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http://www.informaworld.com/smpp/content~content=a916852759~db=all~jumptype=rss

Raised expression of APRIL in Chinese patients with immune thrombocytopenia and its clinical implications

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ESSENCE OF ARTICLE

“Conclusion: APRIL is over expressed in untreated active ITP patients and might be a pathogenic factor of this disorder”

ARTICLE

Abstract

Background: Immune thrombocytopenia (ITP) is an immune-mediated disorder in which destruction of platelets is accelerated by anti-platelet autoimmune antibodies. B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), essential factors for B cell survival are elevated in systemic autoimmune diseases and correlated with clinical findings. High expression of BAFF has been shown in patients with ITP, but the status of APRIL in ITP is still unknown.

Objective: To determine the expression of APRIL and it receptors, B-cell maturation antigen (BCMA) and trans-membrane activator and calcium modulator and cyclophilin ligand interactor (TACI), in patients with ITP, and evaluate the correlation between plasma APRIL levels and platelet accounts or other clinical parameters.

Methods: Plasma samples from 57 patients with ITP, and 30 normal healthy subjects were assayed for APRIL plasma concentration by enzyme linked immunosorbent assay. Real-time quantitative polymerase chain reaction was performed to determine the mRNA expression of APRIL and its receptors (BCMA and TACI) in peripheral blood mononuclear cells (PBMNCs) in 25 normal controls and 34 untreated ITP patients with active disease.

Results: The APRIL levels in the plasma samples from patients with ITP were significantly higher than those from healthy controls ($p = 0.000$). PBMNCs may be a source of the excess APRIL. Treated patients with normal platelet

count have relatively normal plasma APRIL ($p = 0.599$). Plasma APRIL levels in active patients were significantly correlated with platelet counts ($r = - 0.387$ and $p = 0.024$).

Conclusion: APRIL is over expressed in untreated active ITP patients and might be a pathogenic factor of this disorder.

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http://www.ncbi.nlm.nih.gov/sites/entrez

Cas Lek Cesk. 1997 Jul 30;136(15):459-63.

New drugs with positive effects on bones

[Article in Czech]

Zofková I, Kanceva RL.

Endokrinologický ústav, Praha.

ESSENCE OF ARTICLE

"A more potent factor than magnesium is stroncium, which not only activates osteoblats but decreases the number of osteoclasts, thus abolishing bone resorption and enhancing formation."

ARTICLE

The paper concerns the nontraditional treatment of osteoporosis using endogenous substances regulating bone metabolism, and also new drugs. NO in high concentrations decreases the activity of osteoclasts, scavenges superoxides which destroy connective tissue, and activates 1 alpha-hydroxylase in kidneys. Bone metabolism is effectively influenced by donors of NO or by modulators of NO synthase. Osteoclastic function is also inhibited by vitamin K. The administration of the vitamin is indicated in osteoporotic patients with proven vitamin K deficiency. Antiestrogens (tamoxifen), ipriflavon and analogues of wortmannin have antiresorptive activity. Under certain conditions parathyroid hormone (PTH) is anabolic for bone. The positive effect on bone was confirmed with the subcutaneous administration of small doses of PTH simulating physiologic pulsatile secretion, as well as the intact somatotropin-IGF-I (insulin like growth factor-I) axis. PTH is extremely useful, especially in osteoporosis induced by hypoestrinism. Somatotropin (GH) also has an anabolic effect on bone. The hormone stimulates bone metabolism with a prevalence of formation due to direct action on bone, as well as by means of IGF-I. Further growth factors with positive osteoprotic effect are TGF-beta (transforming growth factor-beta), FGF (fibroblast growth factor) and calcium conserving dihomogammalinoleic acid. Magnesium influences bone in different ways. It activates osteoblasts, increases bone mineralization, and enhances the sensitivity of target tissues (incl. bone) to PTH and 1,25(OH)2 vitamin D3, Under certain conditions however, magnesium can stimulate bone resorption. A more potent factor than magnesium is stroncium, which not only activates osteoblats but decreases the number of osteoclasts, thus abolishing bone resorption and enhancing formation. Bicarbonates are also favourable for bone. $NaHCO_3$ together with potassium citrate stimulates osteoblasts and enhances bone mineralisation. In the review other prospective substances are also discussed. The osteoprotic effects of most of these factors were confirmed in vitro

and in studies in animals, but their use in clinical practice is still a matter for investigation. Mutual interactions with classical osteoprotic drugs remain to be established.

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http://www.usatoday.com/news/health/weightloss/2009-11-17-future-obesity-costs_N.htm?csp=34&utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+UsatodaycomHealth-TopStories+(News+-+Health+-+Top+Stories)

Rising obesity will cost U.S. health care $344 billion a year

MOST AND LEAST OBESE

States with the highest and lowest obesity rates in 2009:

Lowest

1. Colorado

2. (tie) Connecticut

Massachusetts

4. Rhode Island

5. (tie) Hawaii

Utah

7. Vermont

8. New Jersey

9. (tie) California

Montana

Highest

1. Mississippi

2. Alabama

3. West Virginia

4. Tennessee

5. Oklahoma

6. South Carolina

7. Kentucky

8. (tie) Arkansas Michigan

North Carolina

Source: America's Health Rankings (americashealthrankings.org)

-

By Nanci Hellmich, USA TODAY

If Americans continue to pack on pounds, obesity will cost the USA about $344 billion in medical-related expenses by 2018, eating up about 21% of health-care spending, says the first analysis to estimate the future medical costs of excess weight.

These calculations are based on the projection that in 10 years 43% of Americans adults may be obese, which is roughly 30 or more pounds over a healthy weight, if obesity continues to rise at the current rate. Extra weight increases the risk of diabetes, heart disease and many types of cancer.

