümbrecht, Germany.

The sampling was done by passing the Starstedt Salivette to the mouth of the participant with a forceps into a hole in the canopy, let the subject turn it into his mouth for 2 minutes, and collect the cotton wool swab via the same hole in the canopy.

As soon as they were collected, the Starstedt Salivette cottons were centrifuged at 3500 rpm during 10 minutes. The collected saliva (around 1mL) was then separated into two ependorf tubes, and frozen at (-80°C).

<u>Analysis of the caffeine content of the saliva samples:</u>

A technique using UPLC-ESI-MS/MS was developed in the scope of this study to analyze salivary caffeine content. It was an improvement in the field, because this technique allowed an analysis of caffeine content in saliva without any pre-treatment of the samples within 10 min. Further information about this method is available in Annex Aa.

Caffeine was quantified by ultra pressure liquid chromatography coupled to electrospray ionization tandem mass spectrometry (UPLC- ESI-MS/MS) on a turbulent flow chromatography system (TLX1 from ThermoFisher) coupled to a 3200 Q TRAP mass spectrometer (AB Sciex). Chromatography separation was realized on a BEH C18 reverse phase column (2.1 x 100 mm, 1.7 μm, Waters Corp.).

MS/MS parameters were optimized to monitor two main caffeine and $3C_{13}$-labelled caffeine transitions reactions. They are, for caffeine: m/z 195→138 (collision energy: 25 eV) and m/z 195→110 (collision energy: 30 eV); and for $3C_{13}$-labelled caffeine (internal standard) transitions: m/z 198→140 (collision energy: 25 eV) and m/z 198→112 (collision energy 30 eV).

Parameter [unit]	Value
Source temperature [°C]	400
Entrance potential [V]	10
Cell exit potential [V]	10
Pressure of the collision gas (nitrogen) [Torr]	5×10^{-3}
Curtain gas (nitrogen) [arbitrary unit]	20
Ion spray voltage [V]	3000
Nebuliser gas (nitrogen), Ion source gas 1 [V]	30
Nebuliser gas (nitrogen) , Ion source gas 2 [V]	20
Declustering potential [V]	51

Table 3A: Optimization of the ESI-MS/MS parameters for quantification of salivary caffeine content

Linearity of calibration through a calibration panel ranging from 0 to 75 μg/mL caffeine was demonstrated. Calibration stability over time was challenged with the same calibrant solutions 10 days apart. The stability over time was necessary to address, because the same calibrant solutions were used on the 3 days of the tests.

During the testing, a calibration curve was derived every 20 test samples to ensure the quality of the data. These calibrations are superimposed to show their reproducibility. The calibration curve was linear over the saliva caffeine concentrations measured (below 2 μg/mL) and calibrations were stable in this range.

Once the method was fully functional, all the samples were defrosted (10h at 5°C). For every sample, 300 μL of saliva was added to 10 μL of $^{13}C_3$-caffeine (100 μg/mL solubilised in water) in a vial. The 288 resulting saliva samples were then injected into the LC-MS instrument, and the whole analysis was realized within 3 days.

4. Heart rate:

Heart rate was measured by using a polar heart rate transmitter (FS1 Polar Electro Europe BV). This device is made of a chest-belt recording heart rate, which emits to an antenna connected to a computer. Heart rate was recorded simultaneously to energy expenditure.

III. **Statistical treatment**

To test the difference of effects on energy expenditure (kcal/min), substrate oxidation (mg/min and no unit) and heart rate (bpm) between the two products, a mixed effects model was used. The model accounted for product (Carbonated or still treatment) and baseline (REE, resting fat and carbohydrate oxidation (mg/min), pre-ingestion RQ and NPRQ (no unit), and resting heart rate respectively) as fixed effects and subject ID as a random effect.

To reinforce the result for the primary outcome, a supportive analysis was performed on energy expenditure: the median of measurements over 5 periods of 24 minutes was calculated and a repeated measures analysis was applied on this data.

