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Metabotype Concept: Flexibility, Usefulness and Meaning in Different Biological Populations

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1. Introduction

Metabolism represents a junction system in biological body receiving cumulated signals from upstream (genome, transcriptome, proteome) and downstream (environment) systems. This median position of the metabolic system makes it to be very sensitive toward internal and external signals resulting in its regulatory role in physiological homeostasy and adaptive responses to endogenous and exogenous factors. These factors have initiation, modulation or pressure effects on the biological organisms and species which adapt, resist or react through different types of metabolisms based on different synthesis and regulation levels of metabolites (Wilson, 2009).

These characteristics give to metabolism a flexibility that may be described by means of four variability criteria (Fig. 1a): presence-absence of metabolites, concentration levels, relative levels or ratios between metabolites, and metabolic profiles characterizing different structural and functional states in biosystems through different metabolites' levels. Presence-absence of metabolites is a qualitative criterion that concerns metabolites that are stimulated by particular internal biological states (species physiology, disease, stress, etc.) or external governing factors (climate, threat, etc.). Beyond this binary aspect of metabolic responses, increase or decrease in concentration levels of some metabolites can be sensitive responses to the degree of a governing implicit factor (e.g. wounding, toxin, pollutant exposure levels, etc.). More precision on association between biological state and governing factor can be extracted from concentration ratios between sensitive metabolites. Complex situations integrating many interactive factors can be characterized by metabolic profiles in which several metabolites levels increase or decrease compared with control conditions or neutral situations.

The four metabolic variability criteria can be used separately or in association to provide reliable pictures on different metabolic phenotypes of a biological system; such pictures characterizing the metabolic phenotypes are called metabotypes (or chemotypes) (Fig. 1a). Identifications of relationships between metabotypes and quantitative or qualitative control factors lead to the concept of metabolic markers. Metabolic markers can be used to anticipate (predict), alert and control responses and states of different components in biological systems. These components include cells, biofluids, tissues, organisms and biological populations or species.
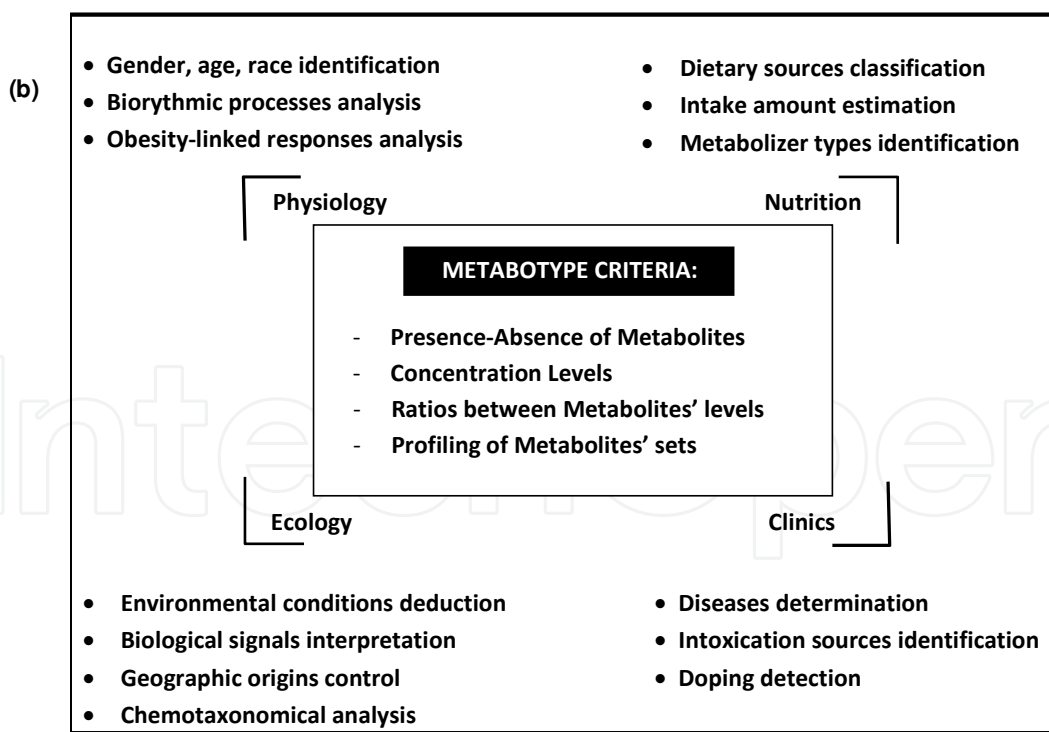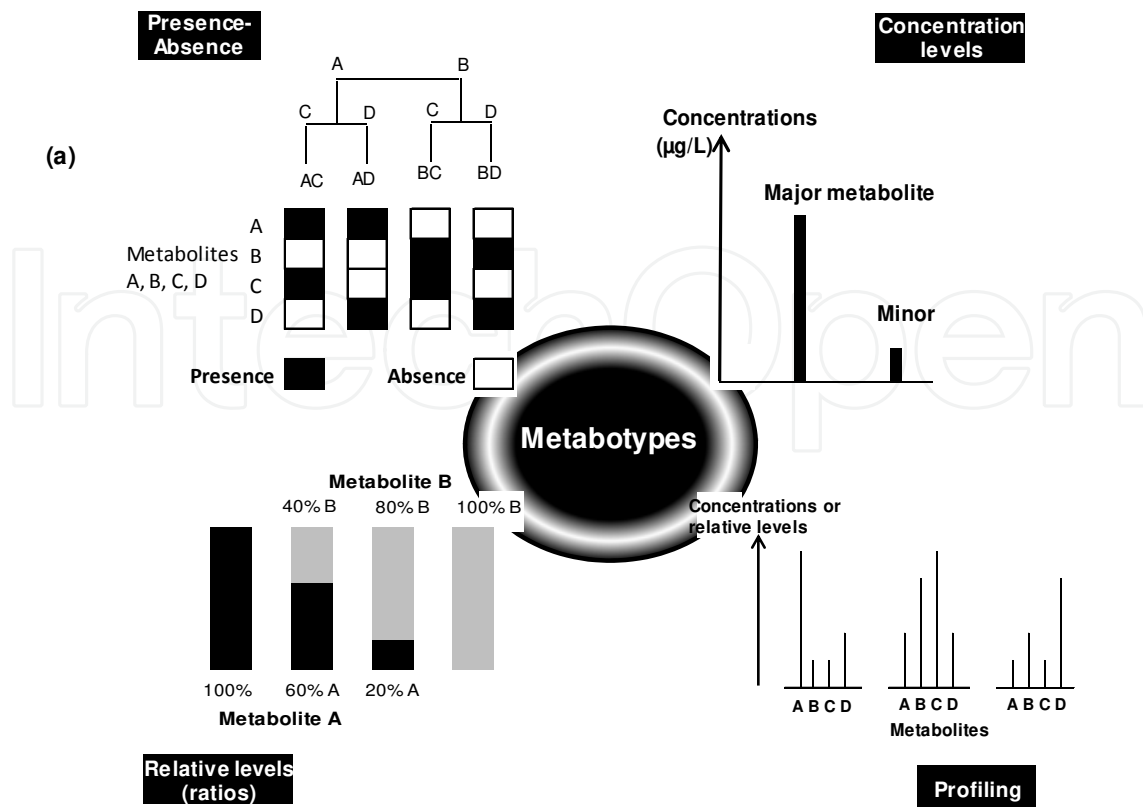
Fig. 1. (a) Schematic representations of four metabotype definitions based on occurrences, concentration levels, ratios and profiles of metabolites in biological matrices. (b) Interest of the four metabotype criteria to develop biomarkers in different biological fields (physiology, nutrition, clinics, ecology).

This chapter illustrates the usefulness of metabotype concept as flexible biomarkers in different biological fields: physiology, clinics, nutrition and ecology (Fig. 1b): in physiology, metabotypes can be markers of biological varieties, gender, aging, biorhythms, etc. (Bell et al., 1991; Bollard et al., 2001; Duncan et al., 2007 ; Holmes et al., 2008). In nutrition, different metabotypes can be correlated to different diets; secondary metabolites, particularly produced by plants, represent strong biomarkers of consumed fruits, herbals and legumes (Kaufman et al., 1997; Yanez, 2008). In clinical field, different metabotypes can be indicator of different disease types, intoxication levels or doping sources (Timbrell, 1998; Kintz et al., 1999; Barderas et al., 2011). In ecology, different plant metabotypes based on secondary metabolites are indicative of different physicochemical conditions (temperature, light, humidity, oxidative stress, etc.) or biological relationships (parasite or herbivore attacks, pollination states, etc.) submitted by the plant (Semmar et al., 2008). Also, metabotype (or chemotype) concept is helpfully used in chemotaxonomy to highlight chemical polymorphism such as chemical varieties of floral colors (Torskangerpoll et al., 2005).

2. Physiological metabotypes

2.1 Metabotypes based on metabolites' occurrences

Presence-absence of metabolites is an efficient metabolomic parameter to characterize different biological species or varieties known to represent different physiological systems (Fig. 2): for instance, benzoic acid is metabolized almost entirely to hippuric acid by primates, rodents and rabbits (mammals) (Fig. 2a). However, it is excreted unchanged and as glucuronide by insects, birds and reptiles (Jones, 1982). Similarly, the excretion of phenylacetic acid (Fig. 2b) as the parent compound or as glutamine, glycine or taurine conjugates is species-dependent (Robertson et al., 2002): for instance, it is excreted as phenylacetyl glutamine in humans and phenylacetylglycine in rats.

Comparison of ^{1}H NMR spectra from control B6C3F1 mouse urines with those of control SD rat urines revealed the presence of guanidinoacetic acid and trimethylamine in mouse but their absence in rat (Bollard et al., 2005).

2.2 Metabotypes based on concentration levels

In animals, several metabolites have been found to quantitatively vary in relation to gender, age, body weight, etc. :

Plasma NMR profiles showed reduced concentration of the triglyceride resonances in female rats compared with male counterparts (Fig.3a) (Stanley, 2002).

In humans, urinary citrate levels have been found to be generally greater in females (Fig.3b) (Hodgkinson, 1962). Moreover, serum cholesterol levels are different between sexes at matched ages, with in general, pre-menopausal females being less susceptible to high cholesterol levels than males (Fig.3c) (Joossen, 1988).