This report comes as the country struggles to find ways to curb medical costs and Congress debates health care legislation.

"Obesity is going to be a leading driver in rising health-care costs," says Kenneth Thorpe, chairman of the department of health policy and management at Emory University in Atlanta. Thorpe did this special analysis on obesity for America's Health Rankings, the 20th annual assessment of the nation's health on a state-by-state basis.

"There is a tsunami of chronic preventable disease about to be unleashed into our medical-care system which is increasingly unaffordable," says Reed Tuckson of United Health Foundation, sponsor of the report with the American Public Health Association and Partnership for Prevention.

Using weight data, Census statistics and medical expenditure information, Thorpe found:

•An obese person will have an average of $8,315 in medical bills a year in 2018 compared with $5,855 for an adult at a healthy weight. That's a difference of $2,460.

•If the percentage of obese adults doesn't change but stays at the current rate of 34%, then excess weight will cost the nation about $198 billion by 2018.

•If the obesity rate continues to rise until 2018, then Colorado may be the only state with less than 30% of residents who are obese.

•More than 50% of the population in several states could be obese by 2018: Oklahoma, Mississippi, Maryland, Kentucky, Ohio and South Dakota.

The report adds to the growing body of evidence of obesity's impact on medical costs. A study released in July showed that obese Americans cost the country about $147 billion in weight-related medical bills in 2008, double what it was a decade ago. It now accounts for about 9.1% of medical spending.

Overall, the United States spends about $1.8 trillion a year in medical costs associated with chronic diseases such as diabetes, heart disease and cancer, and all three are linked to smoking and obesity, the nation's two largest risk factors, according to the America's Health Rankings report.

Smoking is still the No. 1 preventable cause of death in the country, accounting for about 440,000 deaths annually, the report says.

About one in five Americans smoke. More than 3 million people quit smoking this past year. The percentage of people who smoke varies by state, from 9.3% in Utah to more than 25% in Kentucky, Indiana and West Virginia, the study says.

"This report is an urgent call to take much more aggressive action to deal with key disease risk factors such as obesity and smoking," Tuckson says.

Health economist Eric Finkelstein, co-author of The Fattening of America, says medical costs won't go down unless Americans make a serious effort "to slim down by improving their diet and exercise patterns."

For the full report, go to www.americashealthrankings.org

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http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WN2-4WVK2MW-1&_user=10&_coverDate=12%2F31%2F2009&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=f18e4d19f3e7d8f734337182b65f1256

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Post herpetic neuralgia, schwann cell activation and vitamin D

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ESSENCE OF ARTICLE

“ High dose topical vitamin D would appear to offer particular promise because vitamin D has the ability to both reduce glial inflammation and reduce nitric oxide production.”

ARTICLE

Summary

While the underlying pathophysiology of herpes zoster infection has been well characterised, many of the mechanisms relating to the subsequent development of post herpetic neuralgia (PHN) remain uncertain. The dorsal horn atrophy and reduction in skin innervation seen in PHN patients does not adequately explain many clinical features or the efficacy of a number of topical treatments. In the central nervous system the glia, their receptors and their secreted signalling factors are now known to have a major influence on neural function. In the peripheral nervous system, schwann cell activation in response to infection and trauma releases a number of neuroexcitatory substances. Activation of the nervi nervorum in the peripheral nervous system also leads to the release of calcitonin gene related peptide, substance P and nitric oxide. Schwann cell and/or nervi nervorum activation could be an additional mechanism of pain generation in PHN. Such a paradigm shift would mean that drugs useful in the treatment of glial cell activation such as naloxone, naltrexone, minocycline, pentoxifyllline, propentofylline, AV411 (ibudilast) and interleukin 10 could be useful in PHN. These drugs could be used systemically or even topically. High dose topical vitamin D would appear to offer particular promise because vitamin D has the ability to both reduce glial inflammation and reduce nitric oxide production.

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Metabolic Effects of Dark Chocolate Consumption on Energy, Gut Microbiota, and Stress-Related Metabolism in Free-Living Subjects

ESSENCE OF ARTICLE

“Dietary preferences influence basal human metabolism and gut microbiome activity that in turn may have long-term health consequences. The present study reports the metabolic responses of free living subjects to a daily consumption of 40 g of dark chocolate for up to 14 days. A clinical trial was performed on a population of 30 human subjects, who were classified in low and high anxiety traits using validated psychological questionnaires. Biological fluids (urine and blood plasma) were collected during 3 test days at the beginning, midtime and at the end of a 2 week study. NMR and MS-based metabonomics were employed to study global changes in metabolism due to the chocolate consumption. Human subjects with higher anxiety trait showed a distinct metabolic profile indicative of a different energy homeostasis (lactate, citrate, succinate, trans-aconitate, urea, proline), hormonal metabolism (adrenaline, DOPA, 3-methoxy-tyrosine) and gut microbial activity (methylamines, p-cresol sulfate, hippurate). Dark chocolate reduced the urinary excretion of the stress hormone cortisol and catecholamines and partially normalized stress-related differences in energy metabolism (glycine, citrate, trans-aconitate, proline, β-alanine) and gut microbial activities (hippurate and p-cresol sulfate). The study provides strong evidence that a daily consumption of 40 g of dark chocolate during a period of 2 weeks is sufficient to modify the metabolism of free living and healthy human subjects, as per variation of both host and gut microbial metabolism.”