In order to asses if there is a beverage effect (change score of energy expenditure, substrate oxidation, RQ, NPRQ and heart rate) post-ingestion compared to resting values were analyzed by a one sample t-test. Statistics were performed by subtracting pre-ingestion median to post-ingestion median for each subject. These differences were then averaged over all the subjects. Therefore, the graphics display means (all the subjects), but both means and medians are shown in the tables.

Finally, the effect of both products on C_{max} (µg/mL) and AUC (µg.min/mL) of caffeine concentration in saliva were compared by a paired t-test. The difference in time to reach maximal concentration (min) was addressed by applying a log-rank test.

The data were not cleaned prior to statistical treatment, but the periods in which the participant drank the treatment, chew the salivettes, spoke or went to the toilets were discarded and not taken into account in the statistics.

The beverage codes were broken after the protocol and intention-to-treat data sets had been defined and the primary outcome had been analyzed. The codes were: A, Still treatment; and B, Carbonated study treatment.

For the statistical analysis, software R version 9.2.9 (Lucent Technologies) was used.

P value < 0.05 is considered to be significant.

1. Energy expenditure

The primary outcome is the change, between treatments, in the median of post-ingestion energy expenditure PIEE (kcal/min) to resting energy expenditure REE (kcal/min), averaged on all subjects.

A secondary outcome is the treatment effect, compared to baseline: PIEE-REE for each product (kcal/min).

2. Substrate oxidation

A secondary outcome is the change, between treatments, in the median of post-ingestion carbohydrate oxidation (mg/min) to pre-ingestion carbohydrate oxidation (mg/min), averaged on all subjects.

Another secondary outcome is the treatment effect, compared to baseline: the median of post-ingestion carbohydrate oxidation (mg/min) - pre-ingestion carbohydrate oxidation (mg/min) for each treatment, averaged on all subjects.

Fat oxidation was analyzed as for carbohydrate utilization.

Change in RQ (post-ingestion RQ (no unit) - pre-ingestion RQ (no unit)) was also analyzed for each treatment, as an additional insight in substrate oxidation. The treatment effect itself was also triggered, compared to baseline.

NPRQ was addressed identically as RQ.

3. Caffeine absorption

A secondary outcome is the difference, between treatments, in maximum concentration (C_{max} (µg/mL)) and time to reach this concentration (T_{max} (min)) as well as AUC (min.µg/mL) of caffeine concentration in saliva in post-ingestion period compared to resting period.

4. Heart rate

Another secondary outcome is the change, between treatments, in heart rate (bpm) in post-ingestion period compared to resting period.

Additional secondary outcome is the treatment effect, compared to baseline: the median of post-ingestion heart rate (bpm) - pre-ingestion heart rate (bpm) for each product, averaged on all subjects.

Annex Aa:

UPLC method to assess salivary caffeine concentration

Quantitative analysis of caffeine content from saliva samples:

Caffeine was quantified by ultra pressure liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) on a turbulent flow chromatography system (TLX1 from ThermoFisher) coupled to a 3200 Q TRAP mass spectrometer (ABSciex). Each saliva sample was analyzed in duplicates. Chromatography separation was realized on a BEH C18 reverse phase column (2.1 x 100 mm, 1.7 µm. Waters Corp.). LC solvents used were: A) water containing 0.05% acetic acid and B) acetonitrile containing 0.05% acetic acid. A linear gradient was used as the following: 0 min (

A 5 µL injection was achieved from the saliva samples. MS source parameters were optimized as the following: spray voltage: 3000 V, source temperature: 400°C, nebuliser gases (nitrogen): GS1: 30 and GS2: 20, curtain gas (nitrogen): 20, declustering potential: 51 V, collision gas (nitrogen) 5 mTorr, entrance and collision cell exit potentials: EP: 10 and CXP: 10 V. Data acquisition was realized in positive electrospray ionization mode using selected reaction monitoring (SRM) by following the transition reactions of m/z 195→138 and 195→110 (respective collision energies of 25 and 30 eV) for caffeine and m/z 198→140 and 198→112 (respective collision energies of 25 and 30 eV). The dwell time was fixed at 20 msec for each transition with an inter-scan delay of 5 msec.