Oestrogenic hormones are known to exhibit some control over the kidney: in humans, glycosuria (glucose in urine) is common in pregnant females (Fig.3d) (Davison and Hytten, 1975). In the rat, pregnancy is associated with an increased glucose filtration rate and decreased urine flow rate (Bishop and Green, 1980).
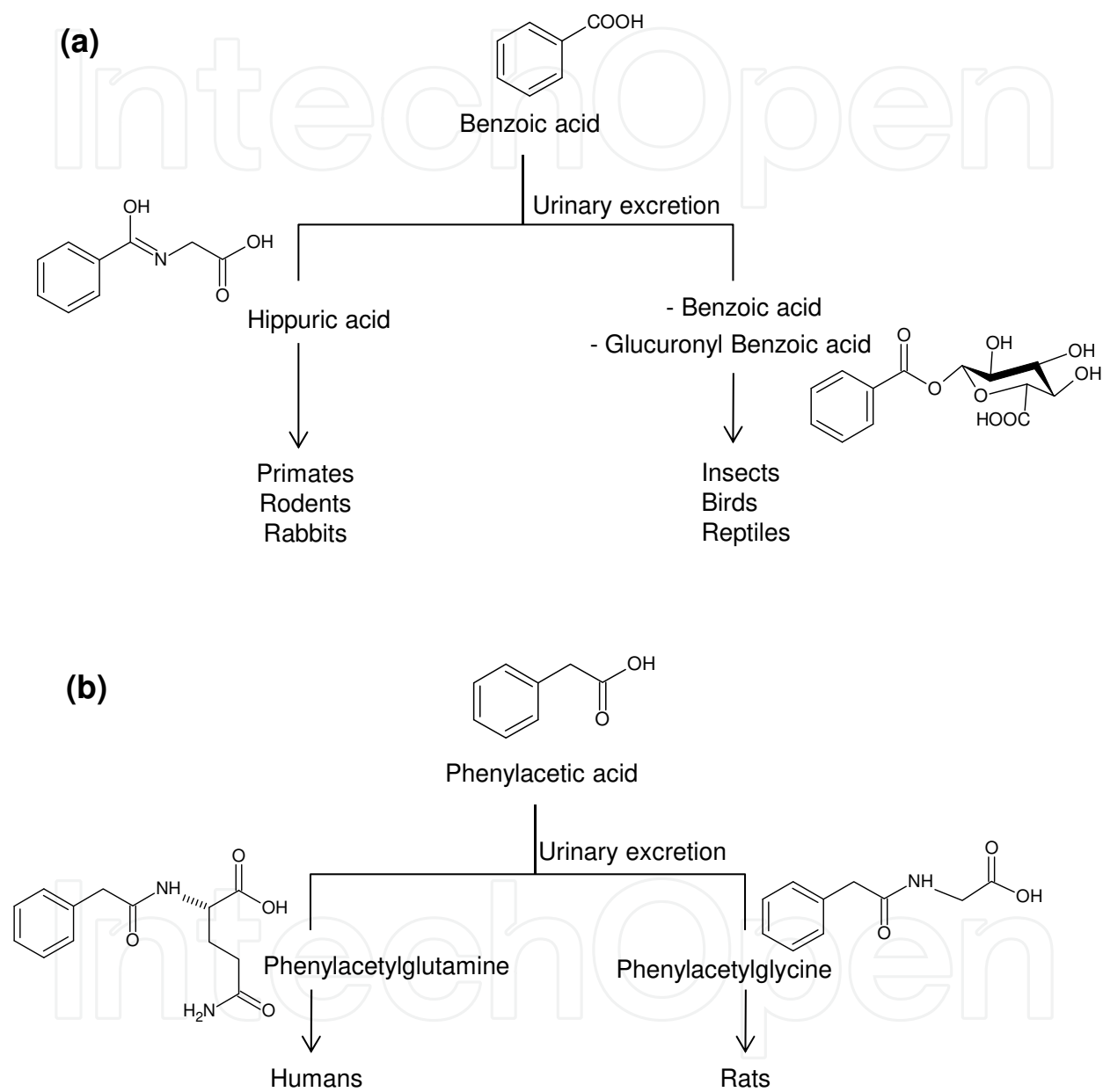
Fig. 2. Metabolic derivatives of benzoic acid (a) and phenylacetic acid (b) excreted in urine of human and animal species and from which specific metabotypes can be defined.
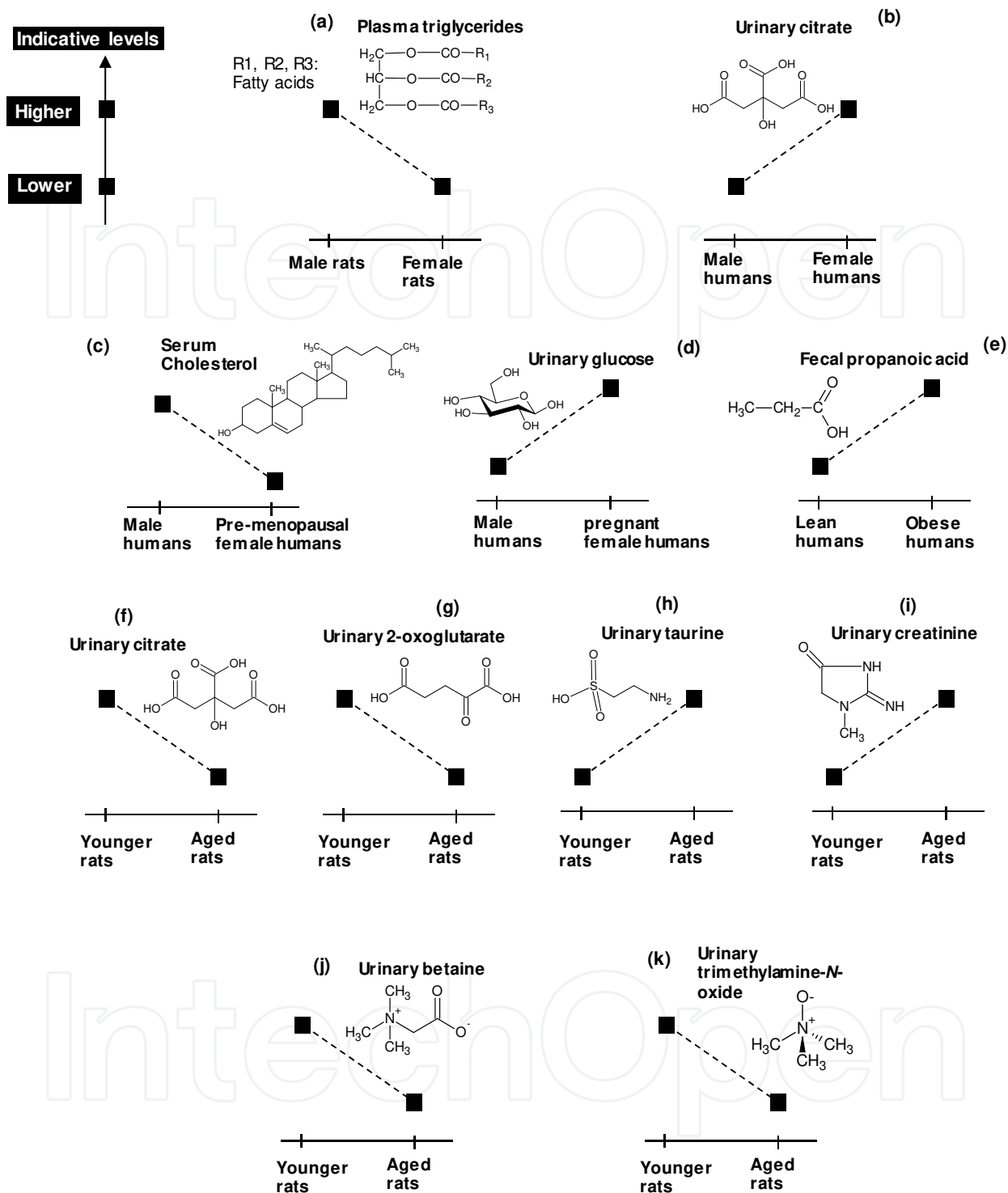
Fig. 3. Quantitative variations of different metabolites in different biological matrices (serum, plasma, urine) in relation to different physiological factors (gender, age, sexual maturity degree, body weight).

Differences in the concentrations of stool short-chain fatty acids (SCFA) between the lean, overweight and obese human subjects have been shown to be important (Schwiertz et al., 2010): the mean of total SCFA concentration in fecal samples of obese volunteers was by

more than 20% higher in total than of lean volunteers. The highest increase was seen for propionate (Fig. 3e) with 41%.

The age is known to result in numerous physiological changes that are reflected by physical and metabolic changes:

Previous studies have shown that ageing rats decrease their excretion of citrate and 2-oxoglutarate versus increase in their taurine and creatinine output (Fig.3f-i) (Bell et al., 1991). The increase in creatinine with age (up to 6 months) may be associated with the increase output from the muscle of larger rats as well as an age-related increase in the glomerular filtration rate (the rat kidney becomes fully functional at 3 months).

Moreover, in young rats (1 month or less), the urinary excretion of trimethylglycine (betaine) and trimethylamine-*N*-oxide was higher than in older rats (Fig. 3j, k). The increased level of betaine and trimethylamine-*N*-oxide was higher than in older rats. The increased level of betaine in the urine of young rats may be due to high choline levels (Bell et al., 1991). Betaine (or *N*-trimethylglycine) results from oxidation of choline; it represents a reservoir of methyl groups and plays the role of methyl donor in the synthesis of methionine in mammals.

In plant world, secondary metabolites showed significant variations in relation to species, age and maturity level: for instance, among two birch species, Silver Birch (*Betula pendula*) does not emit sesquiterpenes (SQT), while Downy Birch (*B. pubescens*) does (Hakola et al., 2001). Moreover, older trees *B. pubescens* emitted greater quantities and higher proportions of SQT than younger ones. In the common snapdragon, *Antirrhinum majus*, the emission of methyl benzoate (MeBA) is increased in pollination period (Dudareva et al., 2000); this may serve as guide for bees to find their way inside the flower. After pollination, emission of MeBA decreases dramatically.

SCFA are produced by the intestinal microbiota which represent a large part of bacteria belonging to the phyla of Firmicus, Bacteroides, Actinobacteria, Proeobacteria and Verrumicrobia (Zoetendal et al., 2008). Some phyla were characterized by high level production of some metabolites: for instance, Bacteroides phylum produces high levels of acetate and propionate, whereas several members of the Firmicus phylum produce high amount of butyrates (Maslowski et al., 2009).

2.3 Metabotypes based on ratios between concentration levels

Metabolic ratios between concentration levels of structurally close metabolites have been used to characterize different biological states in human populations:

Menstruation affects the *N*-oxidation of trimethylamine resulting in a fall in the ratio of trimethylamine-*N*-oxide on trimethylamine in the urine (Fig. 4a) (Zhang et al., 1996).

A metabolomic study on different body weight human subjects showed that leaner people had higher ratios of acetate to butyrate and propionate (Fig. 4b) (Schwiertz et al., 2010, Duncan et al., 2007).

It has been shown that in a Caucasian population, the urinary metabolic ratio of 6 *β*-hydroxycortisol to cortisol was significantly increased in females compared to males (Fig. 4c) (Lutz et al., 2010). This ratio is used as an endogenous marker for CYP3A activity.
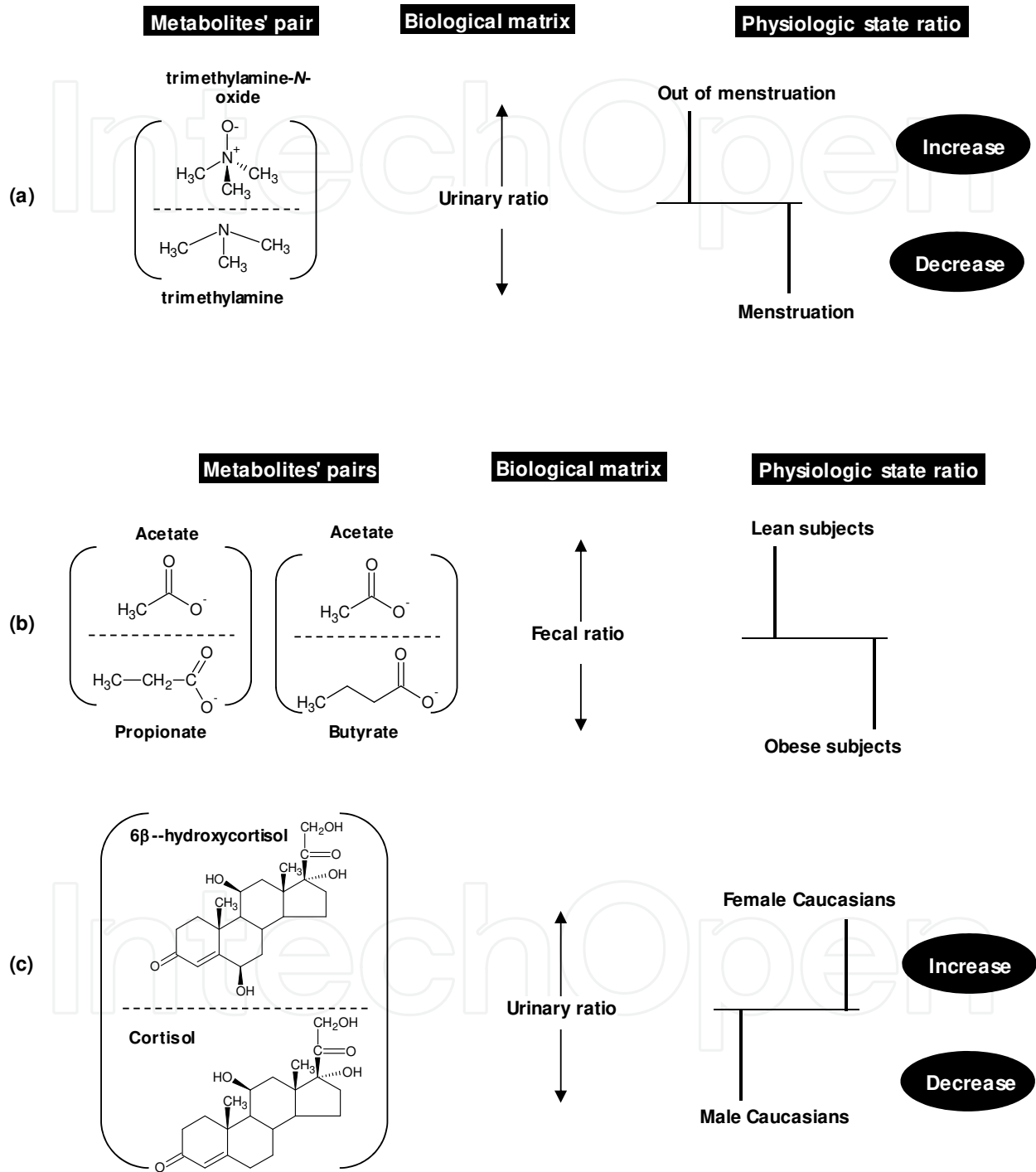
Fig. 4. Metabolic ratios between structurally close metabolites providing biochemical discriminations between different biological states in human populations.

2.4 Metabotypes based on profiling

In animals, gender, species or races have been characterized by specific profiles of organic acid derivatives (amine, tricarboxylic) which are produced at relatively high or low levels:

Metabolic profiling has been applied to characterize genders in rats: elevated levels of bile acid metabolites in urine of female rats reflected increased rate of cholesterol and bile acid synthesis compared to males (Stanley, 2002).

Metabolomic differences between rats and mice have also been highlighted by NMR: ^{1}H-NMR spectra of urines from B6C3F1 mice and Sprague-Dawley (SD) rats revealed consistently higher levels of formate, creatinine, hippurate, dimethylglycine, dimethylamine, fumarate, 2-oxoglutarate and citrate versus lower levels of taurine and betaine (i.e. *N*-trimethylglycine) in the rats compared to mice (Bollard et al., 2005).

^{1}H-NMR spectra of urine samples showed that the genetic strain Alpk:ApfCD mice had relatively   higher levels of 2-oxoglutarate, citrate, trimethylamine-*N*-oxide and guanidinoacetic acid, whilst C57BL107 mice had higher levels of taurine, creatinine, dimethylamine and trimethylamine (Fig. 5) (Gavaghan et al., 1996).
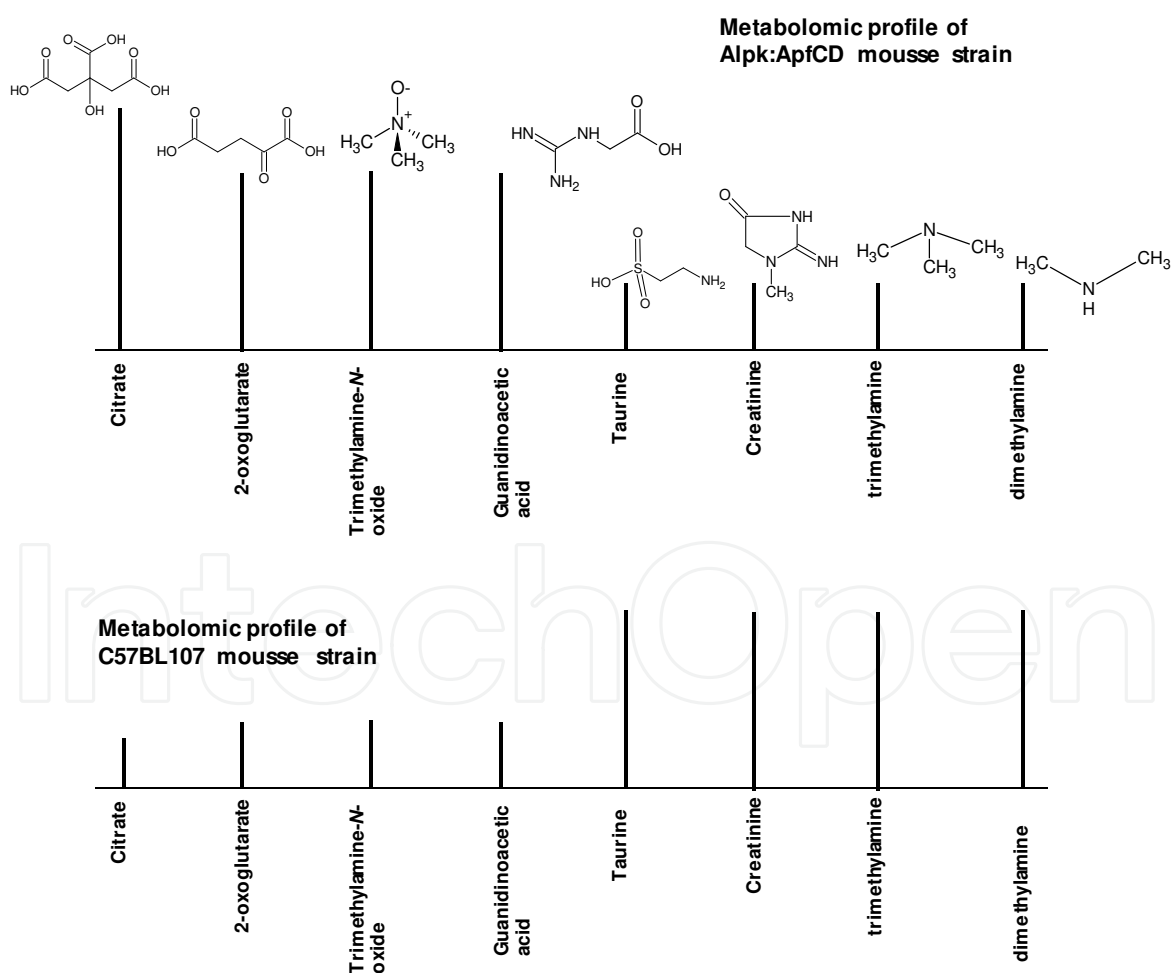

Fig. 5. Metabolomic profiles representing two genetic strains of mice on the basis of relative levels of several metabolites. Bar heights are indicative of relatively higher or lower concentrations depending on mousse strains (Gavaghan et al., 1996).