ARTICLE

Abstract

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Abstract

Dietary preferences influence basal human metabolism and gut microbiome activity that in turn may have long-term health consequences. The present study reports the metabolic responses of free living subjects to a daily consumption of 40 g of dark chocolate for up to 14 days. A clinical trial was performed on a population of 30 human subjects, who were classified in low and high anxiety traits using validated psychological questionnaires. Biological fluids (urine and blood plasma) were collected during 3 test days at the beginning, midtime and at the end of a 2 week study. NMR and MS-based metabonomics were employed to study global changes in metabolism due to the chocolate consumption. Human subjects with higher anxiety trait showed a distinct metabolic profile indicative of a different energy homeostasis (lactate, citrate, succinate, trans-aconitate, urea, proline), hormonal metabolism (adrenaline, DOPA, 3-methoxy-tyrosine) and gut microbial activity (methylamines, p-cresol sulfate, hippurate). Dark chocolate reduced the urinary excretion of the stress hormone cortisol and catecholamines and partially normalized stress-related differences in energy metabolism (glycine, citrate, trans-aconitate, proline, β-alanine) and gut microbial activities (hippurate and p-cresol sulfate). The study provides strong evidence that a daily consumption of 40 g of dark chocolate during a period of 2 weeks is sufficient to modify the metabolism of free living and healthy human subjects, as per variation of both host and gut microbial metabolism.

Metabolic phenotype of mammals results from the combination of multiple genetic, environmental and sociocultural determinants.(1, 2) In man, dietary preferences, lifestyle and genetics influence individual metabolic phenotype, and therefore determine health status and the likelihood to develop diseases.(3) Variations in the dietary pattern affect the metabolism of humans via the key entry points of gut microbiota.(4) Therefore, there is clearly a need to understand human metabolism at the system level with emphasis on the expression of both host and meta-genomes, environmental and lifestyle factors to meet the ultimate goal of providing better health and wellbeing with nutrition. Although predominantly cultural in origin, dietary preferences also result from multiple biological and behavioral processes, which integrate satiety, psychological perception and metabolic effects of foods.(5) We have recently described how dietary preferences can be associated with specific signatures in the metabolic phenotypes of healthy humans, with a metabolic signature based on a modulation of host and gut microbial metabolism.(3)

Perhaps one of the greatest challenges in modern nutrition is to interrogate and classify the critical metabolic interactions between the complex food matrices—containing a wide range of biologically active compounds—and human system metabolism and to understand their role in diverse human disease processes. The sheer complexity of a food matrix, such as dark chocolate, may determine a large variety of effects on the metabolism. Many studies have indeed demonstrated the potential health implications of dark chocolate constituents, but rarely as a whole product. For instance, cocoa is rich in flavonoids, mainly flavan-3-ols (epicatechin, catechin and their oligomers), which were associated with benefits on cardiovascular health by maintaining low blood pressure, improving endothelial function, and by reducing thrombotic state, oxidative and inflammatory states.(6, 7) Benefits of cocoa on improvement of insulin sensitivity and glucose tolerance were also reported.(8, 9) Other biochemically active molecules naturally occurring in chocolate include theobromine, a bitter alkaloid also known to reduce blood pressure, phenylethylamine, a monoamine alkaloid which can act as neurotransmitter, and N-oleoyl- and N-linoleoyl-ethanolamine that slow the rate of anandamide breakdown, a brain neurotransmitter.(10, 11) Therefore, if there is growing evidence on the health benefits associated with chocolate, mechanisms of action of chocolate bioactive components at the molecular levels are poorly understood. This is particularly the case for benefits related to brain health and improvement of stress states where only symptomatic data, such as brain blood flow, are available.(12)

In the present study, we have sought to capture a global view of the metabolic changes associated with chocolate consumption in healthy and free living men and women using metabonomics. Nutrimetabonomics provides a system approach to assess systemic metabolic status of an individual, which encapsulates information on genetic and environmental factors,(1, 13) gut microbiota activity,(14, 15) lifestyle(16) and food habits.(3) Here, we have used proton nuclear magnetic resonance (1H NMR) spectroscopy and mass spectrometry (MS) as complementary analytical platforms for monitoring metabolic changes associated with a daily intake of 40 g of dark chocolate over a

period of 2 weeks in the urine and blood plasma of 30 individuals classified according to their self-reported anxiety trait. We describe the metabolic variations induced by dark chocolate and discuss their association with changes in energy homeostasis, gut microbial activity and the metabolism associated with stress.

Recruitment of Volunteers

This study was conducted by TNO Quality of Life, Zeist (The Netherlands) in accordance with the ethical principles of Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the Medical Ethics Committee METOPP (Medisch-Ethische Toetsing Onderzoek Pati nten en Proefpersonen/medical ethics review of research with patients and test subjects) on April 3, 2006 (The Netherlands). A total of 30 subjects (11 males, 19 females) were enrolled in the study and gave written informed consent (Table 1). The study was designed as a randomized (by age, gender, anxiety trait), parallel, open study. The inclusion of the volunteers was decided upon medical history, age (18−35 years), body mass index (BMI, 18−25 kg•m−2), and blood clinical analyses (Table 1). The exclusion criteria included psychiatric, metabolic, endocrine, gastrointestinal and eating behavior disorders. In addition, smoking, use of medication that may influence appetite and/or sensory functioning, pregnancy, reported slimming or medically prescribed diet, reported unexplained weight loss or gain in the month prior to the screening, alcohol consumption superior to, respectively, 21 and 28 units per week for females and males were also considered as exclusion criteria.