Quantification of caffeine was obtained by plotting the area ratio of the highest SRM signal of caffeine *versus* $^{13}C_3$-caffeine against concentration (m/z 195→110 / 198→112). A 5-data point was used to build the calibration curve, ranging from 0-75 µg/mL of caffeine (100 µg/mL of $^{13}C_3$-caffeine). Standard solutions, solubilised in water, were injected every day to ensure the consistency of the results over time (days). Quantification was realized using Analyst software (version 1.5, AB Sciex).

Another SRM transition was used as qualifier to ensure the integrity of peak integration for both caffeine and its isotopically labelled internal standard. As example, the area ratio of caffeine (m/z 195→140 / 195→110) and $^{13}C_3$-caffeine (m/z 198→140 / 198→112) have to be constant independently of the analyte concentration.

Batch sequence:

Typically, calibrant samples were injected at the beginning and end of each batch, followed by a blank sample. After 20 saliva samples, 2 calibrant standards were injected as quality control samples to check the absence of analytical drift.

Results & Discussion

Optimisation of UPLC-MS/MS conditions

Caffeine and $^{13}C_3$-caffeine standard solutions were infused into the MS and source parameters were optimised to provide the best signal response in positive ionization full scan mode. Once these parameters were set, collision induced dissociation experiments were realized by selecting the protonated species under various collision energies. **Figure 1Aa** depicts the typical CID mass spectra obtained for caffeine and $^{13}C_3$-caffeine.
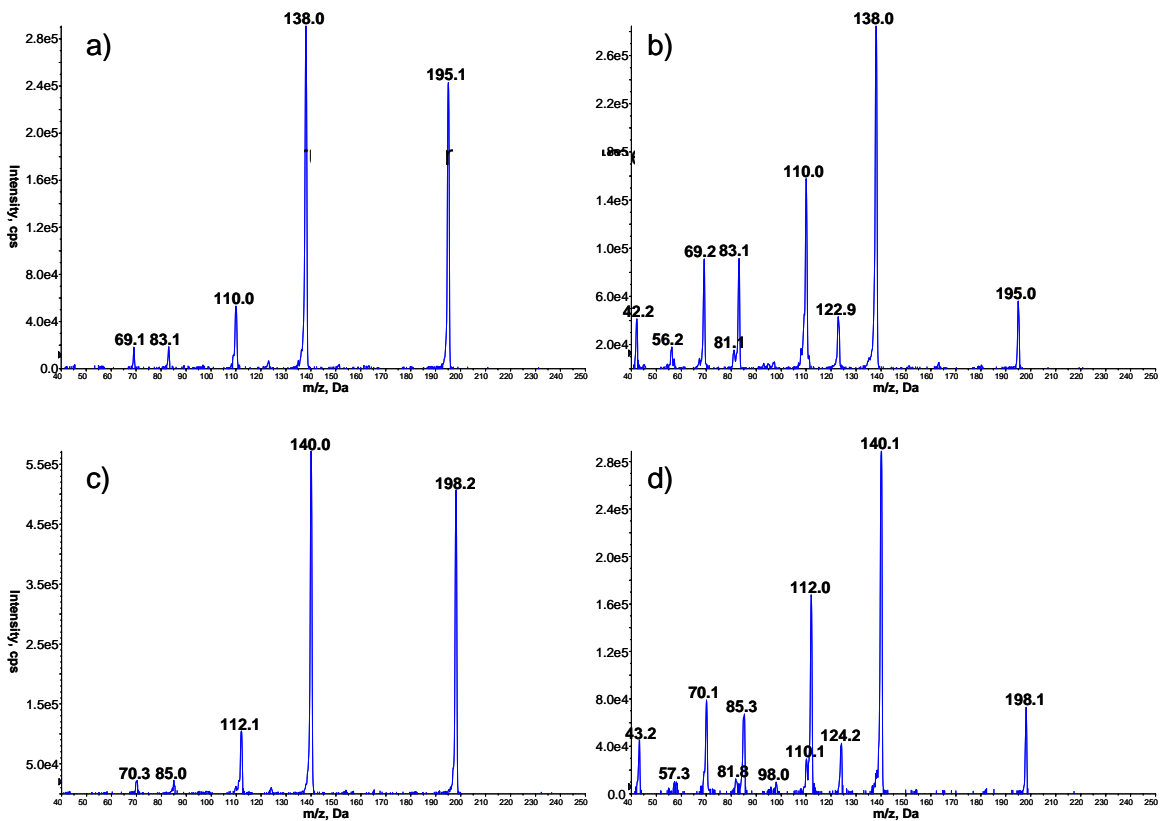
Figure 15Aa: Collision induced dissociation of caffeine (a & b) and $^{13}C_3$-caffeine (c & d). a and c were acquired at a collision energy of 25 eV, whereas b & d at 35 eV.