Metabolic phenotypes analysis has been applied to characterize particular laboratory animal varieties including "germfree" (GF) specimens. GF is the highest quality level of laboratory animals in which there are no any detectable microorganisms in contrast to those commonly known as "SPF", which is merely free of specific pathogens. Germfree animals are especially useful in the researches concerning genetic engineering, cancer, normal intestine flora, immunology and nutrition.

Aqueous extract profiles of gut tissues from GF mice were markedly different from those of conventional mice (Claus et al., 2008) (Fig. 6):

i.   The metabolite profile of the duodenum from GF mice was mainly characterized by higher levels of tauro-conjugated bile acids (TCBAs) and alanine versus lower levels of glycerophosphocoline (GPC) (Fig. 6a) when compared with conventional mice.
ii.   The jejunal tissue of GF group had higher levels of creatine and TCBAs versus lower levels of tyrosine (Fig. 6b).
iii. The ileum of GF mice was characterized by a higher level of TBCAs and lower levels of glutamate, fumarate, lacate, phophocholine and alanine when compared with the ileum from conventional mice (Fig. 6c).
iv.   The metabolic profile of the colon from GF mice revealed a high level in a complex carbohydrate identified as raffinose, and lower levels of lactate, creatine, 5-aminovalerate, propionate, glutamine, myo-inositol, scyllo-inositol, GPC, phosphocholine, choline, formate, uracile and fumarate (Fig. 6d) (Monero and Arus, 1996).

Metabolic profiles of ileum and particularly colon in GF mice were markedly more affected than those of duodenum and jejunum. This reflects the higher microbial loads found in ileum and colon (Dunne, 2001). The lower levels of choline and its phosphorylated derivatives, GPC and phosphocholine (Fig. 6a, c-d) were reported to be likely due to the disturbance of the membrane of colonocytes in GF mice (Claus et al., 2008). Also, the accumulation of the trisaccharide, raffinose, can be a possible consequence of this disruption. In GF animals, raffinose seems to be able to cross the epithelial membrane and accumulates in colonocytes where it induces a rise in osmotic pressure. This phenomenon provokes a well-known signaling cascade that leads to the release of the mobile osmolytes: GPC, myo-inositol and scyllo-inositol.

Beyond static analysis, kinetic metabolic profiling is applied in chronobiology and pharmacokinetics in relation to intrinsic or extrinsic factors (e.g diurnal variations):

In SD rat, ^{1}H-NMR profiles of urinary samples collected during the day showed lower levels of hippurate, taurine, and creatinine together with elevated levels of glucose, succinate, dimethylglycine, glycine, creatine and betaine compared with urine collected during the night (Bollard et al., 2001).

Male rats secrete growth hormone in an "on-off" episodic rhythm between which there are periods when there are no detectable levels of the hormone. Growth hormone secretion in the female rat is "continuous" since hormone levels are always present (Czerniak, 2001).

In women subjects, plasmatic cortisol stimulated by synacthen (synthetic ACTH) showed obesity level-dependent kinetic profiles (Fig. 7) (Semmar et al., 2005a): the secretion and elimination of cortisol were more rapid and higher in the most obese followed by intermediate obese then non-obese subjects.
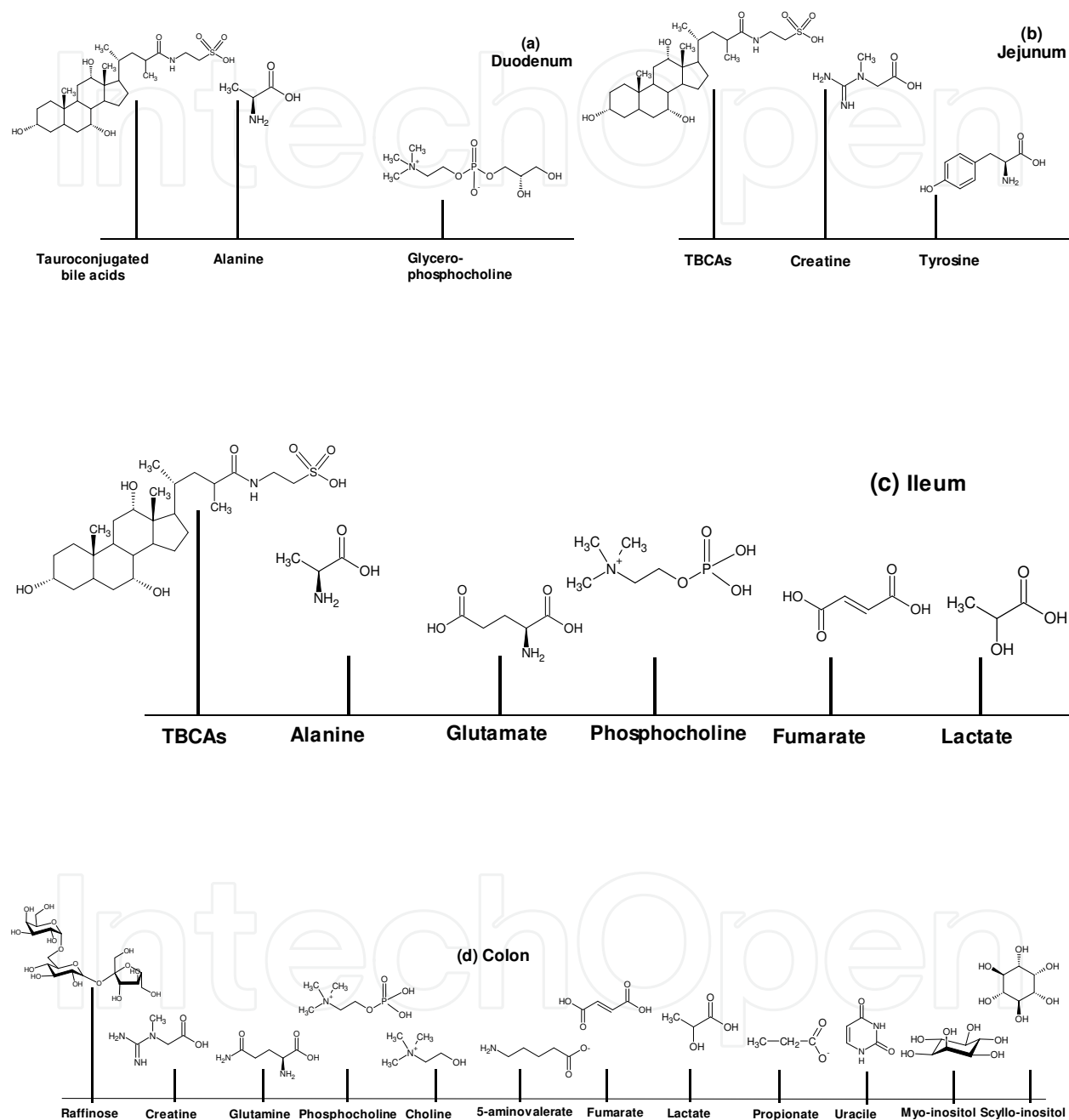
Fig. 6. Metabolomic profiling of different gut tissues of germ free (GF) mice (Monero and Arus, 1996). Bar heights are indicative of relatively higher or lower concentrations in GF compared with conventional mice (details are given in text).
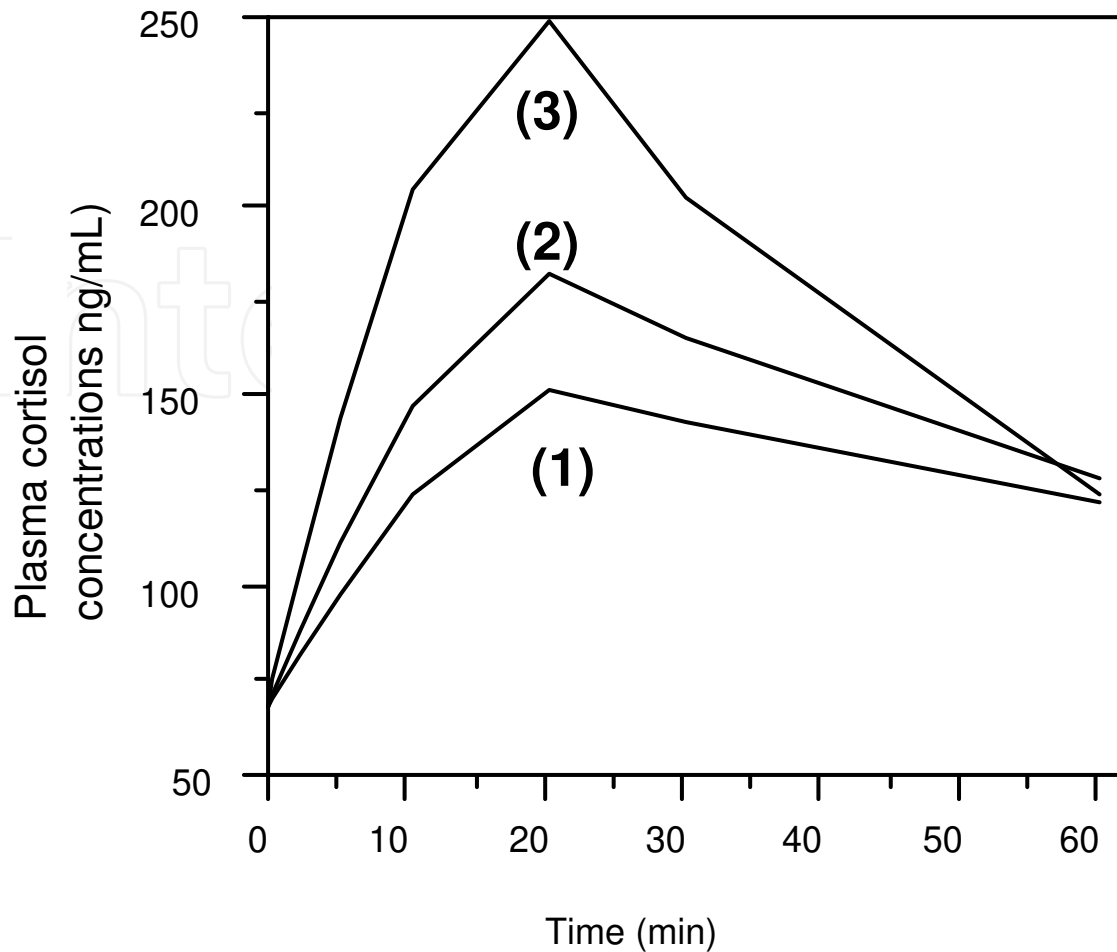
Fig. 7. Kinetic profiles of plasma cortisol concentrations of three body weight levels women populations, highlighting compensatory process between secretion and elimination in relation to obesity levels. (1), (2), (3) corresponds to non-obese, intermediate obese and extreme obese populations, respectively (Semmar et al., 2005).

3. Dietary metabotypes

3.1 Metabotypes based on occurrence of metabolites

Fruits and legumes can be generally characterized by occurrences of specific or abundant secondary metabolites belonging to flavonoids and terpenoids. For instance, in flavonoid class, flavonols, flavones, flavanones, isoflavones, flavanols and anthocyanins are widely present in onions, parsley, citrus fruits, leguminous plants, green tea and blackberry, respectively (Fig. 8) (Majewska et al., 2011; Holden et al., 2005; Kaufman et al., 1997).