Clinical Trial

Participants were asked to avoid consumption of chocolate or chocolate containing products during an 8-day run-in period. The nutritional intervention lasted 2 weeks with a daily intake of 40 g of commercially available dark chocolate (Noir Intense, 74% cocoa solids, Nestl). On day 1 (preintervention) and days 8 and 15 (postintervention), fasting blood plasma and morning spot urine samples were collected. A daily amount of 40 g of dark chocolate was consumed twice per day as a midmorning and a midafternoon snack (20 g each). Participants were divided up into either high or low anxiety trait subgroup according to the evaluation of their dispositional stress as assessed by scoring on the anxiety trait scale of the State-Trait Anxiety Inventory (STAI) test.(17) The STAI is the definitive instrument for measuring anxiety in adults that clearly differentiates between the temporary condition of “anxiety state” and the more general and long-standing status of anxiety trait. The STAI scores from 70 to 78 and from 42 to 64 described low and high anxiety trait, respectively (Table 1). The study included 4 high and 7 low anxiety trait males, and 9 high and 10 low anxiety trait females (Table 1). Metabolic data from subjects having reported adverse events, such as nausea, vomiting, or diarrhea (subjects ID 6, 15, 17, and 44) were excluded from the statistical analysis to avoid introduction of biases in the final outcome of the study.

1H NMR Analysis of Plasma and Urine Samples

Plasma samples (495 μL) were introduced into 5 mm NMR tubes with 55 μL of deuterium oxide (D2O) used as locking substance. Urine samples (500 μL) were adjusted to pH 6.8 using 100 μL of a deuterated phosphate buffer solution (KH2PO4, final concentration of 0.2 M) containing 1 mM of sodium 3-(trimethylsilyl)-[2,2,3,3-2H4]-1-propionate (TSP) into a 5 mm NMR tube.

Metabolic profiles were measured at 300 K on a Bruker Avance II 600 MHz spectrometer equipped with a 5 mm inverse probe (Bruker Biospin, Rheinstetten, Germany). Three types of 1H NMR spectra were registered for each blood plasma sample, including a standard 1H detection with water suppression, a Carr−Purcell−Meiboom−Gill spin−echo with water suppression and a diffusion-edited pulse sequences, as reported previously.(3) The standard spectra were acquired with a relaxation delay of 4 s and a mixing time of 100 ms. CPMG spin−echo spectra were measured using a spin−echo loop time of 19.2 ms and a relaxation delay of 4 s. Diffusion-editing spectra were obtained using a relaxation delay of 1 s, pulsed field gradients set at 46.8 G•cm−1, and a diffusion delay of 120 ms

during which the molecules are allowed to diffuse. Urine spectra were acquired using the standard sequence, with a relaxation delay of 2.5 s and a mixing time of 100 ms.

For each sample, 32 (plasma) and 256 (urine) free induction decays (FIDs) were collected into 64 K data points using a spectral width of 12019.2 Hz, corresponding to an acquisition time of 2.7 s. Prior to Fourier transformation, FIDs were multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz (standard and CPMG spectra) and of 1 Hz (diffusion-edited spectra). The acquired NMR spectra were manually corrected for phase and baseline distortions, and referenced to the chemical shift of α-glucose at δ 5.236 for plasma and of TSP at δ 0.0 for urine using the TOPSPIN (version 2.1, Bruker Biospin, Rheinstetten, Germany) software package.

The metabolite identification was achieved using literature data,(18) and confirmed by 2D 1H NMR spectroscopy experiments performed on selected samples.

Mass Spectrometric Analysis of Plasma and Urine Samples

For mass spectrometry-based metabolite profiling analyses, proteins were removed from plasma and urine samples by precipitation. Subsequently, two nonpolar and two polar fractions were separated for GC-MS and LC-MS/MS analysis, respectively, by adding water and a mixture of ethanol and dichloromethane. For GC-MS analysis, the nonpolar fraction was treated with methanol under acidic conditions to yield the fatty acid methyl esters. Both fractions were further derivatized with O-methyl-hydroxyamine hydrochloride and pyridine to convert oxo-groups to O-methyl-oximes and subsequently with a silylating agent before analysis.(19) In LC-MS analysis, both fractions were reconstituted in appropriate solvent mixtures. HPLC was performed by gradient elution using methanol/water/formic acid on reversed phase separation columns. Mass spectrometric detection technology which allows target and high sensitivity MRM (Multiple Reaction Monitoring) profiling was performed in parallel to a full screen analysis. In the case of urine analysis, a photometric creatinine analysis according to Jaff was performed prior to polar MS analysis and samples diluted to the same creatinine concentration.(20) The polar fraction was applied to each of the systems. For GC-MS and LC-MS/MS profiling, data were normalized to the median of reference samples which were derived from a pool of all pretreatment samples (Day 1) to account for inter- and intrainstrumental variation. Steroids, catecholamines and their metabolites were measured by online SPE-LC-MS/MS (Solid phase extraction-LC-MS/MS).(21) In the case of urine samples, conjugated derivatives of steroids were enzymatically cleaved prior to analyses using a beta-glucuronidase [EC 3.2.1.31] and an arylsulfatase [EC 3.1.6.1] from Helix pomatia. For plasma measurements, absolute quantification was performed by means of stable isotope-labeled standards. The analyses of cortisol in plasma as well as catecholamines and steroids in urine were performed by normalization to pool levels as described for profiling.

Chemometrics

NMR data was converted into 22 K data points over the range of δ 0.2−10.0 and imported into MATLAB environment (The MathWorks, Inc., Natick, MA). Interpolation of all the spectra to the same chemical shift followed by zeroing the intensity values of the water peak from δ 4.68 to 5.10 was performed. The NMR spectra were normalized to a constant total sum of all intensities within the specified range and autoscaled prior to multivariate data analyses (MVA). MS data were normalized to the median of pooled samples and autoscaled before MVA.