Analysis of saliva samples

In order to provide a high throughput of analysis, saliva sample submitted to a centrifugation step was spiked with the isotopically internal standard in an LC vial and directly analyzed by UPLC-MS/MS. These preliminary experiment trials were done to assess if removing the clean-up step before analysis was possible or not. The various SRM traces did not revealed co-eluting peaks (Figure 2Ab).

Moreover, the peaks of caffeine were strong enough and did not reveal any major ion suppression issues, which are sometimes encountered using LC-MS/MS. Therefore, all the saliva samples were processed under similar conditions.
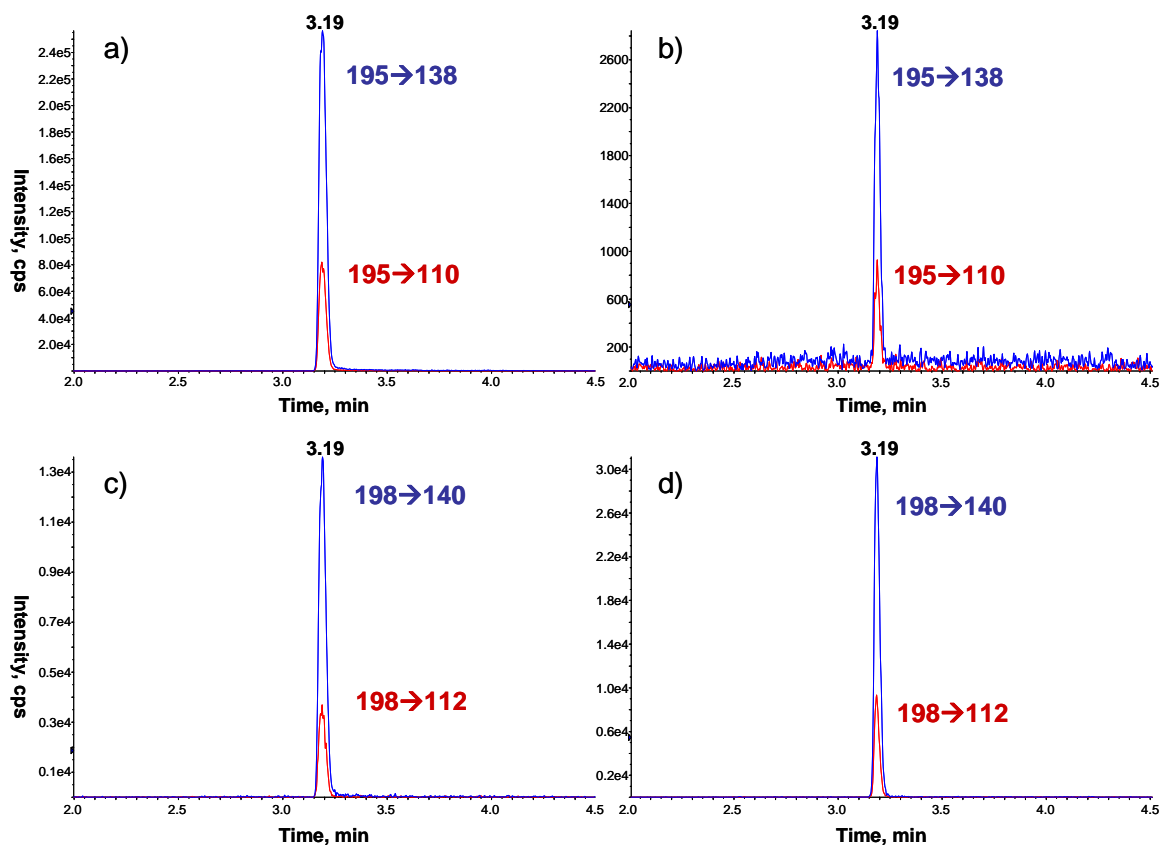

