ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

Contributions of metabolomic changes associated with obesity in humans and dogs in clinical practice

Naïs Auger

Coimbra, julho de 2023

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Index

Index of Figures

Index of Tables

Abbreviations

AT: Adipose Tissue
BCS: Body Condition Score
BIA: Bioelectrical Impedance Analysis
BMI: Body Mass Index
BCAAs: Branched-Chain Amino-Acids
DEXA: Dual X-ray Energy Absorptiometry
D_2O: Deuterium Oxide Dilution
GC: Gas Chromatography
GSH: Glutathione
GST: Glutathione-S-transferase
HDL: High-Density Lipoprotein
IL-6: Interleukin 6
LC: Liquid Chromatography
LysoPC: Lysophosphatidylcholine
MS: Mass Spectrometry
MS/MS: Tandem Mass Spectrometry
NMDA: N-methyl-D-aspartate receptor
NMR: Nuclear Magnetic Resonance
NPY/AGRP: Neuropeptide Y- Agouti-Related Protein
ORMD: Obesity-Related Metabolic Disorders
PC: Phosphatidylcholine
PUFAs: Polyunsaturated fatty acids
SAM: S-adenosylmethionine
SFAs: Saturated Fatty Acids
SCFAs: Short-Chain Fatty Acids
TMA: Trimethylamine
TMAO: Trimethylamine N-Oxide
TNF-α: Tumor Necrosis Factor-alpha
VFAs: Volatile fatty acids
VLDL: Very-Low-Density Lipoprotein
WC: Waist Circumferences

Contributions of metabolomic changes associated with obesity in humans and dogs in clinical practice

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Resumo

A metabolómica é uma área de estudo que se centra na análise de metabolitos em sistemas biológicos e fornece informações valiosas sobre as alterações metabólicas associadas à obesidade e ao excesso de peso, tanto em seres humanos como em cães.

Nos seres humanos, os estudos metabolómicos identificaram vários metabolitos-chave que são influenciados pela obesidade e pelo excesso de peso. Alguns destes metabolitos incluem ácidos gordos, aminoácidos, triglicéridos e acilcarnitinas. Por outro lado, em cão, verificou-se que metabolitos semelhantes são influenciados pela obesidade e pelo excesso de peso. No entanto, podem existir algumas diferenças específicas da espécie no perfil metabolómico. Os cães com obesidade demonstram frequentemente alterações no metabolismo dos ácidos gordos, incluindo níveis aumentados de ácidos gordos livres e triglicéridos circulantes. A desregulação dos aminoácidos, como a elevação dos aminoácidos de cadeia ramificada (do inglês, *branched-chain amino-acid*, BCAAs), também foi observada em cães obesos. Além disso, as alterações nos perfis de acilcarnitina e nos níveis de lisofosfatidilcolinas são comparáveis às observadas nos seres humanos.

É importante notar que os perfis metabolómicos podem variar em função de fatores como a dieta, o contexto genético e a variação individual. Por conseguinte, é necessário haver mais investigação para compreender plenamente as alterações metabolómicas associadas à obesidade e ao excesso de peso em seres humanos e no cão.

Palavras-chave: cão, diagnóstico translacional, metabolómica, modelo espontâneo, obesidade

Abstract

Metabolomics is a field of study that focuses on the comprehensive analysis of metabolites in biological systems and provides valuable insights into the metabolic changes associated with obesity and overweight, both in humans and dogs.

In humans, metabolomics studies have identified several key metabolites that are influenced by obesity and overweight. Some of these metabolites include fatty acids**,** amino acids**,** triglycerides and acylcarnitines. On the other hand, in dogs, similar metabolites have been found to be influenced by obesity and overweight. Nevertheless, there may be some species-specific differences in the metabolomic profile. Dogs with obesity often demonstrate alterations in fatty acid metabolism, including increased levels of circulating free fatty acids and triglycerides. Dysregulation of amino acids, such as elevated branched-chain amino-acid (BCAAs), has also been observed in obese dogs. Additionally, changes in acylcarnitine profiles and lysophosphatidylcholines levels are comparable to those seen in humans.

It is important to note that metabolomic profiles can vary depending on factors such as diet, genetic background, and individual variation. Therefore, further research is required to fully understand the metabolomic changes associated with obesity and overweight in humans and dogs.