Principal components analysis (PCA)(22) was first performed to visualize the global variance of the data sets and to pinpoint outliers. PCA is an important tool for visualizing data structures and one of the most applied dimensionality reduction methods. The aim of PCA is to represent the original data (X) by a set of new orthogonal variables so-called principal components (PCs) which are linear combinations of the original variables. Because the extracted PCs maximize the data variance, PCA is sensitive to the presence of outliers. The data matrix X is decomposed to a score matrix U and a loading matrix V, plus an error matrix E. The elements of the loadings give information about

the contribution of the original variables (NMR or MS) to each PC and the elements of the scores provide information about metabolic similarities and dissimilarities between samples.

Partial Least Squares (PLS) and Orthogonal PLS (O-PLS) discriminant analyses (PLS-DA and O-PLS-DA) were also applied for detailed classification purposes.(23) In PLS-DA, a dependent variable y is modeled using latent variables, maximizing the covariance between X (NMR or GC/LC-MS data) and y. Variable y is a binary vector with value 0 for one class and value 1 for the other class under study; in this paper, y is related to time- and anxiety trait-dependent metabolic variations after dark chocolate supplementation.

In particular, O-PLS-DA, which is a modification of PLS, separates the systematic variation in X into two parts, one linearly related to y and representing the between class variation, and another one orthogonal to y and representing the within class variation. In other words, it provides a way to filter out metabolic information (NMR or GC/LC-MS data) that is not correlated to the predefined classes (time, anxiety trait). The robustness of statistical models was assessed using the standard 7-fold cross validation method. Validity of the model against overfitting was determined by computing the total explained variance of X and y (R2(X), R2(Y)) and the cross-validated predictive ability (Q2(Y)) values of the models as reported in Tables 2 and 3. Negative or very low values of the Q2(Y) indicate that no statistically significant differences were observed. Influential variables that are correlated to the group separation are identified using the variable coefficients according to a previously published method.(24) S-plot was used to visualize the variable influence in the MS data models.(25) In addition, a Student's t test with an alpha of 0.05 was performed on GC/LC-MS variables and one representative NMR signal areas representative of influential metabolites.

Chemometric analysis was performed using the SIMCA-P+ (version 12.0, Umetrics AB, Umeå, Sweden) software package and in-house developed MATLAB routines.

NMR and MS Metabolic Profiling of Blood Plasma and Urine

A wide range of amino and organic acids, ketone bodies, sugars, osmolytes, saturated and unsaturated fatty acids and triglycerides were detected using 1H NMR spectroscopy and GC/LC-MS analysis of blood plasma. Holistic NMR plasma profiles dominated by low molecular weight components (CPMG spectra) and macromolecules (diffusion-edited spectra) were complemented by GC/LC-MS semiquantitative measures of 148 targeted metabolites. Similarly, the 1H NMR urine profiles exhibited a set of resonances arising from major low molecular weight molecules, such as ketone bodies, organic acids, amino acids, and aromatic metabolites were completed with 157 targeted metabolites measured with LC-MS.

Overview of Metabolic Variations by PCA

PCA was first applied to assess the inherent similarity of the urine and plasma metabolic profiles using 4 PCs. For urine, PC1–4 explained, respectively, 12, 7, 6, and 5% of the total variance present in the NMR data and 17, 8, 7, and 6% in the MS data (Supplementary Figure 1A,B). The urine samples from the subject 21 were removed from the statistical analysis due to a statistically dominant dilution effects in the NMR profile. For plasma, PC1-4 explained 23.9, 8.7, 5.3, and 4.5% of the total variance, respectively, in the NMR data and 13.8, 9.0, 6.5, and 5.6% in the MS data (Supplementary Figure 1C,D). PCA highlighted that interindividual variability of the metabolic profiles of urine and plasma tended to be greater than intraindividual variations (Supplementary Figure 1). Analysis of NMR-derived models revealed that interindividual differences were associated with variations in the urinary levels of creatinine, trimethylamine-N-oxide (TMAO), hippurate, citrate, and lactate, and plasma composition in lipoproteins, lipids, phosphocholine and glucose. Investigations of the MS-derived models indicated that the main source of metabolic variations between subjects was due to changes in the urinary excretion of xylose, lactate, glycerate, lysine and 4-dihydroxyhippurate, and plasma concentrations of serotonine, corticosterone, 3,4-

hydroxyphenylacetate, and homovanillate. Interpretation of the PCA scores plot did not reveal any distribution of the samples according to age, time, BMI and self-reported anxiety. However, a separation trend due to gender differences could be observed and was particularly marked in MS urinary data along the second PC.

Additional investigations were performed using O-PLS-DA to maximize the separation between the groups of samples (high and low anxiety trait or time of sample collection) and identify class-specific metabolites (Tables 2 and 3).

Daily Consumption of Dark Chocolate Induces a Specific Metabolic Signature

Supervised chemometric analyses of the urine NMR and MS data revealed statistically significant time-dependent changes, as noted by the positive value of the model predictability parameter Q2(Y). Interpretation of the urine O-PLS-DA scores plots (Figures 1 and 2) and model descriptors (Table 2) indicated that a 1 week daily intake of dark chocolate by free living subjects is reflected in the metabolic profiles as assessed by NMR spectroscopy. The metabolic changes became even more significant after 2 weeks of consumption, as observed by the greater value of Q2(Y) value and a clearer separation of the two groups of samples in the scores plot (Table 2, Figures 1 and 2). Interpretation of the corresponding O-PLS-DA coefficients plots indicated that, after 1 week, chocolate consumption resulted in increased levels of 4-hydroxyphenylacetate and several unassigned metabolites giving resonances at δ 7.85 (s), 8.03 (s), 8.22 (s), 3.0 (s), 3.39 (s), 3.91 (m) and 3.53(s) (Table 4, Figure 1). These metabolic changes were associated with downward trends in creatinine, and an unassigned aromatic metabolite giving resonances at δ 7.51 (m) and 7.70 (m). Comparison of samples obtained at baseline and after 2 weeks of treatment revealed greater metabolic changes in both endogenous and gut microbial metabolism. In particular, after 2 weeks of chocolate consumption, these metabolic changes were maintained and were associated with additional decreased levels of phenylacetylglutamine and p-cresol sulfate (Table 4).