Figure 16Ab: SRM transitions observed for calibrant (a-c) and saliva sample (b & d). a & b depict the caffeine traces whereas c & d its corresponding internal standard.

Quantification of caffeine

As initial trial, a broad range of caffeine concentration was used to build the calibration curve (ranging from 0-75 µg/mL). A good linearity was obtained from 0-25 µg/mL, which was ideal for the caffeine present in the saliva samples.

Once the method was fully functional, all the samples were defrosted (10h at 5°C). For every sample, 300 µL of saliva was added to 10 µL of $^{13}C_3$-caffeine (100 µg/mL solubilised in water) in a vial. The 288 resulting saliva samples were then injected into the LC-MS instrument, and the whole analysis was realized within 3 days.

Annex B:

Data and Results

The characteristics of energy expenditure, substrate oxidation, caffeine concentration and heart rate measurements during pre- and post-ingestion periods for both treatments are presented in the following tables.

1. Energy expenditure

Energy expenditure (kcal/min)					
Product	Period	Median	Mean	St. dev.	Range
	Pre	1.03	1.07	0.16	0.83 – 1.52
Still treatment	Post	1.08	1.12	0.17	0.73 – 2.05
	Pre	1.02	1.06	0.16	0.72 – 1.71
Carbonated treatment	Post	1.06	1.11	0.20	0.80 – 2.65

Table 1B: Energy expenditure, n = 12

2. Substrate oxidation (fat and carbohydrate oxidations, RQ and NPRQ)

Fat oxidation (mg/min)					
Product	Period	Median	Mean	St. dev.	Range
Still treatment	Pre	56.6	52.9	19.7	-4.6 – 93.9
	Post	66.4	68.5	19.8	-1.2 – 198.6
Carbonated treatment	Pre	57.8	54.4	19.4	10.3 – 109.1
	Post	56.7	58.6	25.4	-14.6 – 244.7

Table 2B: Fat oxidation, n = 12

Carbohydrate oxidation (mg/min)					
Product	Period	Median	Mean	St. dev.	Range
Still treatment	Pre	88.3	92.2	46.4	17.4 – 241.4
	Post	64.8	67.7	33.8	-55.3 – 269.0
Carbonated treatment	Pre	84.1	84.9	37.5	12.6 – 215.7
	Post	82.5	88.4	45.9	-136.1 – 354.3

Table 3B: Carbohydrate oxidation, n = 12

On top of these measurements presented in the thesis, we have measured respiratory quotient and non-protein respiratory quotient.