Keywords: canine, metabolomics, obesity, spontaneous model, translational diagnostic

Introduction

The prevalence and incidence of obesity in pet dogs and people has only increased since the 20th century related to sedentary lifestyles and dietary changes (Eleanor Raffan, 2017). Indeed, in 2014, 38.8% of dogs were overweight or obese in France (Wakshlag & Loftus, 2014). Nowadays, around the world, people have reached a prevalence of 39% of overweight (Wallis & Raffan, 2020), and 10% to 20% of obesity is reported in companions' animals (Lefebvre 2020). In humans, it is estimated that by 2030, over 2.16 billion of individuals will be overweight, while 1.12 billion will be obese, being, therefore, one of the major health concerns for the 21st century (Ataey *et al.*, 2020).

Obesity is defined as an excess of adipose tissue in the body (Rangel-Huerta *et al.*, 2019) and a pathology that leads to a chronic and generalized state of inflammation (Lefebvre, 2020). In veterinary medicine, a dog is considered overweight when Body Condition Score (BCS) exceeds 10% to 20% of his healthy weight, and obese when BCS is up to 30% respectively (Ward *et al.*, 2019). In humans, obesity is characterized by a Body Mass Index (BMI), a ratio of body weight (kg) and height squared (m^2), greater than, or equal to, 30 and overweight is defined as a BMI between 25.0 to 29.9 (World Health Organization 2018).

It was reported, in a research study performed in the United States, the positive correlation of owner's BMI with their dog's BCS; this correlation is also extended to predisposing factors for obesity, such as sedentary lifestyles, unhealthy eating habits and genetic susceptibility (Linder *et al.*, 2021). In this thesis, it was assumed that Europe follows the same profile USA does (Linder *et al.*, 2021; Wakshlag & Loftus, 2014), although no studies were performed to confirm this tendency (Linder *et al.*, 2021; Wakshlag & Loftus, 2014). However, this simple and useful index statement did not assess the metabolic alterations frequently associated with obesity (Cirulli *et al.*, 2019; Cline *et al.*, 2021). Moreover, the characterization of obesity is subjective and variable, and precise techniques are still not available for common practice (Brooks *et al.*, 2014; Saleem Ansari, 2020).

Advanced research on regulation of fat, characterization methods for identifying obesity and his clinical management have paved the way for deeper investigation into the underlying mechanisms through metabolomics (Richard *et al.*, 2020; Tvarijonaviciute *et al.*, 2020). In addition to metabolic pathways implicated in the pathogenesis of obesity, humans and dogs shared also comorbidities of such a disease (Verkest, 2014). Under obese state both humans and dogs suffer from metabolic syndrome, including insulin resistance, hypertension, or hyperlipidemia (Tvarijonaviciute *et al.*, 2020). However, dogs do not register type II diabetes or atherosclerosis unlike humans (Verkest, 2014). Indeed, vascular complications are more described in humans than in dogs although, in both species, there are similar complications such as cardiovascular diseases and respiratory disorders (I.Jeusette *et al.*, 2004).

Veterinary medicine studies could thus contribute to the development of early obesity markers with high impact in animal and human health (Osto & Lutz, 2015). In the literature, some molecular variations, obtained from analytical samples such as saliva, plasma, urine and feces, are common in both obese humans and dogs and can be considered as ''metabolomic syndrome''. An example is the increase of taurine,

branched-chain amid acids. On the contrary, the occurrence of a decrease in metabolites such as butyrate and trigonelline are described. Furthermore, some controversy has been noticed in molecules such as acyl-carnitines and lysophosphatidylcholines. Since metabolomic studies in dogs are still scarce compared to human studies, therefore the metabolites report is not exhaustive.

The present bibliographic review aims to highlight a parallelism between canine and human obesity research through metabolomics as a translational tool for the study of obesity and overweight. Indeed, from clinical studies, metabolomics provides fundamental knowledge to better understand, prevent, and find potential targets for a more precise characterization of this pathology. Moreover, this study aims to reinforce the usefulness of veterinary spontaneous models of disease for future experimental studies in obese dogs, as a valuable and reliable model for obese human research.