Intriguingly, MS-based metabolic profiling showed that chocolate-induced metabolic effects were statistically significant only in subjects with inherent high anxiety trait (Tables 2 and 4). Interpretation of the loadings plot showed that chocolate consumption was associated with decreased levels of normetanephrine, adrenaline, corticosterone, noradrenaline, progesterone, leucine, cortisol and asparagine, and an increase of glucose-6-phosphate, cystine and threonic acid (Figure 2). Additional analyses of the urinary NMR data indicated that the effects of dark chocolate consumption on urinary excretion of aromatic compounds were similar in both high and low anxiety trait subjects (Tables 2 and 4).

Additional chemometric analyses of blood plasma metabolic profiles did not reveal statistically significant effects of dark chocolate over the time as assessed by NMR or MS (Table 2).

Anxiety Trait is Associated with a Specific Human Metabolic Signature

Chemometric analysis of urinary NMR and MS profiles revealed significant compositional changes associated with self-reported anxiety trait (Table 3, Figures 3 and 4). For NMR data, analysis of the coefficients plots indicated that subjects with a higher anxiety trait were characterized with higher urinary excretion of hippurate, glycine, citrate, and lower levels of methyl-succinate, trans-aconitate, and a series of unassigned signals, most likely arising from a polyol, and a signal at δ 1.24 (Table 5, Figure 3). These metabolic changes were also associated with trends toward higher urine levels of succinate, lactate and urea, and trends toward lower urinary excretion of trimethylamine (TMA), and p-cresol sulfate (Table 5).

For MS data, high anxiety trait subjects showed higher urinary concentrations of glycine, 3-methoxytyrosine, β-alanine, proline, 3,4-dihydroxyphenylalanine (DOPA), adrenaline, an upward trend of lactate, and lower levels of p-cresol sulfate, aconitate, and a downward trend of arabitol when compared to low anxiety trait individuals (Table 5, Figure 4). Structure annotation of p-cresol sulfate metabolite is based on strong analytical evidence (combinations of chromatography, MS, chemical reactions, deuterium-labeling, database and literature search, comparison to similar/homologue/isomeric reference compounds).

Analysis of the blood plasma NMR and MS profiles reveal subtle but significant anxiety trait related metabolic differences at the baseline and after 2 weeks of dietary intervention (Table 3). Interpretation of the NMR coefficients plots indicated that high anxiety subjects tended to have a relative higher level of choline, and lower concentrations of glycine and glutamine compared to low anxiety individuals (Table 5). In addition, after 2 weeks of treatment, high anxiety trait individuals showed increased concentrations of glutamate and choline, and decreased levels of acetate in plasma (Table 5). Analysis of the S-plots derived from MS data analysis showed increased levels of lycopene and β-carotene in high anxiety trait subjects at baseline, and the higher plasma concentration of β-carotene was still observed after 2 weeks of treatment with dark chocolate (Table 5). Moreover, at 2 weeks post-treatment, subjects with a high self-reported anxiety trait also showed increased levels of normetanephrine (Table 5).

Effects of Dark Chocolate Consumption on Anxiety Trait Related Metabolism

The anxiety trait-related metabolic differences observed in urine were significantly reduced following 1 and 2 weeks intervention with dark chocolate, as noted with the low/negative value of the Q2(Y) parameter and the loss of statistically significant differences between the groups (Tables 3 and 5). The NMR and MS signals corresponding to the influential metabolites identified by chemometrics were analyzed using a Student t test (Table 5) and displayed using box-and-whiskers plots in order to explore their changes overtime (Figure 5). The changes of hippurate, p-cresol sulfate, glycine, citrate, trans-aconitate, proline, DOPA, and β-alanine showed similar trends from high anxiety trait individuals toward low anxiety trait subjects and further support a normalization of the metabolic profiles (Table 5). Interestingly, anxiety trait-related metabolic signatures in blood plasma were maintained over the duration of the clinical trial, except for lycopene for which the difference was not significant following the dietary intervention. In addition, t tests were performed on the contrasts for the second ((t1 − t0)high vs (t1 − t0)low) and third time points ((t1 − t0)high vs (t1 − t0)low) to assess the metabolic relationships between anxiety trait and dark chocolate consumption (Supplementary Table 1). The results validated significant relationships between anxiety trait level, chocolate consumption and the urinary excretion of 3-methoxytyrosine, adrenaline, glycine and trans-aconitate, as well as plasma levels of acetate (Supplementary Table 1).

Figure 5. Time-dependent metabolic differences between high and low anxiety trait individuals Comparison of MS signals of plasma β-carotene, urine p-cresol sulfate and glycine, and area normalized intensities of representative signals of hippurate, trans-aconitate, and citrate from 1H NMR urine metabolic profiles displayed using box-and-whisker plots. For metabolites identified by 1H NMR spectroscopy, data are presented as area normalized intensities (a.u.) as follows: 1 × 10−2 a.u. for citrate, 1 × 10−4 a.u. for trans-aconitate and 1 × 10−1 a.u. for hippurate. Median values are highlighted by dashed and solid lines. Statistical significance of differences with time and anxiety trait levels is reported in Tables 4 and 5.