Respiratory quotient (no unit)

Product	Period	Median	Mean	St. dev.	Range
Still treatment	Pre	0.83	0.83	0.05	0.75 – 0.98
	Post	0.80	0.80	0.03	0.68 – 0.97
Carbonated treatment	Pre	0.82	0.83	0.04	0.75 – 0.93
	Post	0.82	0.82	0.05	0.64 – 1.01

Table 4B: Respiratory quotient, n = 12

Non-protein respiratory quotient (no unit)

Product	Period	Median	Mean	St. dev.	Range
Still treatment	Pre	0.83	0.83	0.06	0.73 – 1.02
	Post	0.79	0.79	0.04	0.67 – 1.01
Carbonated treatment	Pre	0.82	0.82	0.05	0.73 – 0.95
	Post	0.82	0.82	0.06	0.62 – 1.05

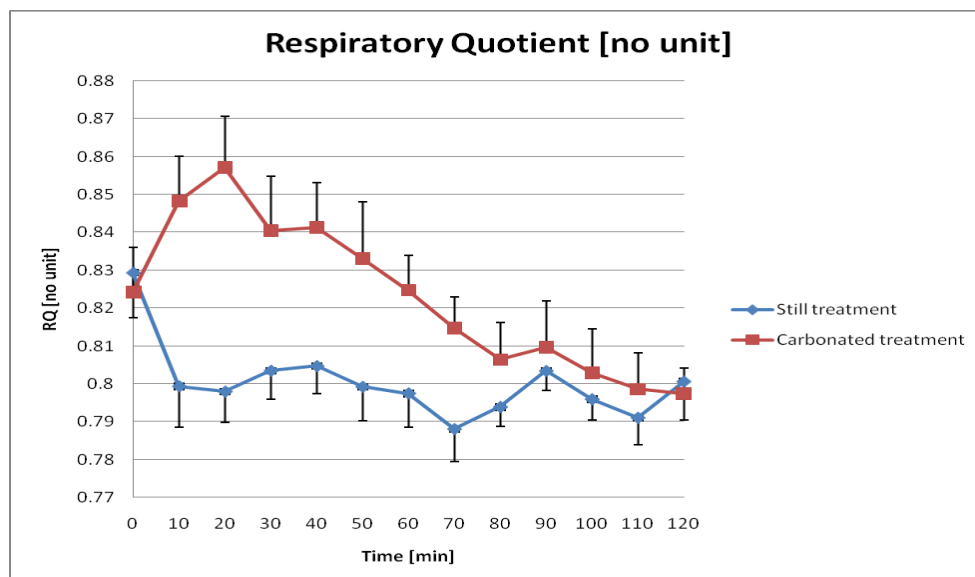Table 5B: Non-protein respiratory quotient, n = 12
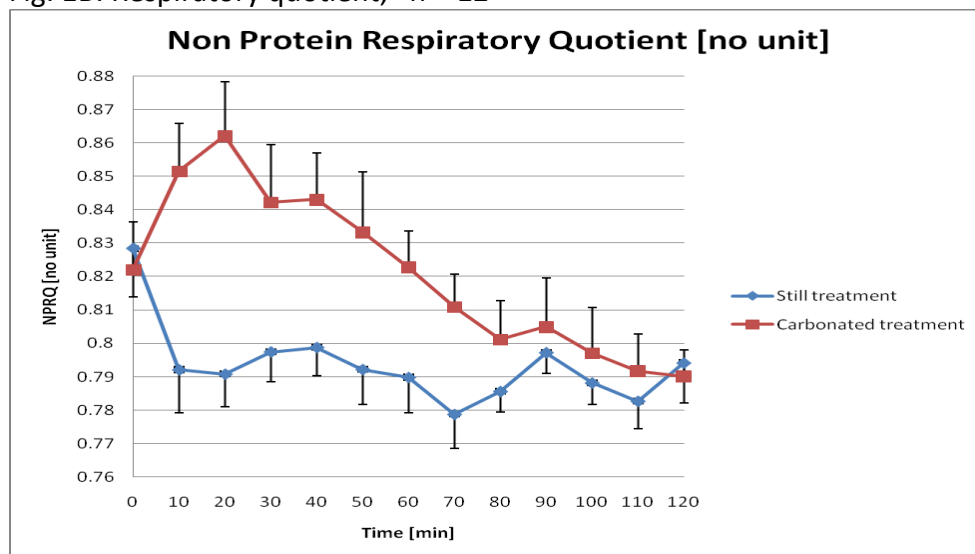