The author reviewed the literature, selecting articles from PubMed using the following keywords: **canine, metabolomics, obesity, spontaneous model, translational diagnostic**. Overall, 50 studies were analyzed, from 2004 to 2023, where, selected based on the following criteria: **more recent**, published in **high impact journals**, pronounced **synthesized, complete** and **well organized reviews**, following the **European Guidelines**. The selected bibliography has a relative balance in number regarding human and dog obesity. In the second part (2. Metabolome in dog and human obesity, page 14), twenty studies were included, five of them used an untargeted metabolomic approach and two used targeted metabolomic approach; eight were without metabolomic precision because they were reviews of published articles. Five studies refer to genomics and proteomics. Every experimental study used two groups: a control group composed of lean individuals, and an experimental group composed of obese individuals (Muñoz-Prieto *et al.*, 2021). Only one exception was made by *Lucena et al.*, with both groups composed of obese dogs and differed by the presence of obesity related metabolic disorders (ORMD) (Lucena *et al.*, 2019). The majority of dog breeds studied were Labrador Retrievers or Beagles (Muñoz-Prieto *et al.*, 2021). The definition of obesity was mainly based on BCS nine-point scale in dogs and BMI in humans (Apper *et al.*, 2020; Rangel-Huerta *et al.*, 2019).

The thesis starts by pointing out the similarities between obesity in humans and dogs, including regulation of fat and diagnostic methods for identification of obesity. This has paved the way for deeper investigation into the underlying mechanisms through metabolomics. Secondly, analytical alterations on metabolomes from several biological samples, such as blood, urine, or saliva, from obese and lean individuals have been described and provide valuable insights into the alterations of metabolome associated with obesity. Further, by analyzing the metabolites produced by the gut microbiota, researchers can gain insights into how these microorganisms can contribute to metabolic changes associated with obesity.

Besides species and breed differences and predispositions, the ‘’metabolomic syndrome’’ may be a more precise methodology to characterized obesity in both humans and dogs.

Poster Publications: “Metabolomics: a promising translational tool for the diagnosis of obesity and overweight”, I Congress BioMedLab, 10-12 march at Alfândega do Porto organized by *Associação Portuguesa de Ciências Biomédicas Laboratoriais*.

1. Basic concepts on obesity similarities between humans and dogs

1.1 Etiopathogenesis

1.1.1. Fat regulation physiology

Understanding the physiology of homeostatic pathways of adipose tissue is a key point that allowed exciting research in this area to begin (Jeusette *et al.*, 2004; Richard *et al.*, 2020). Initially, it was thought that obesity was based on glucostatic and lipostatic models indicating that glucose or lipids from the diet were responsible for appetite or satiety (Wakshlag & Loftus, 2014).

Afterwards, researchers began to understand the importance of hormonal influence and the impact of some gene's (*e.g.*, genes encoding for pro-opiomelanocortin protein or melanocortin-4 receptor) on the appetite center, located in the hypothalamus (Wallis & Raffan, 2020). Adipose tissue (AT) represents an energy reserve of the organism (Richard *et al.*, 2020). The storage of triglycerides, synthesized by the liver, are contained in the adipocytes and fatty acids can be released, later, according to energy expenditure and fasting (Richard *et al.*, 2020). AT is central for the regulation of systemic lipid metabolism, and nutritional and hormonal cues served to balance lipid storage and breakdown within the fat cell (Lefebvre, 2020). Depending on the increase or multiplication of adipocytes, two types of obesity could be distinguished: when adipocytes multiplied in number – hyperplastic obesity – during growth or puberty, and adipocytes enlargement – hypertrophic obesity – on adulthood (Lefebvre, 2020). Besides the energy storage function, AT is also considered to be an endocrine organ (Richard *et al.*, 2020). In fact, adipocytes secrete cytokines and hormones (*e.g.*, adiponectin, leptin, Tumor Necrosis Factor-alpha (TNF-α), Interleukin 6 (IL-6)), depending on the number of adipocytes, though influencing long distant organs (Richard *et al.*, 2020). Indeed, appetite regulation (Figure 1) is carried out in the short or long term via appetite stimulating hormones (*e.g.*, ghrelin) or inhibiting hormones (*e.g.*, leptin, cholecystokinin) (Eleanor Raffan, 2017). Plasma leptin concentrations thus correlates strongly with obesity in dogs and in humans (Wakshlag & Loftus, 2014). A disruption of the lepto-melanocortin axis leads to obesity in humans and in dogs (Eleanor Raffan, 2017; Richard *et al.*, 2020). Subsequently, it was realized that some factors influence these discoveries upstream (Cline *et al.*, 2021) and play a preponderant role in the appearance of obesity (Wallis & Raffan, 2020).