Among the flavonols, quercetin is widely present in the plant world. It occurs as different glycosidic forms with quercetin-3-rhamnoglucoside (or rutin) being one of the most widespread forms (Fig. 9d). The different forms of quercetin glycosides have been found to be good markers in food quality control: in onions, quercetin is bound to one or two glucoses to give quercetin-4'-glucoside and quercetin-3,4'-glucoside (Fig. 9c); apples and berries, however, have been characterized by the occurrence of quercetin-3-galactoside and quercetin-3- arabinoside, respectively (Fig. 9b, a) (Kühnau, 1976; Zheng et al., 2003).
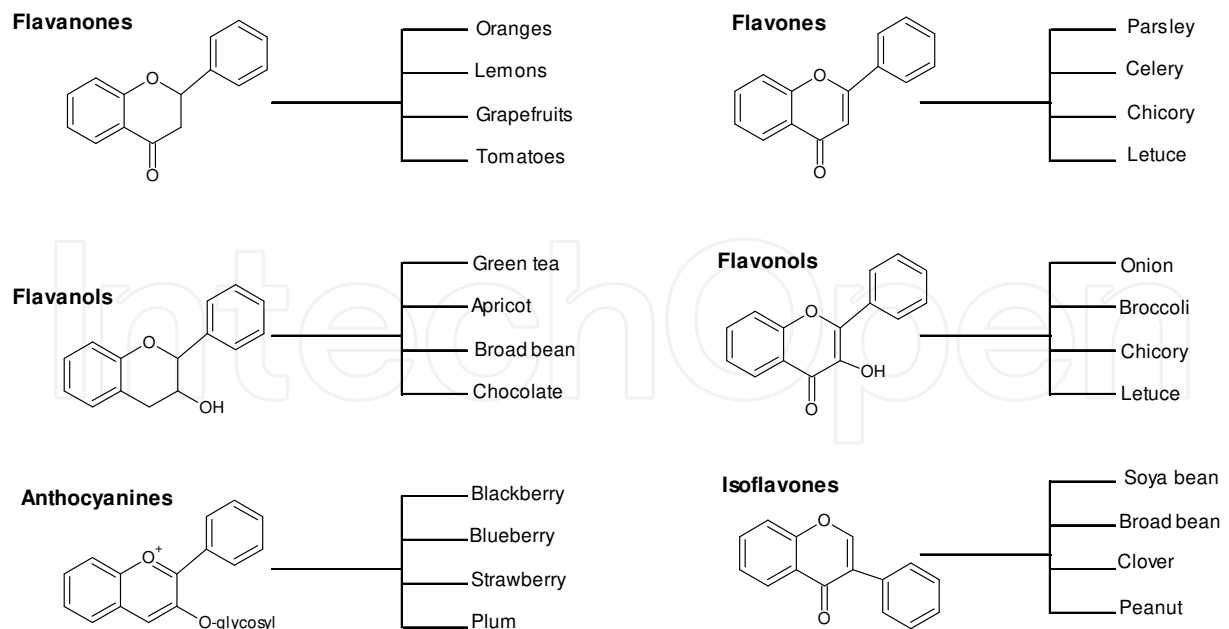
Fig. 8. Metabolic characterization of different dietary plants on the basis of high occurrences of produced flavonoid classes in their tissues.
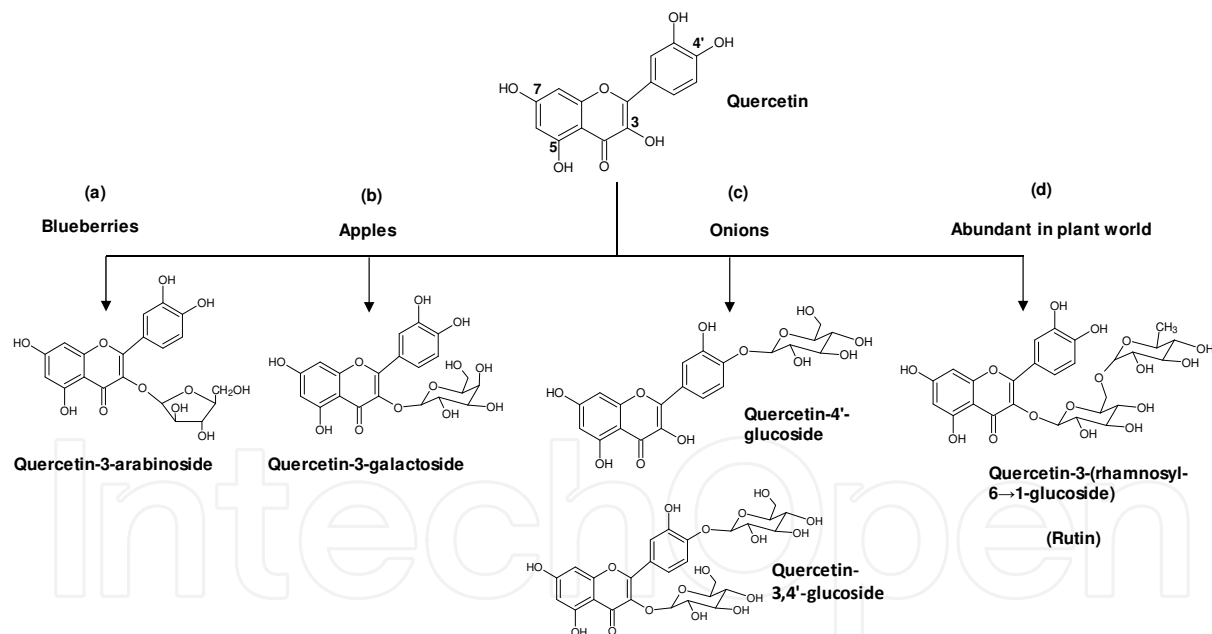

Fig. 9. Metabolic characterization of some dietary plants on the basis of abundant quercetin glycosides in their tissues.

Flavanones are flavonoids particularly abundant in citrus and vary qualitatively in relation to fruit types (Mouly et al., 1998; Kawaii et al., 1999; Gattuso et al., 2007): The lemon (*Citrus limon*) can be distinguished by production of eriocitrin and hesperidin (Fig. 10 a), whereas in grapefruits, naringin predominates in presence of narirutin (Fig. 10c). In oranges (*Citrus sinensis*) and mandarins (*Citrus reticulata*), hesperidin is the major flavanone in presence of narirutin (Fig. 10b).
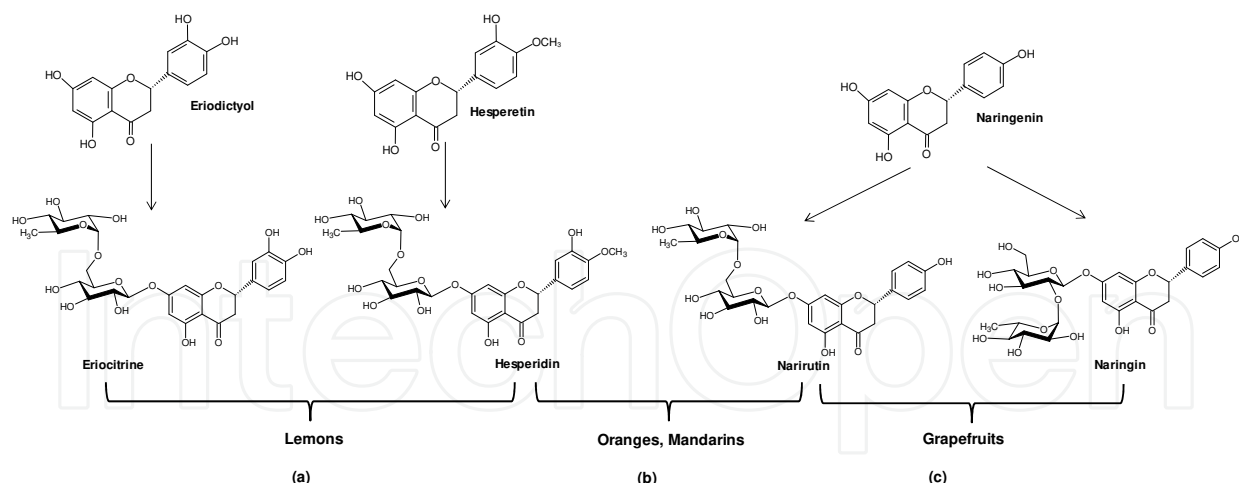
Fig. 10. Metabolomic distinction between different *Citrus* species based on major flavanone glycosides in fruit juices.

3.2 Metabotypes based on concentration levels

High amounts or concentrations of secondary metabolites have been used to control dietary plant varieties as well as to diagnose animal or human diets:

Onions are rich source of quercetin-4′-*O*-glucoside and quercetin-3,4′-*O*-diglucoside. Mullen et al. (2006) reported that 270 g of lightly fried onions contains 275 µmol of flavonol glucosides with the main constituents being 143 µmol of the 4′-*O*-glucoside and 107 µmol of the 3,4′-*O*-diglucoside (Fig. 11).
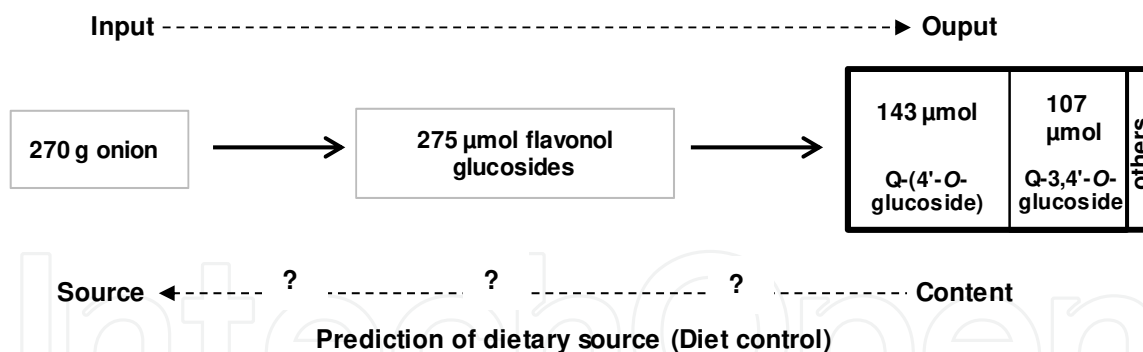

Fig. 11. Abundance of flavonol glucosides in onion particularly dominated by 4′-*O*-glucoside and 3,4′-glucoside of quercetin (Q) (Mullen et al., 2006).

In human subjects, plasma quercetin was found to be a good marker of dietary intake because its concentrations increase with increasing ingested dose (Radtke et al., 2002). In a strictly controlled dietary intervention study, 77 health human subjects consumed either 170 or 850 g of fruits, vegetable and berries daily. Quercetin intake was calculated to be 3 to 24 mg/d on the respective diets. The mean ± SD of plasma quercetin concentration was 78 ± 56 nmol/L during the habitual diet; it decreased to 70% during the low-vegetable diet and increased to 170% during the high-vegetable diet (Freese et al., 2002) (Fig. 12).
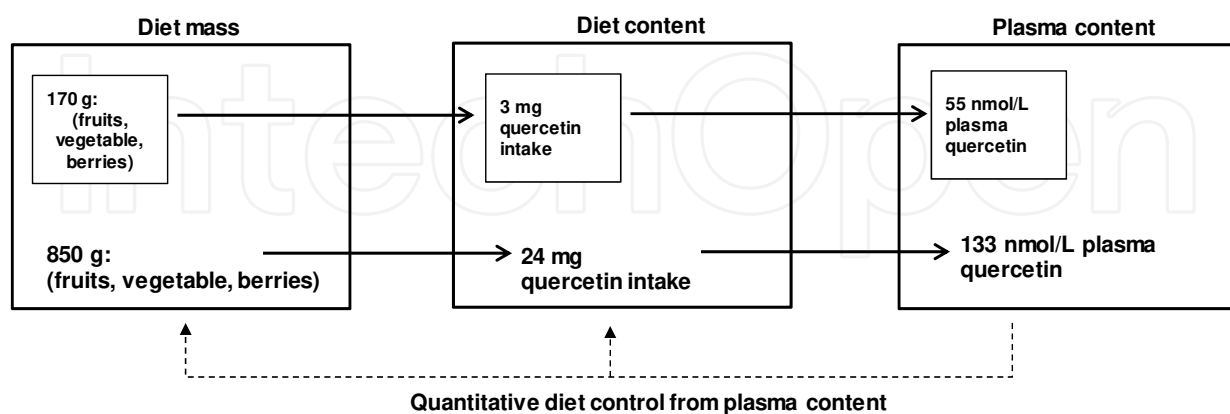
Fig. 12. Increase in plasma quercetin concentration (nmol/L) in relation to diet mass (g) and its total quercetin content (mg) in human population (Freese et al., 2002).

Ingestion of 200 mL of coffee has been reported to increase the plasma conjugated caffeic acid level in human subjects (Nardini et al., 2002).

Following consumption of both green and black tea, human urinary samples showed significant increases in level of hippuric acid and 4-hydroxyphenylacetic acid (Mulder et al., 2005).

Excretion of creatinine in the urine of rats, per unit of skeletal muscle mass, was found to be promoted by food deprivation (Rikimaru et al., 1989).

3.3 Metabotypes based on ratios between concentration levels

Legumes are known to be important dietary sources of isoflavones (Liggins et al., 2000). Women subjects having consumed isoflavones from soymilk powder were classified into two excretion levels according to the relative contents of daidzein and genistein recovered in feces and urine (Fig. 13) (Xu et al., 1995): in strong isoflavone excreters, the percentages of daidzein and genistein recovered in feces were 10 and 20 times greater than those weak excreters. Isoflavones recovered in urine for 48h revealed excretions of daidzein and genistein which were 2 and 3.5 times in high excreters than low excreters.