Fig. 1B: Respiratory quotient,   n = 12

Fig. 2B: Non-protein respiratory quotient, n = 12

Consistent with their effects on substrate oxidation, the treatments affected RQ and NPRQ differently. Indeed, compared to baseline, still treatment significantly decrease RQ and NPRQ (by 0.03, p = 0.006 and 0.04, p = 0.007, respectively). Compared to baseline, carbonated treatment did not significantly affect RQ and NPRQ (p = 0.576 and p = 0.611, respectively). Comparing both products, carbonated treatment significantly increased RQ (0.02, p = 0.003) and NPRQ (0.03, p = 0.003).

3. Salivary caffeine kinetics

Caffeine concentration: AUC (min.ug/ml), C_{max}(ug/ml), T_{max}(min)					
Product	Measure	Median	Mean	St. Dev.	Range
Still treatment	AUC	135.1	137.8	14.5	116.2 – 160.1
	C_{max}	1.38	1.45	0.30	1.09 – 2.10
	T_{max}	15.0	27.5	30.6	15.0 – 120.0
Carbonated treatment	AUC	144.9	147.9	17.1	129.5 – 185.2
	C_{max}	1.57	1.65	0.33	1.18 – 2.23
	T_{max}	15.0	25.0	16.1	15.0 – 60.0

Table 6B: Caffeine concentration in saliva, n = 12

4. Heart rate

Heart rate (bpm)					
Product	Period	Median	Mean	St. dev.	Range
Still treatment	Pre	64.0	66.2	13.6	49.0 – 153.0
	Post	63.0	66.9	18.0	42.0 – 175.0
Carbonated treatment	Pre	64.0	64.7	8.5	46.0 – 92.0
	Post	61.0	61.7	9.2	41.0 – 114.0

Table 7B: Heart rate, n = 12

Annex C :

Dossier Santé

Tout d'abord, je tenais à vous remercier chaleureusement pour votre participation au projet. Le but du présent dossier est de vous communiquer les résultats des examens que vous avez effectués dans le cadre de cette étude: dépense énergétique et fréquence cardiaque. Vous serez invité au group meeting de « Energy and Metabolic Health » où je présenterai les résultats de l'étude, et pourrez avoir accès au R&D report dans Documentum.

Dépense énergétique de repos:

La mesure de la dépense énergétique au repos permet d'évaluer la quantité de calories brulées lorsque vous êtes dans une situation minimale d'activité. Cela correspond à la quantité de calories utilisés par votre corps lorsque vous êtes couché et immobile. Dans ces conditions, la valeur obtenue représente la quantité de calories utilisées par vos organes et vos muscles. Lorsqu'elle est extrapolée sur 24h, cette valeur représente vos besoins caloriques quotidiens minimaux, auxquels s'ajoute la quantité de calories utilisées par votre corps pour réaliser toutes les autres activités de votre vie quotidienne. La dépense énergétique de repos peut ensuite être multipliée par un facteur d'activité (voir tableau « niveaux d'activité physique » ci-dessous), représentatif de votre niveau d'activité (sportives et non-sportives) pratiquées au quotidien. La valeur ainsi obtenue est une estimation de votre dépense énergétique totale qui correspond à la quantité de calories qui, lorsque consommées, devraient vous permettre de garder un poids constant. Cette valeur vous est donnée à titre indicatif et est sujette au changement, selon votre niveau d'activité qui peut varier au quotidien.