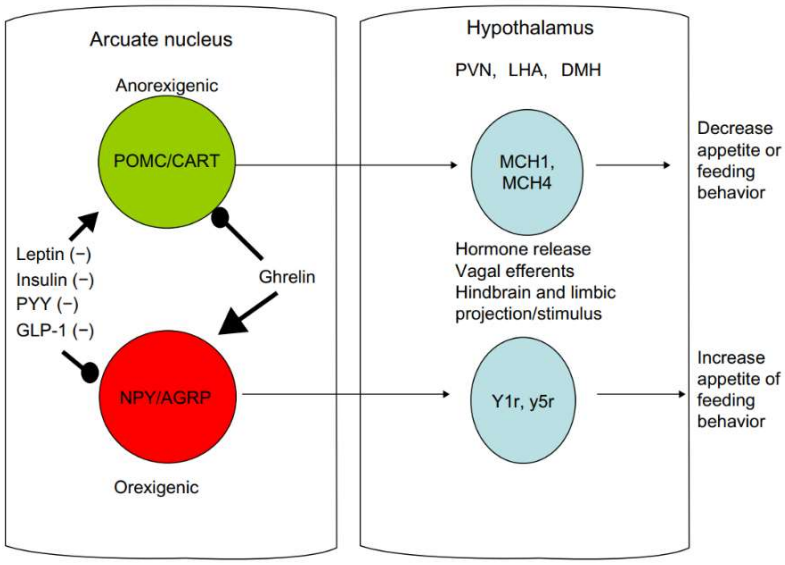

Figure 1 – Hormonal appetite regulation. Appetite regulation is carried out by short or long term via appetite stimulating hormones (*e.g.*, ghrelin) or inhibiting hormones such as leptin, which stimulates satiety in both humans and dogs. Indeed, appetite inhibiting hormones act on POMC/CART nucleus and stimulate release of MCH 1 and 4 which triggers decrease of appetite. Respectively, stimulating hormones act on NPY/AGR nucleus and stimulate release of neuropeptide 1 and 5 increasing appetite behavior. POMC/CART, pro-opiomelanocortin protein/cocaine amphetamine related transcripts; PYY, peptide YY; GPL-1, glucagon-like peptide 1; NPY/AGRP, neuropeptide Y/agouti-related protein; MCH 1 and 4, melanin-concentrating hormones; Y1r, Y5r, neuropeptide Y receptors 1 and 5; PVN, paraventricular nucleus; LHA, lateral hypothalamic area; DMH, dorsomedial hypothalamus. Adapted from *Wakshlag & Loftus, 2014.*

1.1.2. Obesity endogenous predisposing factors

There are several endogenous factors such as age and reproductive *status* induce a decrease of metabolic rate and consequently a greater weight gain (Lefebvre, 2020). It was observed that spayed dogs are predisposed to overweight in the two years following their sterilization because of the reduction in energy requirements (Cline *et al.*, 2021; Wakshlag & Loftus, 2014). Finally, genetic factores must be considered, since different genes were identified in metabolic obesity (Wallis & Raffan, 2020). Indeed, mutations were detected in obese patients in the genes encoding for pro-opiomelanocortin protein (POMC) and melanocortin-4 receptor (MC4) (Wallis & Raffan, 2020). The genes concerned were mostly involved in the lepto-melanocortin axis (Wakshlag & Loftus, 2014; Wallis & Raffan, 2020). These mutations were even observed in some breeds of dogs which are “naturally” predisposed to obesity (Eleanor Raffan, 2017), like Labradors and Golden retrievers, while other breeds are considered resistant like greyhounds and whippets, among others (Wallis & Raffan, 2020). Nowadays, a genetic test is available for retrievers to detect the POMC gene mutation, allowing owners to prevent the onset of obesity (Wallis & Raffan, 2020). *Wallis & Raffan* showed that the genetic basis for canine obesity is still being investigated, but some believe it will follow the same pattern humans and other animal species did (Wallis & Raffan, 2020). In humans, genetic inheritance defined the tendency to become obese by 40 to 70% (Osto & Lutz, 2015). *Osto et al.,* reviewed two studies that emphasized the heritability of

obesity predisposing genes is not sufficient to induce obesity symptoms (Bouchard et al., 1990; Kopelman P.G, 2000; Osto & Lutz, 2015). For the authors, external factors are as important as internal factors.