In analogous study, human subjects consumed a single dose of strawberries (250 g), raspberries (225 g) and walnuts (35 g), all of which contain ellagitannins (hydrolysable tannins). Intakes resulted in urinary excretion of a derivative, urolithin B-3-*O*-glucuronide, in quantities equivalent to 2.8% (strawberries), 3.4% (rasperries) and 16.6% (walnuts) regarding the ingested ellagitannins (Fig. 14) (Cerda et al., 2005).
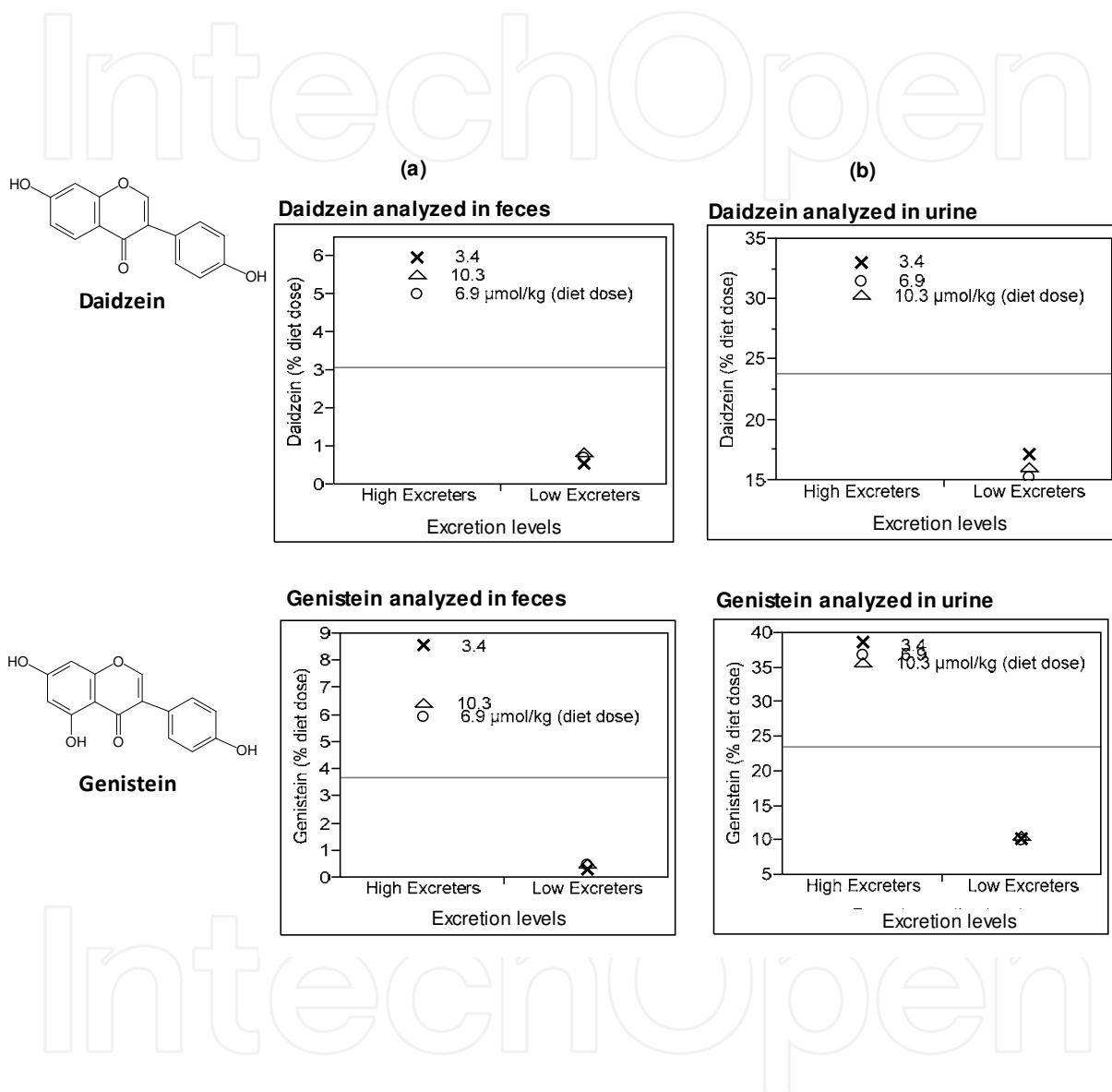
Fig. 13. Metabolomic classification of human subjects into two excretion levels of isoflavones (daizein and genistein) according to percentages of faecal and urinary isoflavones compared to diet dose. Low and high excreters had low and high excretion percentages of isoflavones, respectively.
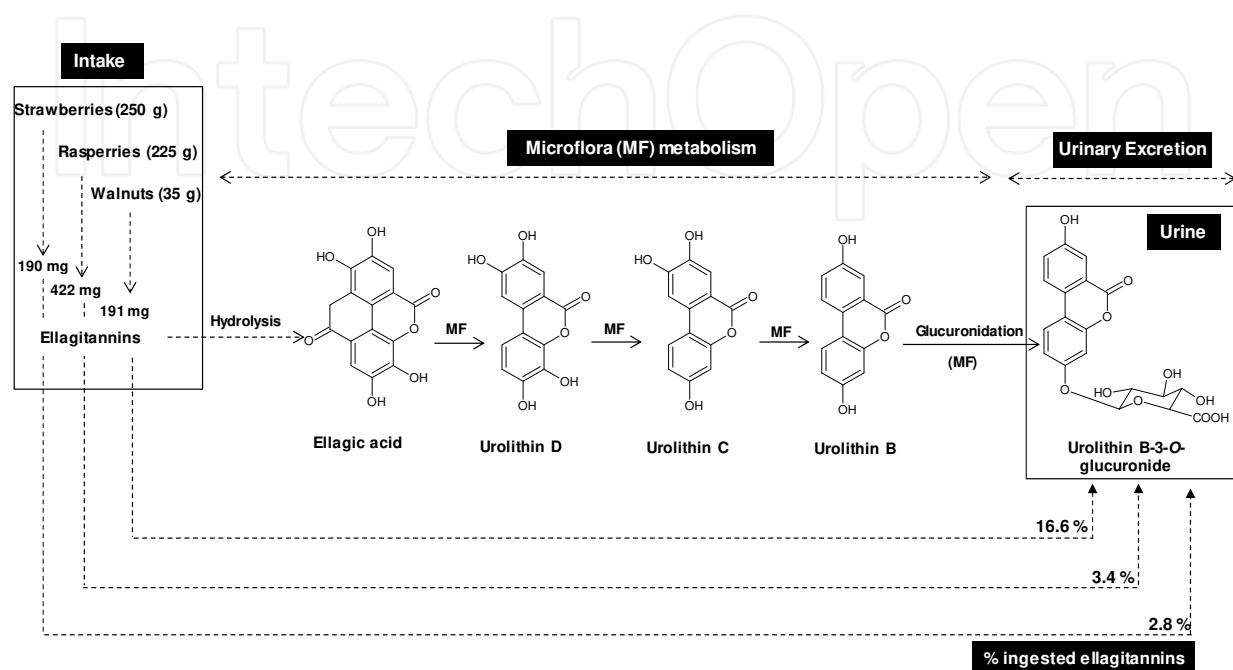
Fig. 14. Characterization of three tannin-rich diets by the excretion percentages of a urinary metabolite (urolithin B-3-*O*-glucuronide) referred to the ingested dose of ellagitannins (Cerda et al., 2005).

3.4 Metabotypes based on profiling

Flavanone enantiomers profiles have been analysed in different fruit juices and their concentrations have been found to be efficient biomarkers of the plant dietary source (Fig. 15) (Yanez et al. 2008):

-   Orange juices contained the highest concentrations of (2R)- and (2S)-hesperidin;
-   Conventional and organic grapefruit juices contained the highest concentrations of R(+)- and S(-)-hesperetin, and (2R)- and (2S)-naringin.
-   Conventional and organic tomato juices showed the highest levels of R(+)- and S(-)-naringenin. Also, the chemical profiles of tomato juices showed the co-occurrences of the eight enantiomers. Organic juice can be distinguished by relatively higher levels of naringin enantiomers.
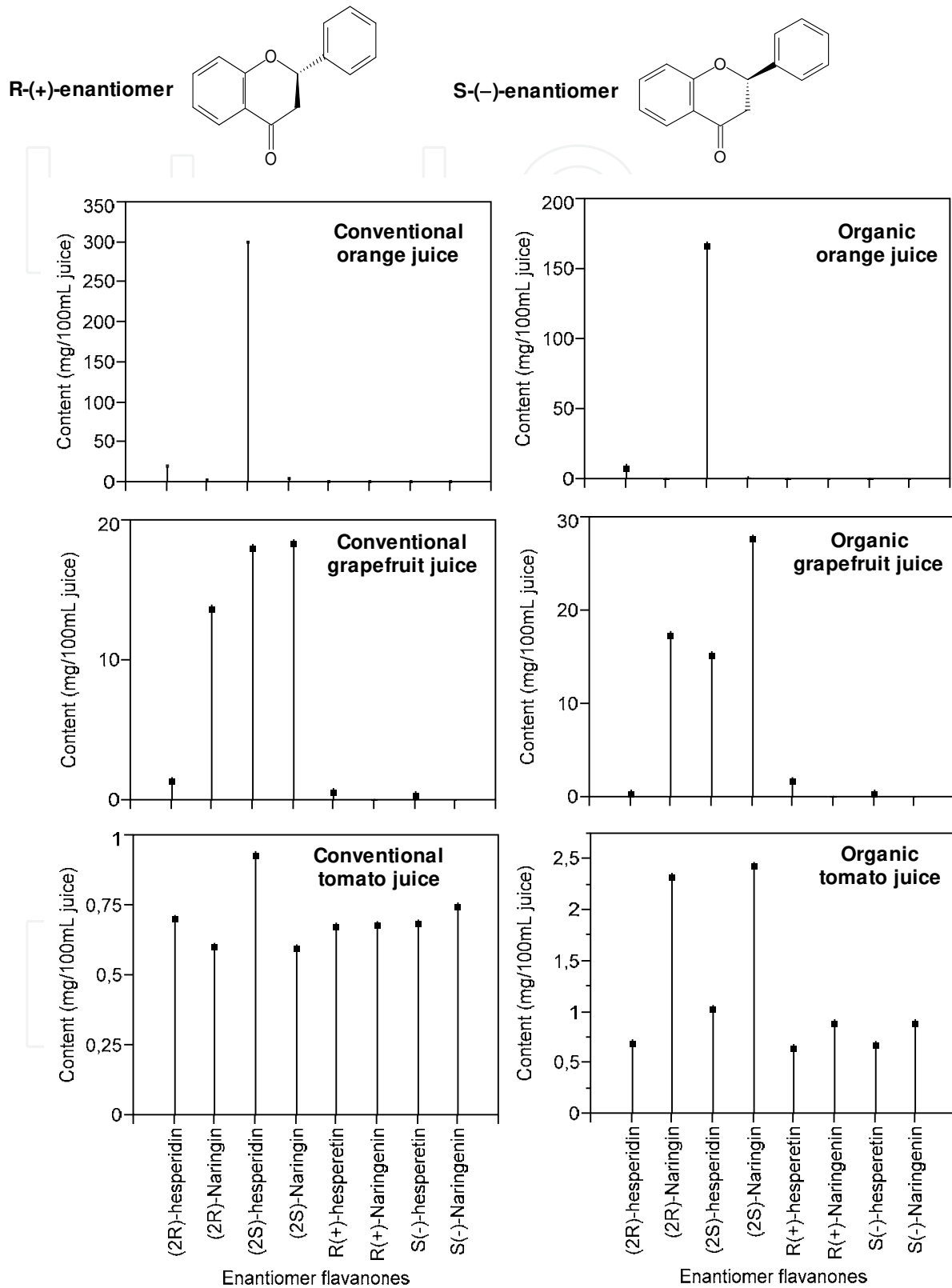
Fig. 15. Metabolomic characterization of different fruit juices by their chiral flavanone profiles.

Human subjects ingesting three times two cups of coffee at 4-h intervals had urinary profiles containing ferulic, isoferulic, dihydroferulic, 3-methoxy-4-hydroxybenzoic, hippuric and 3-hydroxyhippuric acids (Rechner et al., 2001).

After ingestion of 270 g of lightly fried onion by human subjects, plasma and urine samples collected over 24h showed very different metabolic profiles of concentrations (Fig. 16) (Mullen et al., 2006):
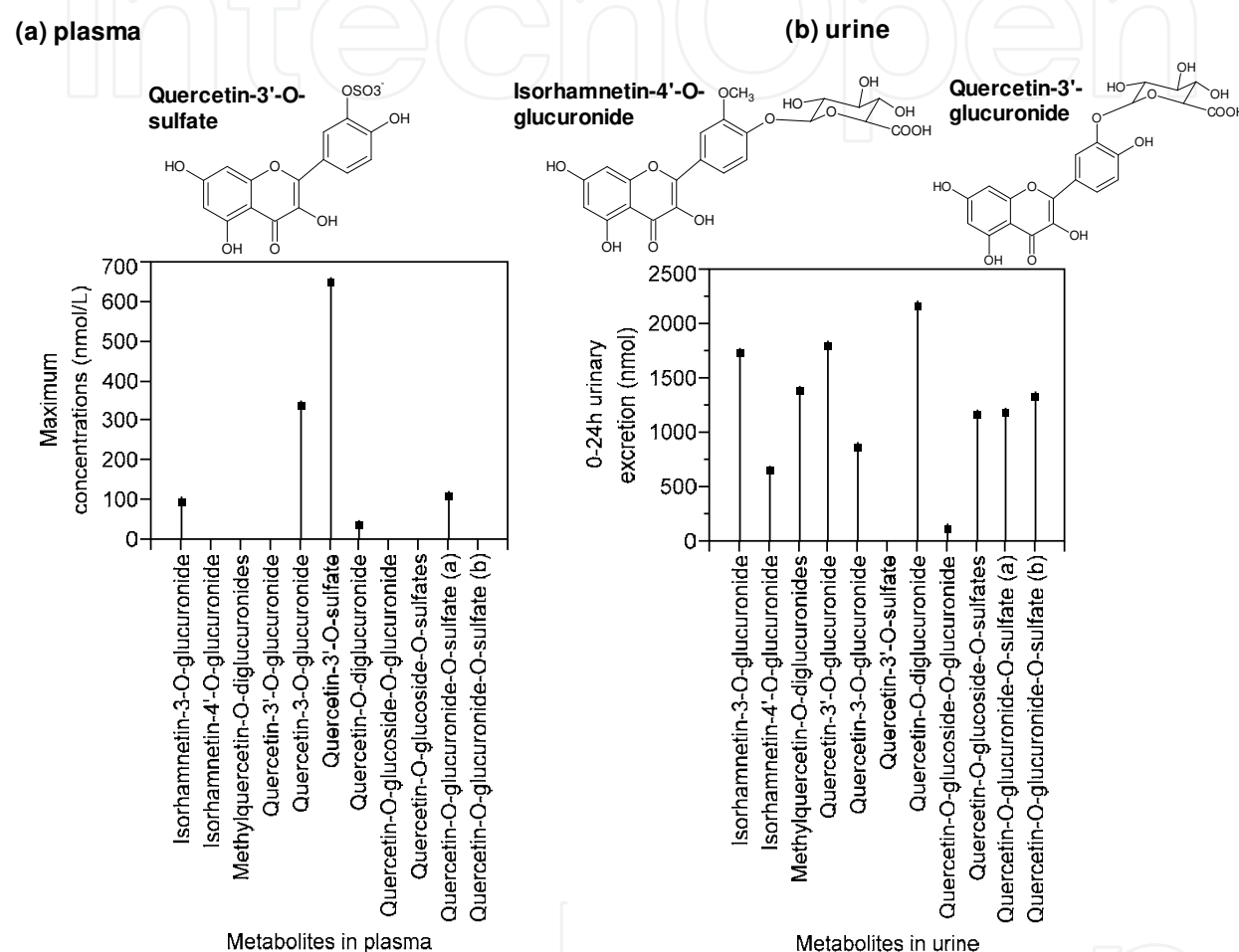

Fig. 16. Metabolic profiles of conjugated quercetin metabolites in plasma (a) and urine (b) following ingestion of lightly fried onion (270 g) by health human subjects (Mullen et al., 2006).