In the present study, NMR- and MS-based metabolic profiling are shown as complementary techniques to provide a comprehensive modeling of the biological response of a free living population to a daily consumption of dark chocolate. The overview of urine and plasma metabolite profiles revealed that interindividual differences were greater than intraindividual variations, which illustrates the strong influence of lifestyle and genetics on individual metabolic phenotypes. Such metabolic variations make the study of the metabolic effects of dark chocolate in free-living subjects difficult when using nonsupervised chemometric methods. Here, metabonomics is applied to pinpoint modulation of the host and gut microbial metabolism in response to daily consumption of dark chocolate with emphasis on stress-associated metabolic changes.

Self-Reported Anxiety Trait is Associated with Specific Urine and Plasma Metabolic Signatures

In the current experiment, individuals were classified according to their dispositional stress as assessed by scoring on the well-validated anxiety trait scale of the State-Trait Anxiety Inventory (STAI) test.(17) The chemometric modeling of metabolite variations in relation to anxiety trait levels revealed different physiological processes in the absence of a specific nutritional intervention. Others have provided evidence that chronic and acute stress may contribute to the disruption of metabolic homeostasis, and subsequently to individual susceptibility to diseases.(26) In particular, the individual response to chronic stress is tightly connected to the hypothalamic-pituitary-adrenal metabolic axis and the sympathoadrenal system.(27) Our observations described systemic changes in hormonal metabolism of high anxiety trait individuals when compared to low anxiety trait subjects, as observed by MS with a higher urinary excretion of adrenaline, DOPA and 3-methoxytyrosine, two intermediates in dopamine synthesis.(28) The first step in the catecholamine metabolism is the hydroxylation of the amino acid tyrosine to DOPA, by the rate-limiting enzyme in catecholamine biosynthesis tyrosine hydroxylase. DOPA is then decarboxylated to dopamine which is the direct precursor to noradrenaline and adrenaline. It is well-described that physical and mental stress simulates the release of adrenaline via the sympathetic nervous system and synthesis of the adrenocorticotropic hormone that enhances the activity of specific enzymes, including tyrosine hydroxylase.(29) Therefore, the concomitant increased urinary levels of DOPA, 3-methoxy-tyrosine and adrenaline highlight a greater synthesis of catecholamines in subjects stratified with high anxiety trait, with inferred effects on energy metabolism.(27)

The results obtained by NMR and MS also demonstrated a functional relationship between anxiety trait levels and several pathways involving the tricarboxylic acid cycle (citrate, succinate, aconitate), gluconeogenetic pathways (lactate), urea cycle (urea, proline), and protection against oxidative stress (plasma concentrations of lycopene and β-carotene). In particular, the anticorrelated variation of citrate and trans-acotinate suggested additional variations in renal tubular pH and aconitase activity.(30) Therefore, the observed metabolic changes were consistent with the stress-mediated modulation of gluconeogenesis by catecholamines.(27) Moreover, NMR-based metabolic profiling of urine showed that high anxiety trait individuals tended to have lower urinary concentrations of polyols, including arabitol an intermediate in the pentose and glucuronate metabolism, which may also reflect a modulation of energy metabolism as a function of dispositional stress.

Nowadays, there is strong evidence that life stress impacts directly on gastrointestinal function in animals and humans via modulation of key physiological parameters, such as intestinal permeability and secretion and release of biological mediators.(31, 32) Changes of gastrointestinal functional ecology are intimately linked to gut microbial populations and activities,(33) and abnormal microbiota composition is often observed in the development of irritable bowel syndromes.(34) Urine of mammals contains many polar cometabolites resulting from gut microbial-mammalian metabolic interactions.(1, 35) Therefore, metabolic monitoring of urinary excretion of many aromatic compounds (e.g., phenolics, indoles and benzoyl derivatives), methylamines, short chain fatty acids and their hydroxylation products provides indirect information on the gut microbial metabolic activities.(36, 37) For instance, multivariate statistical modeling of urine and blood plasma indicated a modulation of choline metabolism, that is, high circulating levels of plasma choline and low urinary excretion of trimethylamine, coprocessed by the gut microbiota from dietary compounds containing choline.(37) Moreover, differences in anxiety trait levels were associated with differential urinary excretion of p-cresol sulfate and hippurate. These changes reflected different gut microbial metabolism of aromatic amino acids.(14, 36) Certain aromatic compounds, such as benzoate and phenylacetate, that can be coprocessed by the gut microbiota are well-characterized glycine and glutamine level reducing agents.(38, 39) Both NMR- and MS-based metabolite profiling of urine revealed relatively higher excretion of glycine in high anxiety trait individuals, with inferred relationships with amino acid interconversion, and benzoate metabolism. Additional blood plasma metabolic variations at baseline and at the end of the study, that is, reduction of circulating levels of plasma glycine and glutamine/glutamate, may be functionally related to changes of benzoate and phenylacetate metabolism in response to bacterial processing of dark chocolate.

The Biological Response of Free Living Subjects to a Daily Consumption of Dark Chocolate was Dependent on Self-Reported Anxiety Trait

The metabolic response to chocolate intervention in the whole cohort revealed that a daily intake of dark chocolate resulted in subtle and cumulative metabolic effects on the urinary excretion of gut microbial cometabolites over a 2 weeks period. Increased levels of 4-hydroxyphenylacetate and decreased content of phenylacetylglutamine and p-cresol sulfate reflected the adaptation of gut microbiota to process dark chocolate content and its active ingredients, such as phenylethylamine, N-oleoyl- and N-linoleoyl-ethanolamine, theobromine, flavonoids (epicatechin, catechin and their oligomers).(36, 37, 40) In particular, urinary excretion of 4-hydroxyphenylacetate and hippurate was previously ascribed to intake of polyphenols-rich products such as chocolate.(41) These observations are therefore complementary to our preliminary investigations of metabolic signatures associated to chocolate dietary habits.(3)