Epigenetics should also be taken into account to explain the increase of obesity prevalence in recent years (Durrer Schutz *et al.*, 2019). To our knowledge, no epigenetics studies have been conducted on dogs regarding this subject and may constitute a very important field of research (Durrer Schutz *et al.*, 2019).

1.1.3 Obesity exogenous predisposing factors

Sedentary lifestyle and the recent technologies push humans and dogs towards obesity (Muñoz-Prieto *et al.*, 2018). Indeed, the increasing importance of daily time spent behind screens reduced time for walks or other forms of physical exercise (Muñoz-Prieto *et al.*, 2018).

Beyond the owners' lifestyle (consuming high-dense foods, for example), other actors showed to be considered: the education level including the school and sport programs, the importance of veterinarians in the prevention and recognition of obesity, and consequently, the socioeconomic *status* (access to high caloric fast food), (Ward *et al.*, 2019).

A study of *Lin & Li, 2021*, synthesized very well the perception of dog owners on dog's and human's obesity and the environmental influence by putting forward the influence of "junks" food and an "obesogenic marketing" which promote energy-dense beverages or foods (Lin & Li, 2021). The study of Wakshlag & Loftus, 2014 showed that the BMI in humans is positively correlated to the BCS in dogs (Wakshlag & Loftus, 2014). In other words, obese or overweight owners are prone to have a dog to look alike (Wakshlag & Loftus, 2014). A less recent study deduced the opposite, but this could be explained by biases in people selection because the participating dog's owners were mainly selected from an animal welfare facebook group (Muñoz-Prieto *et al.*, 2018). For clinician in human and veterinary medicine, understanding of these factors is essential to prevent and treat obesity in the context of ''One Health'', considering not only internal factors specific to humans and animals, but also the nutritional, technical and environmental factors that contribute for the development of an ''obesogenic environment'' (Day, 2017).

1.2. Clinical presentation and management of obesity

1.2.1 Obesity diagnosis

Quantification of adiposity or assessing body condition was presented by similar methods in both humans and dogs (Tvarijonaviciute *et al.*, 2020). Clinical methods based on visual observation and palpation, as morphologic scales such as BCS, are used in common veterinary practice thanks to their simplicity, rapidity and reproducibility performance (Tvarijonaviciute *et al.*, 2020). In humans, anthropomorphic measures, such as BMI, commonly practiced in humans, are not really applied to

veterinarian practice (Ward *et al.*, 2019). Unfortunately, obesity diagnosis scoring has subjectivity and variability and other techniques exist but are still not available for common practice (Brooks *et al.*, 2014). *Tvarijonaviciute et al.* summarized different methods of obesity diagnosis in dogs and humans reported in Table 1 (Tvarijonaviciute *et al.*, 2020). A distinction could be made between simple methods, described as "semi-quantitative'', and "quantitative methods"' qualified as more complex, expensive and not applicable in current practice (Tvarijonaviciute *et al.*, 2020). For instance, a biphotonic or dual X-ray absorptiometry (DEXA) determines body composition by differencing bone mineral content, fat mass and lean masse (non-fat tissues) thanks to photons from two energy levels (70 and 140 kVp) absorbed at different degrees relative to type of tissues crossed by X-ray (Witzel *et al.*, 2014). It is one of the most used techniques in research for determining bone density (German *et al.*, 2010).

Table 1 – Pros and cons of quantitative and semi-quantitative methods to obesity measurements. Adapted from Tvarijonaviciute *et al.*, 2020 & German *et al.*, 2010.