Dans le cadre de cette étude, la dépense énergétique de repos à été mesurée à l'aide de la calorimétrie indirecte. Cette technique évalue la concentration d'O_2 et de CO_2 présents dans les gaz expiratoires à jeun et au repos strict.

Votre dépense énergétique de repos, moyennée entre les deux visites, a été évaluée à **1715.5 kcal/jour**.

En vous référant au tableau ci-dessous, vous pouvez multiplier cette valeur par le facteur d'activité correspondant à votre situation. La valeur obtenue est une indication de vos besoins énergétiques caloriques quotidiens.

Par exemple, si votre dépense énergétique de repos a été mesurée à 1500 kcal par jour, et que vous êtes très sédentaire (valeur du niveau d'activité physique : 1.4), vous aurez une dépense énergétique réelle de : 1500 x 1.4 = 2100 kcal par jour.

Niveaux d'activité physique :

Catégorie du style de vie	Valeur du niveau d'activité (sans unités)
Sédentaire ou faible activité	1.40-1.69
Modérément actif ou actif	1.70-1.99
Très actif	2.00-2.40

Source : Food and Agriculture Organization of the United Nations
http://www.fao.org/docrep/007/y5686e/y5686e07.htm

En général, chez l'homme adulte qui pratique un niveau d'activité physique faible, la dépense énergétique totale (dépense énergétique de repos + dépense énergétique des activités) se situe autours de 2500 kcal/jour.

Voici les valeurs de référence pour des hommes adultes :

Calories nécessaires (kcal)	Activité physique		
Age (années)	Faible	Moyenne	Forte
19-30	2500	2700	3000
31-50	2350	2600	2900

Source : santé canada : http://www.hc-sc.gc.ca/fn-an/food-guide-aliment

Voici les valeurs de référence pour des femmes adultes :

Calories nécessaires (kcal)	Activité physique		
Age (années)	Faible	Moyenne	Forte
19-30	1900	2100	2350
31-50	1800	2000	2250

Source : santé canada : http://www.hc-sc.gc.ca/fn-an/food-guide-aliment

Fréquence cardiaque :

Lors de cette étude, seules la fréquence cardiaque de repos, et celle induite par la boisson ont été évaluées. La fréquence cardiaque à l'effort n'a pas été mesurée, bien que ce soit elle qui donne une réelle idée de la santé cardiaque. En effet, la fréquence de repos n'atteste pas de la santé du cœur ; elle est plutôt indicative du degré d'activité physique quotidien et du niveau de stress.

Votre fréquence cardiaque au repos, moyennée entre les deux visites est de : **55.6 battements par minute**.

En général, la fréquence cardiaque au repos est située entre 58 et 74 battements par minute.

N		Repos		Anticipation	
Entrainé	Non entrainé	Entrainé	Non entrainé	Entrainé	Non entrainé
5	4	67	69	148	124
5	4	67	67	130	115
4	4	63	68	129	118
4	4	62	70	122	129
4	4	58	64	118	128
4	4	59	74	108	109

Source : McArdle W.D. et al. "Telemetered cardiac response to selected running events", J. Appl. Physiol, 23:566, 1967

Voici quelques éléments d'interprétation de votre fréquence cardiaque de repos :

Une personne sportive a une fréquence cardiaque de repos plus faible qu'une personne sédentaire.

Par ailleurs, dans la présente étude, les volontaires étaient allongés, ce qui peut entrainer une diminution de la fréquence cardiaque.

Une haute fréquence cardiaque de repos peut être expliquée par l'anticipation d'une situation inconnue, …

J'espère que ce dossier vous aura permis de mieux appréhender vos besoin caloriques journaliers et donné une idée de votre fréquence cardiaque de repos.

Merci encore sincèrement d'avoir pris part à cette étude clinique!