The main plasma metabolite, quercetin-3'-*O*-sulfate, was excreted only in trace quantities in urine while isorhamnetin-*O*-glucuronide and quercetin-*O*-diglucuronide that were minor components in plasma were major urinary metabolites (Fig. 16). Several other metabolites, including quercetin-3'-*O*-glucuronide and isorhamnetin-4'-*O*-glucuronide, which were present in trace quantities or absent from plasma were excreted in urine in substantial amounts.

In two separated human studies, and following the consumption of 200 g of strawberries (Mullen et al., 2008) or 200 g of blackberries (Felgines et al., 2005), the urinary contents were

characterized by pelargonidin and cyanidin metabolites profiles, respectively: following the consumption of strawberries, urine samples were characterized by the predominance of pelargonidin-3-*O*-glucose in presence of pelargonidin-*O*-glucuronides with small quantities of pelargonidin aglycone and a pelargonidin-*O*-sulfate. Following the consumption of blackberries, the urine had a cyanidin-based profile containing unmetabolized cyanidin-3-*O*-glucoside, a cyanidin-*O*-glucuronide and a 3'-*O*-methyl-cyanidin-*O*-glucuronide.

In obese human population, low and medium carbohydrate diets reduced the total SCFA concentrations in fecal matrix, compared with a maintenance (high carbohydrate) diet (Duncan et al., 2007). This decrease concerned also acetate, propionate, and valerate concentrations analysed in saddles (Fig. 17). Low and medium carbohydrate diets can be distinguished by lower butyrate concentrations; such a reduction is more marked under low diet than intermediate one.
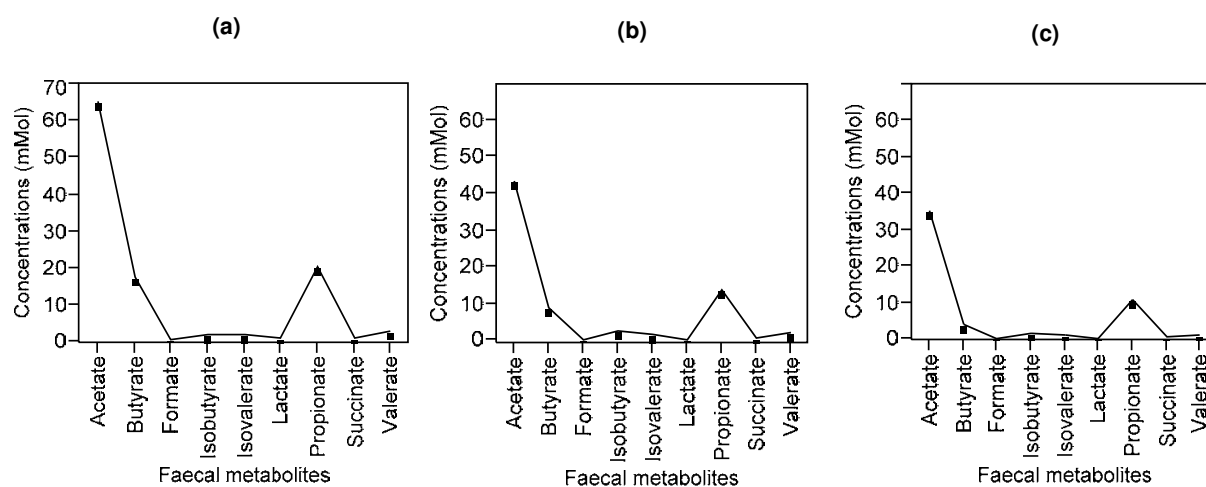

Fig. 17. Variations in concentrations of short chain fatty acids (SCFA) and lactate in stool samples of obese humans in relation to three carbohydrate levels diets (Ducan et al., 2007). (a), (b), (c) correspond to high, medium and low carbohydrate diet, respectively.

A previous study showed that SD rats deprived of water for 48h had elevated levels of creatinine and depleted levels of taurine, hippurate, 2-oxoglutarate, succinate and citrate (Clausing and Gottschalk, 1989). Water deprivation has a direct effect on osmoregulation implying variations in osmoregulators' levels as taurine.

4. Clinical metabotypes

Pathologies are known to induce changes in concentrations, regulation ratios and overall profiles of different metabolites that could be used to diagnose or characterize different diseases. Some examples will be given to illustrate the interest of different metabolomic criteria in clinical cases.

4.1 Metabotypes based on occurrences of metabolites

Occurrences of particular metabolites in biological matrices represent a strong metabolomic parameter to identify intoxication or doping sources:

Among endogenous metabolites, a particular attention has been paid to glutathione conjugates as potential markers of exposure (Van Weli et al., 1992). This is because glutathione (GS-H) detoxifies reactive chemicals (R-X) to which biological systems are exposed. The result of this conjugation (GSR) is the excretion of a variety of sulphured metabolites.

Concerning xenobiotics, chemicals that are reactive or are metabolized to intermediates reacting with DNA are of particular concern in relation to genotoxicity, and therefore, possible carcinogenicity:

In styrene industry, exposure to reactive alkylating agents can be diagnosed by analyzing DNA adducts, such as styrene oxide-O-6-guanine detected in white blood cells of exposed workers (Hemminki and Vodicka., 1995). Exposure to cyanide can be identified by rapid analysis of this toxin in blood of exposed individuals. However, free cyanide disappears rapidly from blood suggesting that a biological sample should be collected quickly, and analysis should be performed as soon as possible. If analysis of cyanide cannot be performed quickly, then cyanide exposure can be identified from the detection of three major markers of cyanide in the blood or urine: cyanide ion (CN-), thiocyanate (SCN-), and 2-aminothiazoline-4-carboxylic acid (ATCA) or its tautomer 2-iminothiazolidine-4-carboxylic acid (ITCA) (Logue et al., 2005).

Adducts such as *N*-(2-hydroxyethyl)valine have also been detected in haemoglobin from hospital workers exposed to ethylene oxide (Van Welie et al., 1992).

In clinical field, DNA adducts have also been detected in the white blood cells and urine of patients treated with anti-cancer drug such as N-methyl-*N*-nitrosourea (Prevost et al., 1996). Also, the *N*-7-guanyl aflatoxin B_1 adduct can be detected in urine, and used as a biomarker of exposure to the carcinogen aflatoxin B_1, which may be present in the diet (Groopman et al., 1994).

Oxidative damage to DNA can be detected by urinary 8-hydroxy-2′-deoxyguanosine (Van Welie et al., 1992). Also, urinary 8-hydroxy-2′-deoxyguanosine has been proposed as a biomarker of oxidative stress in humans (Bianchini et al., 1996).

In sport, doping controls are generally carried out on the basis of two complementary tests: (i) urine analysis and blood analysis provide short-term information on drug use by an individual; (ii) however, long term histories are accessible through hair analysis, because drug appears to be incorporated into the hair. For instance, in complement of testosterone determination, the identification of unique testosterone esters in hair enables an unambiguous charge for doping because the esters are certainly exogenous substances (Gaillard et al., 1999).

For drugs of abuse like cocaine and opiates, the threshold dose for detecting cocaine in hair appears to be approximately 25-35 mg cocaine, administered intravenously (Henderson, et al.; Kintz et al., 1999). Once incorporated into hair, a single dose of cocaine can be detected for 2 to 6 months. Codeine was detected in hair for 8 weeks after a single oral dose of 60 mg (Thieme et al., 2000).

Following intranasal absorption of 1.5 mg/kg BW of cocaine hydrochloride by humans, urinary excretion of unchanged cocaine was detected for only 8h (maximal excretion within

2 h) (Hamilton et al., 1977). However, benzoylecgonine (the major hepatic metabolite of cocaine) was generally detected in urine for 48 to 72 h (maximal excretion 4 to 8 h following cocaine administration).

4.2 Metabotypes based on concentration levels

The NMR spectrum of a biofluid can be conveniently thought of as a series of 'biomarker windows', which are spectral regions that contain signals from metabolites associated with specific targets for toxicity or disease (Fig. 18). For instance, metabolic response of the multimammate mousse (*Mastomys natalensis*) to 2-bromoethanamine (C_2H_6BrN) and propyleneimine (C_3H_7N) treatment was the induction of taurinuria (Fig. 18a). Taurine is amino acid known to protect renal medullary cells from osmotic stress and therefore may accumulate in the inner medulla of the *Mastomys*, protecting it from nephrotoxicity (Holmes et al. 1997).
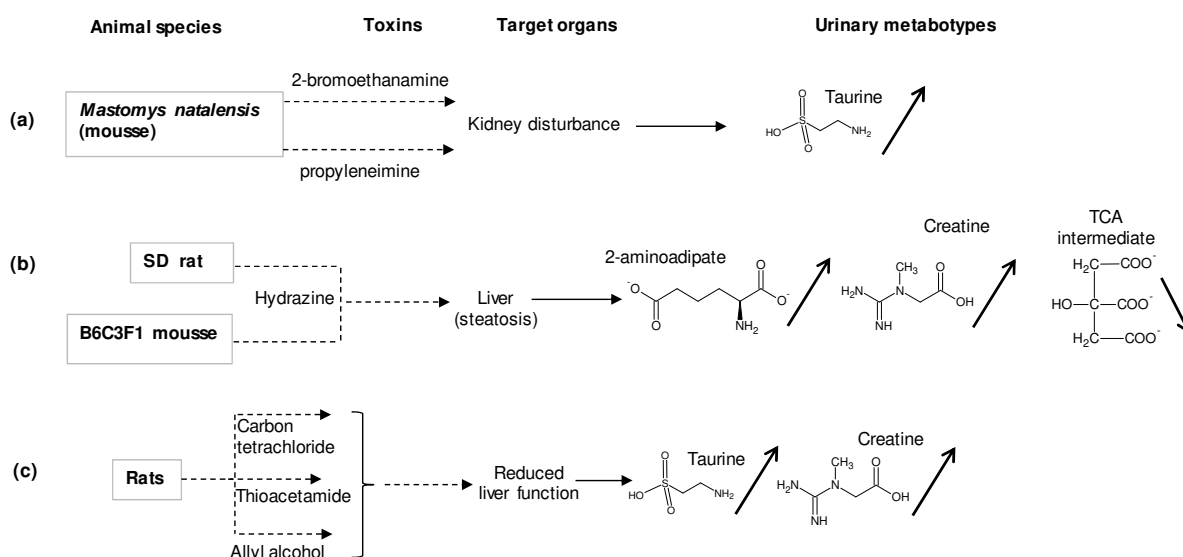

Fig. 18. Variation of urinary concentrations of metabolites, indicating kidney or liver perturbations in animal species previously exposed to some toxins.

Combining ^{1}H-NMR urine spectra with multivariate statistical analysis, metabolic responses of SD rats and B6C3F1 mice to hydrazine (NH_2NH_2) exposure were investigated (Bollard et al., 2005). Several common metabolic responses to hydrazine consisted of elevated levels of 2-aminoadipate and creatine versus depletion of the TCA cycle intermediates in the urine (Fig. 18b). Combined increases in taurine and creatine concentrations in urine have been associated with reduced liver function after exposure of rats to carbon tetrachloride (CCl_4), thioacetamide (C_2H_5NS) and allyl alcohol (C_3H_6O) (Fig. 18c) (Holmes et al., 1998).

In oncology, human prostatic epithelial cells are unique in accumulating zinc that blocks citrate degradation. In cancer cells, however, the prostate tissue contains low levels of citrate because it does not accumulate zinc and because most of citrate is used for fatty acid synthesis. 1H-NMR of prostate tissues confirmed the dramatic decrease in citrate levels in prostate gland during malignancy (Raina et al., 2009). A recent study showed a lower risk of

developing high-grade prostate cancer for men with low serum cholesterol levels (Platz et al., 2009). Inversely, high levels of cholesterol in metastasic bone tissue (>70mg/g tissue) were found to be revelator of prostate cancer, compared to normal bone tissue (50-60mg cholesterol/g tissue) as well as to bone metastases from other cancers (<70mg/g) (Thysell et al., 2010).

4.3 Metabotypes based on ratios between concentration levels

Induction of cytochrome P450 isozymes may be used as biomarker of the effect of exposure of many species to a variety of chemicals, such as organochlorine compounds and polycyclic hydrocarbons. There are well-established urinary markers for cytochrome P450 induction, such as increased ratio of 6-β-hydroxycortisol/17-hydroxycorticosteroids (6β-OHF/17-OHCS) (Hugget et al., 1992). For instance, in a recent study, serum carbamazepine level was inversely associated with the urinary 6β-OHF/17-OHCS ratio (Konishi et al., 2004).

Elevated urinary ratio of creatine/creatinine has been proposed as marker of testicular damage (Timbrell et al., 1994).

Important increase of the ratio of lactate level on glucose level can be a biomarker of cancer cells in biological body: the conversion of glucose to lactate in the presence of oxygen represents a critic aerobic pathway that allows cancer cells to proliferate rapidly (Kim and Milner, 2011; Mazurek et al., 2011). Cancer cells metabolize glucose and glutamine more than normal cells to support the *de novo* biosynthesis of nucleotides and energy required for the high rate of cell proliferation.

4.4 Metabotypes based on profiling

Metabolomic profiling has been used to reliably identify different diseases including cancers and cardiovascular disturbances:

Concerning breast cancer, malignant cells (MDA-MB-435) content showed significant increase in glutathione (GSH) , *m*-inositol, creatine and phosphocholine concentrations and decrease in isoleucine, leucine, valine, and taurine concentrations, compared to normal mammary epithelial cells (MCF-10A) (Fig. 19) (Yang et al., 2007).