Quantitative methods	**In humans**		**In dogs**	
	Pros	**Cons**	**Pros**	**Cons**
DEXA	Accurate estimation of body fat, non-invasive	Expensive	Accurate estimation of body fat	Require anesthesia or sedation; expensive
D_2O	Accurate estimation of body fat	Depends on hydration *status*	Accurate estimation of body fat	Depends on hydration *status*, complex; expensive
BIA	Non-invasive, practical, cheap	Depends on hydration *status,* menstrual cycle, body positioning and temperature	Correlates with DEXA	Inaccuracy, depends on hydration *status*
Semi-quantitative methods	**In humans**		**In dogs**	
	Pros	**Cons**	**Pros**	**Cons**
Body weight	Easy to perform, repeatable at any time, objective	Did not quantify fat *versus* lean mass	Easy to perform	Did not quantify fat *versus* lean mass
Anthropometric measures (ex. BMI, WC)	Easy to perform	Subjective	Easy to perform	Subjective, not really used
Morphologic scales (ex. BCS)	Not used		Easy to perform, correlated with DEXA	Subjective

BCS, Body Condition Score; BMI, Body Mass Index; DEXA, Dual-Energy X-ray Absorptiometry; D_2O, Deuterium Oxide Dilution; BIA, Bioelectrical Impedance Analysis; WC, Waist Circumferences.

Then, by Fourier-transform infrared spectrophotometry, it is possible to determine «deuterium oxide dilution (D_2O) », based on the indirect measurement of water inside the body through the detection of a stable isotope of hydrogen (deuterium) (Hooper *et al.*, 2020). It is assumed that water is mostly non-associated with adipose tissues (Tvarijonaviciute *et al.*, 2020). Thus, by determining the amount of water in the body, the fat mass is deduced thanks to different equations (Hooper *et al.*, 2020). A third method, named Bioelectrical Impedance Analysis (BIA) explores body conductivity (German *et al.*, 2010). Electrodes and electric current are applied allowing to obtain a volume of water knowing that fluids are conductors (German *et al.*, 2010). By means of an equation, the body fat mass is reached (German *et al.*, 2010). This technique also assumes that fat mass doesn't contain water (German *et al.*, 2010).

1.2.2 Weight management in humans and dogs

The European Journal of Obesity published weight management guidelines in humans (Durrer Schutz *et al.*, 2019). Some similarities in management of obesity in dogs have been identified by comparing these guidelines with those from the American Animals Hospitalization Association (AAHA) in obesity (Cline *et al.*, 2021). Indeed, obese management of dogs or humans requires a detailed history (lifestyle activity level, feeding management), a clinical examination (determination of BMI and WC in humans *versus* BCS and muscle condition score in dogs) and detection of comorbidities (Cline *et al.*, 2021; Durrer Schutz *et al.*, 2019). The motivation to join a weight management program (patient *versus* dog's owner) is success-dependent of weight management plan and communication between clinical team and patients or dog's owners. In humans, weight management is based on three main points: lifestyle modifications (nutrition, physical activity and psychological aspects such as self-esteem or stress); pharmacotherapy and bariatric surgery (Durrer Schutz *et al.*, 2019). In contrast with humans, studies and clinical practice in dogs were only focused on nutritional plan and lifestyle modifications (Cline *et al.*, 2021). Two appetite suppressant molecules have been used in dogs and removed because of insufficient clinical demand (German, 2016). Surgical approach has been residual and very few studies have been published in dogs (only two from 2014-2020), (Guo et al., 2014; Vedrine et al., 2020).

1.2.3 Metabolomics as a diagnostic tool

Metabolomics is the study of metabolic changes and reveals the signature of disease-related small molecules (Ellero-Simatos *et al.*, 2019). It enables the identification and measurement of metabolites from biological fluids like plasma, urine and saliva and presents potential benefits in sensitivity and specificity when compared to conventional diagnostic procedures (Gulston *et al.*, 2007). This innovative field, which appeared in the 1980s has been widely used in medical, nutrition and environmental research (Nicholson & Lindon, 2008). The acquisition of metabolic profile is very complex and is through many stages involving many actors (Söder *et al.*, 2019). The general process of metabolomics studies includes sample collection and preparation, but the preparation depends on the analytical method used (Bermudez Sanchez *et al.*, 2021).

Table 2 summarizes and compares some characteristics between two popular analytical tools, Nuclear Magnetic Resonance (NMR) and Mass spectrometry (MS) (Wishart, 2019). After preparing samples, targeted or untargeted metabolomics is performed (Rangel-Huerta *et al.*, 2019). Targeted metabolomics measures defined groups of chemically characterized metabolites (Muñoz-Prieto *et al.*, 2021). Untargeted and targeted metabolomics are, by far, complementary (Rangel-Huerta *et al.*, 2019). Untargeted metabolomics detects and analyses all the measurable analytes in a sample including chemical unknowns (Muñoz-Prieto *et al.*, 2021). Complexity of such techniques and sample analysis were not analyzed in this review.