Our observations indicated that the metabolic impact of a daily intake of dark chocolate was strongly dependent on the dispositional stress state of the individuals, as noted with statistically significant metabolic effects only in subjects with inherent high anxiety trait. Consumption of dark chocolate resulted in the decrease of the levels of catecholamines (adrenaline, noradrenaline, normetanephrine), corticosterone, and the stress hormone cortisol in the urine from subjects with high dispositional stress. Chronic stress is correlated with increases in stress hormones cortisol and catecholamines,(42-45) and our results suggest potential beneficial implications of dark chocolate consumption for reduction of mental and/or physical stress and improvement of the metabolic response to stress. Moreover, the anxiety trait-related metabolic differences observed in urine (e.g., levels of hippurate, p-cresol sulfate, glycine, citrate, trans-aconitate, proline, DOPA, and β-alanine) tended to be normalized toward the levels observed in low anxiety trait subjects, whereas metabolic signatures in blood plasma were maintained over the duration of the clinical trial. Therefore, our observations provided additional evidence that consumption of dark chocolate may beneficially impact on stress-associated metabolism as observed through a partial normalization of stress-related differences in energy metabolism and gut microbial activities.

Our study in free living and healthy humans demonstrates a link between metabolic phenotype of individuals and specific dietary patterns. The current observations strongly support the idea that specific foods impact on human metabolism through the modulation of gut microbial activities. The daily consumption of dark chocolate resulted in a significant modification of the metabolism of healthy and free living human volunteers with potential long-term consequences on human health within only 2 weeks treatment. This was observable through the reduction of levels of stress-associated hormones and normalization of the systemic stress metabolic signatures. Therefore, subtle changes in dietary habits are likely to modulate the metabolic status of free-living individuals that might be associated with long-term health consequences, in particular via the activity of the symbiotic bacterial partners.

Abbreviations:

CPMG, Carr–Purcell–Meiboom–Gill; MS, mass spectrometry; NMR, nuclear magnetic resonance; O-PLS-DA, orthogonal projection to latent structure discriminant analysis; PCA, principal component analysis; PLS-DA, projection to latent structure discriminant analysis.

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Supporting Information

Supplementary Figure 1, principal component analysis of biological matrices; Supplementary Table 1, summary of contrast test statistics to assess metabolic relationships between anxiety trait levels and chocolate consumption in urine and plasma. This material is available free of charge via the Internet at http://pubs.acs.org.

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Byline: The Dallas Morning News

This is your brain on bonbons:

New research suggests that chocolate packs chemicals similar to the active ingredient also active pharmaceutical ingredient (or API), is the substance in a drug that is pharmaceutically active. Some medications may contain more than one active ingredient.

..... Click the link for more information. in marijuana.

While this doesn't mean that M&Ms can deliver the same side effects

Effects of a proposed project on other parts of the firm. as the illicit drug illicit drug Street drug, see there , the compounds may ``participate in the subjective feelings associated with eating chocolate,'' scientists said in a study released today.

Not that chocolate was previously considered drug-free. The treat contains a respectable helping of caffeine and theobromine the•o•bro•mine

A bitter, colorless alkaloid found in chocolate products and used as a diuretic, vasodilator, and myocardial stimulant.

theobromine

an alkaloid prepared from dried ripe seed of the tropical American tree , a cousin of caffeine. But chocolate seems to inspire a devotion that transcends the need for these stimulants, researchers observed in the journal Nature.

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``There is something about chocolate that people like it way beyond its texture, taste and smell," said Daniele Piomelli of the Neurosciences Institute The Neurosciences Institute is a nonprofit research institute that is focused upon "high risk - high payoff" research designed to discover the biological basis of higher-brain function in humans and other animals. in San Diego San Diego (săn dēā`gō), city (1990 pop. 1,110,549), seat of San Diego co., S Calif., on San Diego Bay; inc. 1850. San Diego includes the unincorporated communities of La Jolla and Spring Valley. Coronado is across the bay. . Last year, for example, Americans on average ate more than 11 pounds of chocolate per person, a greater amount than any other confection con•fec•tion

A sweetened medicinal compound. Also called electuary. .

In the study, which may give new meaning to the notion of inhaling a candy bar, Piomelli and his colleagues report that chocolate contains three compounds called cannabinoids Cannabinoids

The chemical compounds that are the active principles in marijuana.

Mentioned in: Marijuana , the same class of substances responsible for marijuana's sense of euphoria. However, studies with rat brain cells indicate that the Hershey-variety cannabinoids might only amplify the potency of natural cannabinoids already present in the brain, slowing their breakdown.

While not saying that the experiments are - scientifically speaking - fudged, other researchers were cautious about the new work.

Just because the chemicals are present in the chocolate doesn't mean they can survive the digestive process, let alone travel to the brain, said Bruce Stillings, senior vice president of scientific affairs for the Chocolate Manufacturers Association.

``There have been many theories over the years as to why people love chocolate," Stillings said. ``None of them have shown to be true."

There is also no evidence that chocolate's cannabinoids occur in high enough concentrations to matter biologically, or that they have the chemical stability to hang around in the body long enough to cause some pharmacological

effect, said Michael Walker, a professor of psychology and neuroscience at Brown University in Providence, R.I., who studies cannabinoids.

``While the findings from Piomelli's laboratory are very interesting, it would be premature to conclude that eating chocolate leads to anything like a marijuana high,'' he said.

Piomelli, who describes himself as a ``moderate chocolate consumer,'' acknowledges these concerns. On a larger scale, he said he hopes this research will encourage other scientists to examine deeper questions about food chemistry.

``It is intriguing and important to understand why people crave certain types of foods,'' he said.