However, free choline and glycerophosphocholine were below the detection level in MDA-MB-435.

In patient suffering from coronary artery disease or left ventricular dysfunction, preischemia state was characterized by higher alanine levels versus lower concentrations of glucose, lactate, free fatty acids, total ketones, 3-hydroxybutyrate, pyruvate, leucine and glutamate analysed in the coronary sinus compared with arterial sample contents (Turer et al., 2009).

In patients suffering from non-ST-segment elevation acute coronary syndrome, plasma samples showed decrease in citric acid, 4-hydroxyproline, aspartic acid and fructose versus increase in lactate, urea, glucose and valine, compared to control healthy subjects (Vallejo et al., 2009).
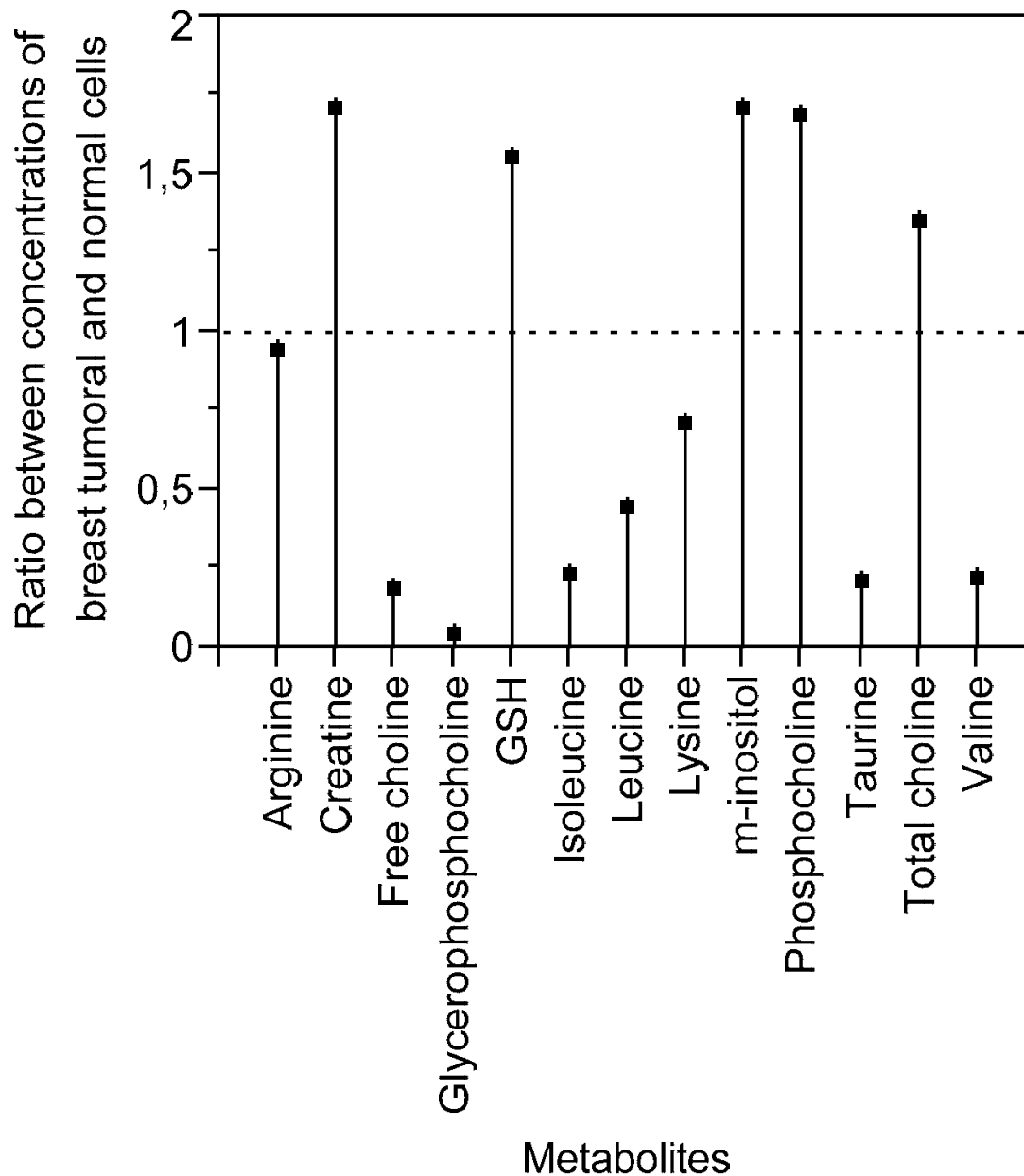
Fig. 19. Metabolomic profile characterizing breast tumor cells (MDA-MB-435) from normal cells (MCF-10A) based on ratios between metabolite concentrations MDA-MB-435/MCF-10A. High metabolic regulations are indicated by ratios >1, and inversely.

5. Metabotypes linked to biodiversity and environment conditions

5.1 Metabotypes based on occurrences of metabolites

In plant world, presence-absence of some secondary metabolites have high chemotaxonomic values. For instance, in monocotyledons, some families (Poaceae and Cyperaceae) are characterized by *C*-glycosyl flavonoids, i.e. flavonoids in which aglycone and saccharidic moiety have a *C-C* link, in addition to the *C-O* link which is abundant in the plant world (Fig.20a) (Semmar, 2010).
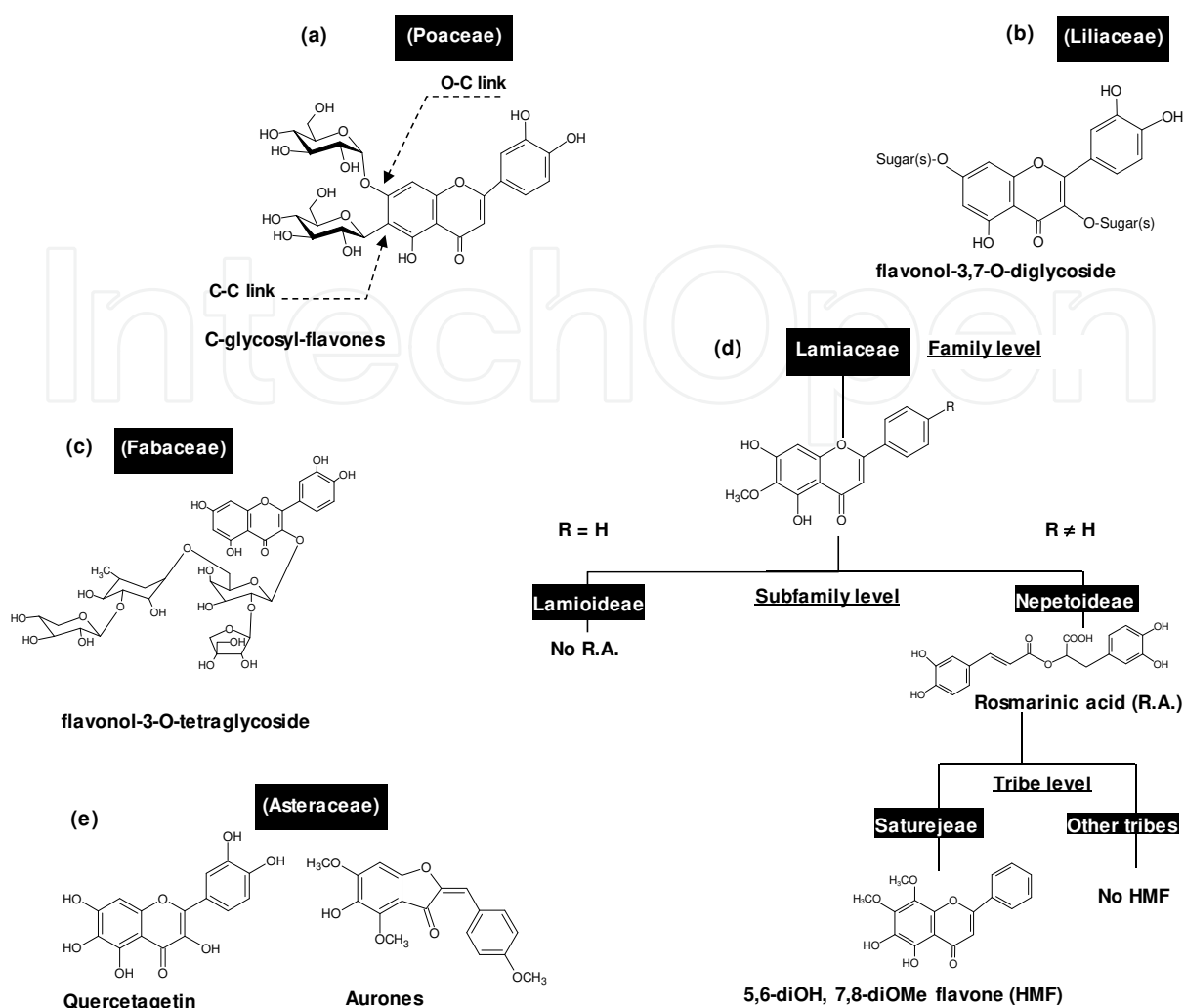
Fig. 20. Phytochemical characterizations of different plant taxons on the basis of specific or abundant phenolic compounds in their tissues.

Apart the link type between aglycone and sugar, glycosylation degree provides good chemotaxonomical criterion to a general characterization of plant families. For instance, leaf-tissues of Liliaceae species (monocotyledons) were characterized by the presence of di- and tri-*O*-glycosides of flavonoids, and a rare occurrence of monoglycosides (Williams, 1975). Flavonol 3, 7-diglycosides seem to be common constituents of the Liliaceae (Fig.20b) (Budzianowski, 1991; Williams, 1975). The family Fabaceae (dicotyledons) has been shown to be productive of multiglycosylated flavonols (Fig.20c) (Semmar, 2010).

Within the Lamiaceae family (dicotyledons), the presence-absence of rosmarinic acid (Fig. 20d) has been shown to be an excellent chemotaxonomic marker because of its presence in the subfamily Nepetoideae and absence in the subfamily Lamioideae (Janicsák et al., 1999; Harborne, 1966b). Members (tribes and genera) of these two subfamilies have been phytochemically characterized by the presence-absence of hydroxyl and methyl groups substituted on the A-ring of flavone aglycones. For instance, the presence of 5,7-dihydroxy-6-methoxyflavones with a substituted B-ring is characteristic of the subfamily Nepetoideae (Fig. 20d), particularly of *Salvia*, *Rosmarinus* and *Ocimum* species (Tomás-Barberán and Wollenweber, 1990); in Lamioideae, a 5,7-dihydroxy 6-methyl ether flavone has been found

in the genus *Scutellaria* but with unsubstituted B-ring (Fig. 20d). Moreover, in the subfamily Nepetoideae, the genera *Thymus*, *Satureja*, *Micromeria*, *Acinos*, *Calamintha*, *Origanum* and *Mentha* were characterized by the production of the 5,6-dihydroxy-,7,8-dimethoxyflavone (Tomás-Barberán and Wollenberg, 1990); all these genera belong to the tribe Saturejeae (Fig. 20d).

Among the dictotyledons, the family Asteraceae has been characterized by the production of aurones and quercetagetin which is a flavonol almost entirely found in this family (Fig. 20e) (Iwashina, 2000).

In *Tulipa* (Liliaceae), the flower colors are fundamentally defined by the anthocyanidin type: orange and flesh pink colors are linked to pelargonidin, black-red and red-orange are due to cyanidin, and black-blue-violet-purple are governed by delphinidin (Fig. 21) (Shibata and Ishikura, 1960; Torskangerpoll et al., 2005).

Moreover in tulips, the shade of flower tepals was showed to be dependent on some chemical substitutions of anthocyanins: substitutions of anthocyanins by aromatic acyl groups have been reported to be responsible for bluing effect (Torskangerpoll et al., 2005). Also, combinations of cyanidin and pelargonidin with carotenoids generally induced attractive red and orange colours (van Eijk et al., 1987).

5.2 Metabotypes based on concentration levels

In plant world, accumulation of anthocyanins has been shown to be a good marker of cold stress: low temperatures have been shown to induce anthocyanin synthesis in many plant species, e.g. in leaves of *Arabidopsis thaliana* (Leyva et al., 1995), *Cotinus coggygria* (Oren-Shamir and Levi-Nissim, 1997), *Pinus banksiana* (Krol et al., 1995), etc. .
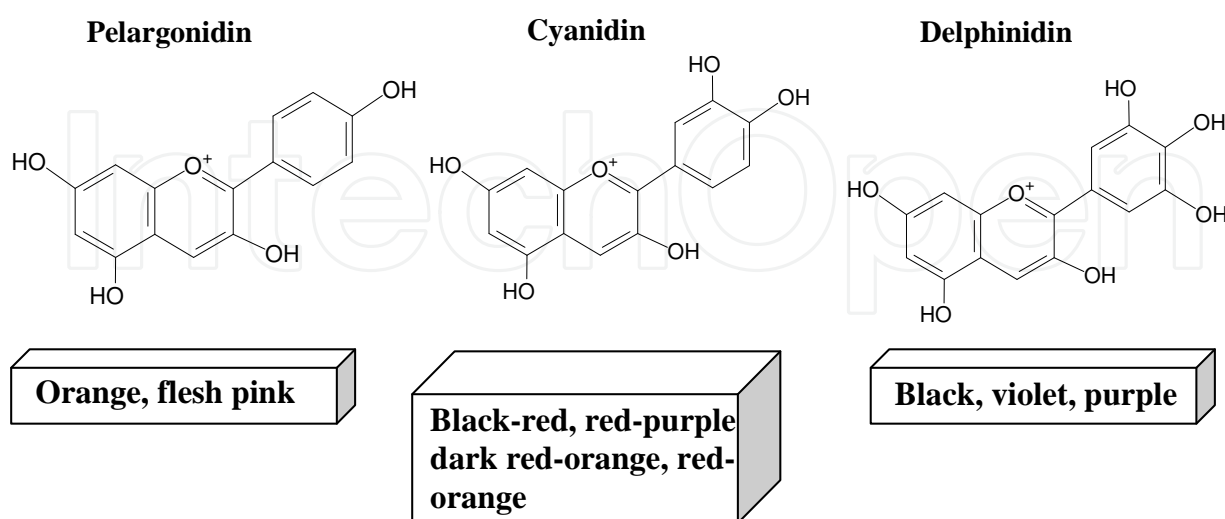

Fig. 21. Variation of tulip flower colours in relation to occurrence and abundance of anthocyanidin type.