Table 2 – Pros (normal) and cons (*italics*) associated with NMR and MS techniques in metabolomics. Adapted from Wishart, 2019.

NMR	MS
✓ Non-destructive sample	× *Destructive sample*
✓ Excellent reproducibility	× *Moderate reproducibility*
✓ Simple sample preparation	× *Complex sample preparation*
× *Poor to moderate sensitivity*	✓ Excellent sensitivity
× *Very expensive instrumentation*	✓ Moderate expensive instrumentation
× *Small spectral database*	✓ Large spectral databases

The *«fingerprint»* of metabolites was realized by analytical techniques giving rise to a spectrum whose peaks intensity correspond to the relative abundance of ions composing each molecule and enables to determine the structure of a metabolite (Nicholson & Lindon, 2008). The majority of studies listed here used NMR, Liquid Chromatography-Mass Spectrometry (LC-MS), Gas Chromatography-Mass spectrometry (GC-MS), combined MS/NMR or tandem mass spectrometry (MS/MS) as analytical techniques. Two studies used Ultra Performance Liquid Chromatography-Mass Spectrometry because particles size column is smaller than two microns making it more quantitative and accurate than High Performance Liquid Chromatography-MS (Zhao & Lin, 2015). The combination of techniques enlarged the spectrum of metabolite identification and provided a greater detection and interpretation of metabolites in the context of obesity by the qualitative and quantitative information they provided (Gulston *et al.*, 2007). In NMR, the sample is subjected to an intense electromagnetic field which is absorbed by the atomic nuclei of the molecules and the relaxation phase would characterize them and give information about the structure of metabolites (Gulston *et al.*, 2007). This technique alone is one of the oldest used but had low sensitivity compared to MS (Gulston *et al.*, 2007). In MS, the sample is fragmented because of energy received and the mass of these fragments is analyzed (Gulston *et al.*, 2007). MS is coupled to LC or GS before being performed in order to separate small molecules from macromolecules (Gulston *et al.*, 2007). Usually, metabolomics deals with low weight molecules, most often less than 1500 Da (Rangel-Huerta *et al.*, 2019). The MS/MS acquired samples between 25 to 1000 Dalton against 60 to 1000 for MS alone (Qu *et al.*, 2022).

After obtaining massive information, it is necessary to process the data using data processing platforms or software like XCSM Online or ChenomX (Roger *et al.*, 2022). Figure 2 demonstrates the several hurdles to overcome in acquiring and analyzing metabolites (Zhang *et al.*, 2017) like numerous statistical steps and analysis performed with statistical tools such as Principal Coordinate Analysis or Partial Least Squares-Discriminant Analysis in order to give meaning to the results (Ellero-Simatos *et al.*, 2019).