Apart anthocyanins, sesquiterpenes (SQT) were found to be reliable indicator of thermic stress in emitting plant species: for instance, in young orange tree, emission of β-caryophyllene (β-Car) increased 5-6 fold for air temperature increase of 10°C (Hansen and Seufert, 1999). Temperature experiment performed on young corn plants showed that the proportion of β-Car (as a percentage of total emitted biogenic volatile organic compounds) was maximal at 37°C (Gouinguené and Turlings, 2002).

SQT levels have been also found to be biomarkers of diurnal and seasonal rhythmicity: β-Car emissions from various *Citrus* varieties were found to increase during the morning with generally a concentration peak around noon (Ciccioli et al., 1999). In potatos, emitted SQT increased steadily throughout the day and peaked in the afternoon (Agelopoulos et al., 2000). In Finnish scots pine, β-Car emissions exhibited significantly seasonal variability, with maximum emissions observed during summer months (Tarvainen et al., 2005).

Volatile terpenes (isoprenes, monoterpenes and sesquiterpenes) were found to be good markers of water stress in some plants. In *Pinus halepensis* (Alepo pine), water stress induced monoterpenes emissions by the leaves (Ormeño et al., 2007a). In young orange tree, severe drought reduced β-Car emissions to 6% of pre-drought levels, but emissions were unaffected by mild drought conditions (Hansen and Seufert, 1999).

Different works concluded relationships between metabolic variability in plants and soil composition: higher concentrations of aluminium in soil resulted in increase in exuded phenolic compounds by the roots of maize (Kidd et al., 2001). Aluminium resistant variety of maize exuded 15-fold higher level of flavonoids when pre-treated with silicon than when no such pre-treatment was applied. In scots pine (*Pinus sylvestris* L.), tree exposed to nickel had higher concentrations of condensed tannins compared with control (Roitto et al., 2005). Calcareous soils stimulated emissions of α-humulene from Aleppo pine, whereas siliceous soils favored α-humulene and β-bourbonene from Rock Rose (Ormeño et al., 2007b).

In plant world, emissions of some volatile compounds were found to be positively correlated to biotic disturbance such as parasite or herbivory: in Black Sage, SQT emissions increase significantly under infection with aphids (Arey et al., 1995). In corn seedlings, SQT emissions increase as a response to caterpillar feeding, and it has been demonstrated that such emission attracted wasps which parasitize caterpillars (Turlings et al., 1995).

5.3 Metabotypes based on ratios between concentration levels

Several plant species have been biogenically characterized by the ratios of individual sesquiterpenes (SQT) relative to the overall emitted (volatile) SQT (Duhl et al., 2008). In SQT emission profiles, β-caryophyllene (β-Car) was the most frequently reported and abundant; α- and β- farnesene as α-humulene are also prominent components to observed profiles (Fig. 22). The results concern studies where the plants were not disturbed because, disturbance is known to affect the variability of emitted SQT blends:
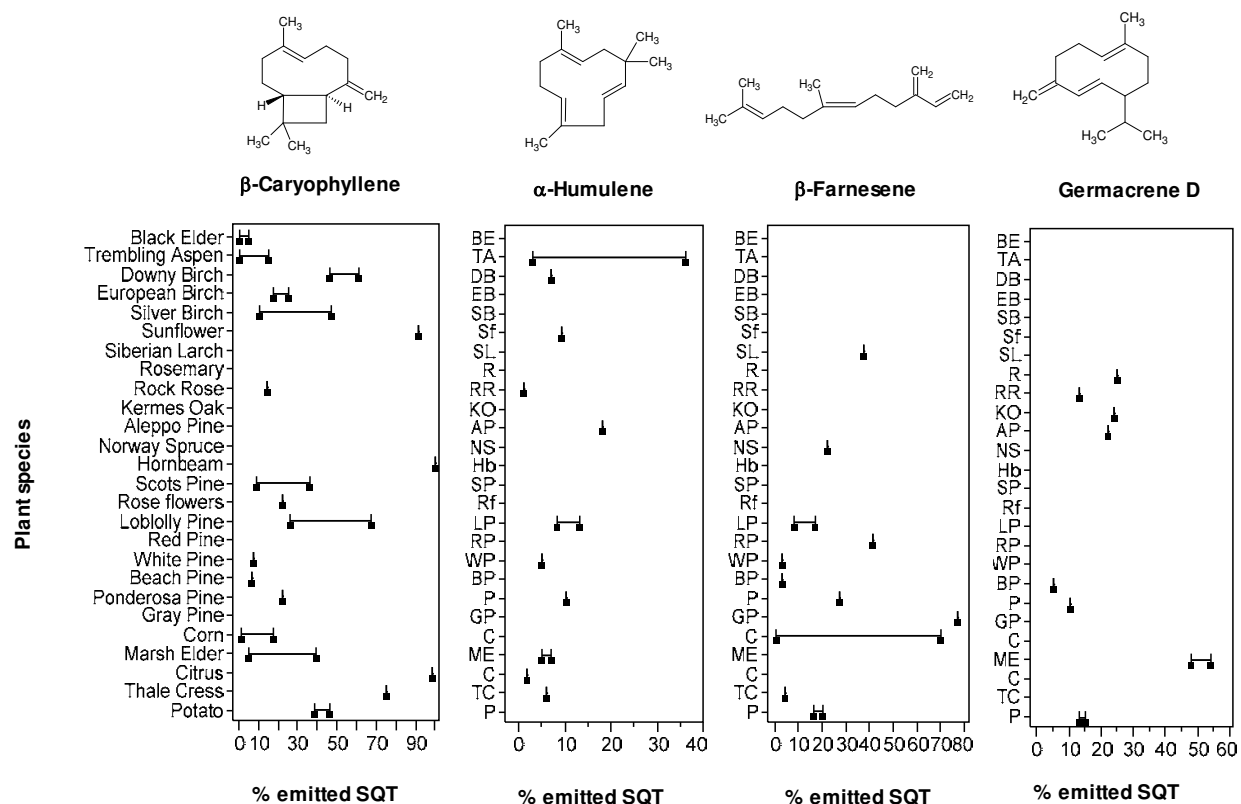
Fig. 22. Characterization of different plant species by the percentages (%) of some volatile sesquiterpenes (SQT) (β-caryophyllene, α-humulene, β-farnesene, germacrene D) referred to the overall SQT emissions.

SQT ratios showed that plant species can be characterized by dominance or relatively high levels of some SQT : sunflower, hormbeam and citrus species are highly productive of *β*-caryophyllene (*β*-car) (>90%). The SQT pool of gray pine seems to be dominated by high relative levels of β-farnesene (77%); the corn shows wide inter-individual variation range of β-farnesene going from 0 to 70%. Marsh elder can be characterized by germacrene D representing 48 to 54% of overall emitted SQT. Trembling aspen seems to emit relatively more α-humulene with high inter-individual variability (3-36%). Apart from *α*-humulene, *β*-humulene (not presented) is also frequent in nature and was reported to represent 55-57% of SQT in red and white pines (Duhl et al., 2008).

In tulips (Liliaceae), the flower colors acquire higher variability governed by the relative levels of mixed anthocyanins (Shibata and Ishikura, 1960): cultivars having "magenta nuances" showed anthocyanin content in which the relative amounts of cyanidin 3-rutinoside increased at the expense of delphinidin-3-rutinoside. Garden varieties with blue nuance (black, black-purple, fade-sky, violet and purple) have relatively high content of delphinidin type in tepals (i.e. the delphinidin content was more than 50% of the total anthocyanin content). Orange colored tepals were to a large extent correlated with high relative amounts of the pelargonidin derivatives at the expense of the two other aglycone types.

Apart from the chemotaxonomic characterization of plants, metabolic ratios were analysed in relation to different environmental conditions to characterize adaptive responses of biological species:

By opposition to monoterpene emissions, sesquiterpene emissions were found to be reduced by water deficit in *Pinus halepensis* among other typical Mediterranean species (Ormeno et al., 2007a). On the basis of negative effect on sesquiterpene emissions versus positive effect on monoterpene emissions, the drought stress can induce a shift in terpene composition leading to an increase in the ratio monoterpenes/sesquiterpenes in plant.

5.4 Metabotypes based on profiling

Metabolic profiling is used in chemotaxonomy to highlight chemical polymorphisms in plant species leading to better understand the biochemical origins of biodiversity:

For instance, analysis of flavonoid glycosides in the leaves of *Astragalus caprinus* (Fabaceae) highlighted four chemotypes (Chtp) characterized by high relative levels of different compounds among 14 in all (Fig. 23) (Semmar et al., 2005b): Chtp I was exclusively characterized by the presence of diglycosides of methylated flavonols (rhamnazine, rhamnocitrin) (11-14) which are acylated by a methyl-glutaric acid. Chtp II was characterized by high regulation of a tetraglycoside of quercetin (1). Chtp III was characterized by high regulations of a tetraglycoside of kaempferol (2) and its acylated derivatives (acylated by *p*-coumaric or ferrulic acid) (6-10). Chtp IV was characterized by a relatively high regulation of a triglycoside of kaempferol (5).

Correlation analyses between the relative levels of the 14 flavonoids showed positive trends between flavonoids based on a same aglycone and negative trends between flavonoids having different aglycones (kaempferol, quercetin and methylated aglycones) (Semmar et al., 2007). This compatibility between statistical correlations and chemical structures helped to analyse the network of metabolic links between flavonol glycosides (Fig. 23).
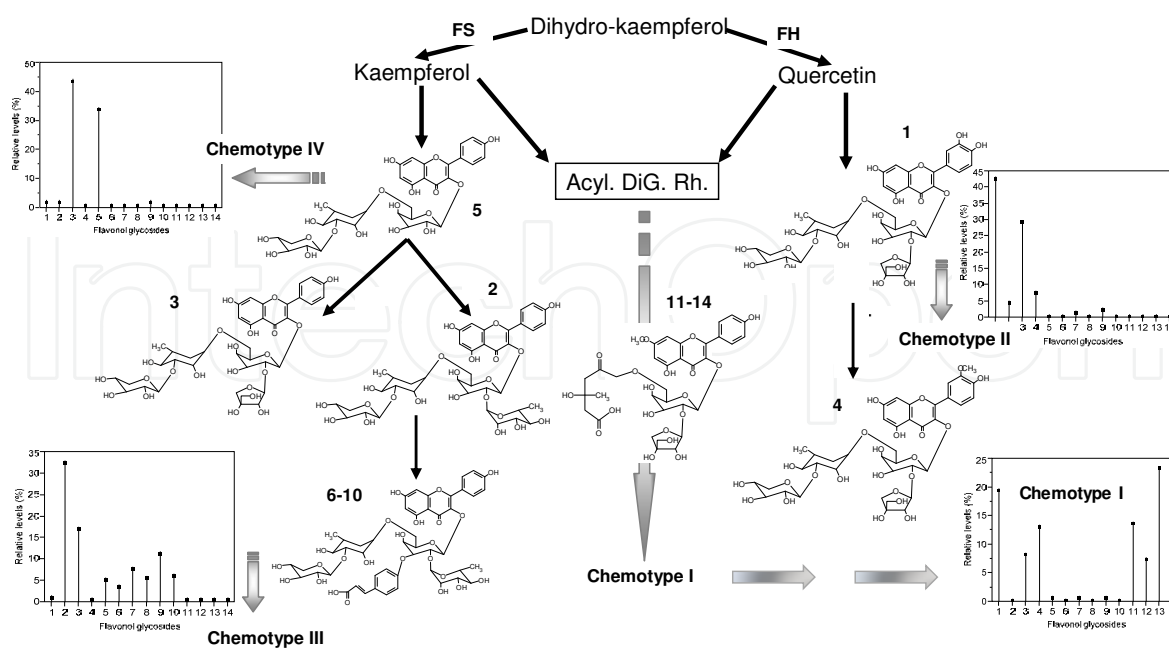

Fig. 23. Four chemotypes of *Astragalus caprinus* (Fabaceae) based on different metabolic regulations between flavonol glycosides pathways. FS: Flavonol Synthase; FH: Flavonol Hydroxylase; Acyl. Dig. Rh.: Acylated Diglycosyl of Rhamnazin or Rhamnocitrin

Sampling of 404 plants of *A. caprinus* from North to South of Tunisia highlighted a significant link between metabotypes' (chemotypes') abundances and geographical area (climatic conditions): Chemotype I rich in less hydrophilic compounds was abundant in the south (arid). The north (humid) seemed to offer favorable conditions to chemotypes III and IV (both based on kaempferol derivatives). The center of Tunisia, with intermediate climatic conditions, allowed a co-evolution of the four chemotypes with a relatively higher abundance for chemotype II (based on quercetin metabolism).

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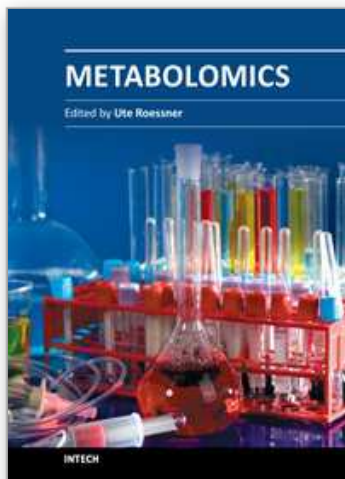
Metabolomics

Edited by Dr Ute Roessner

Metabolomics is a rapidly emerging field in life sciences, which aims to identify and quantify metabolites in a biological system. Analytical chemistry is combined with sophisticated informatics and statistics tools to determine and understand metabolic changes upon genetic or environmental perturbations. Together with other 'omics analyses, such as genomics and proteomics, metabolomics plays an important role in functional genomics and systems biology studies in any biological science. This book will provide the reader with summaries of the state-of-the-art of technologies and methodologies, especially in the data analysis and interpretation approaches, as well as give insights into exciting applications of metabolomics in human health studies, safety assessments, and plant and microbial research.

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