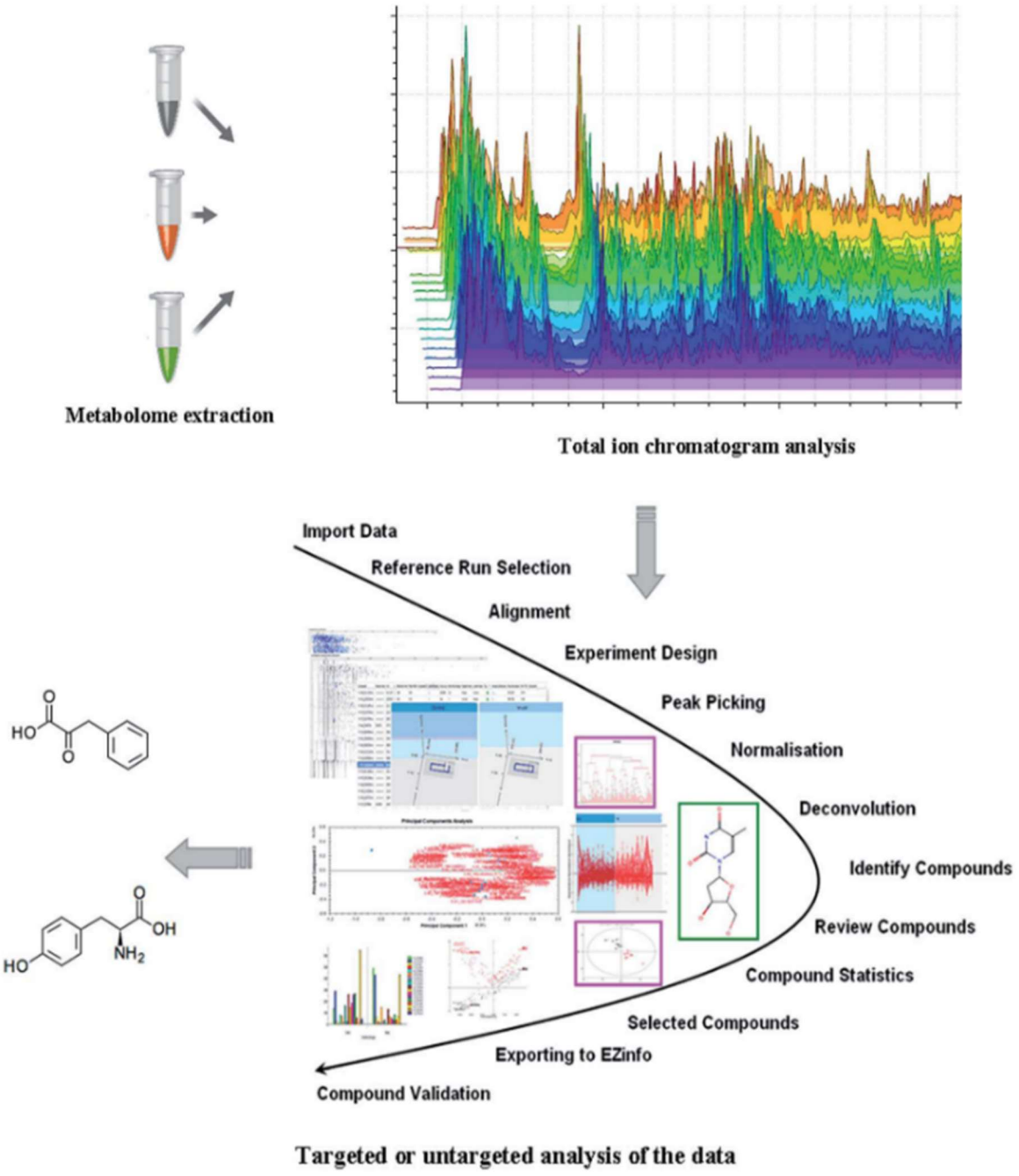

Figure 2 – Example of metabolomics approach and several analytical steps that followed the detection of metabolites. Adapted from *Zhang et al., 2017*.

2. Metabolome in dog and human obesity

2.1 Metabolomics belongs to an integrative approach

2.1.1 Proteomic benefits to metabolomic studies

Metabolites correspond to the small molecular scale (Figure 3). They derive from genes, transcribed into proteins, eventually affected by environmental factors such as pollution or diet (Jin & Ma, 2021). The study of *Lucena et al.* dealt with a proteomic analysis in obese dogs with or without obesity-related-metabolic-disorders (ORMD) and showed an increase of nine proteins implicated in glycolysis, cellular response to stress, transport functions and cytoskeletal dynamics (Lucena *et al.*, 2019). Glutathione S-transferase is one of the oxidative stress inhibiting enzymes increased in obesity (Lucena *et al.*, 2019).

Among changes in salivary metabolites pathways reported by *Muñoz-Prieto et al.*, glutathione metabolism is one of the most represented (Figure 3) (Muñoz-Prieto *et al.*, 2021). Proteomic and metabolomic results could be confronted and bring additional value to establishment of obesity biomarkers and their pathways as the metabolome is the terminal downstream product of the genome and consists of the total complement of all the low molecular weight molecules (metabolites) in a cell, tissue, or organism (Jin & Ma, 2021). Another example was adiponectin, inversely correlated with body mass and known to increase glycolysis and stimulate fatty oxidation (Eleanor Raffan, 2017). Lower concentrations of this protein were detected in plasma from obese individuals and could therefore be related to glucose and fatty acid metabolism (Osto & Lutz, 2015). Similarly, leptin influences lipid metabolism by stimulating beta-oxidation and inhibiting lipogenesis (Osto & Lutz, 2015).

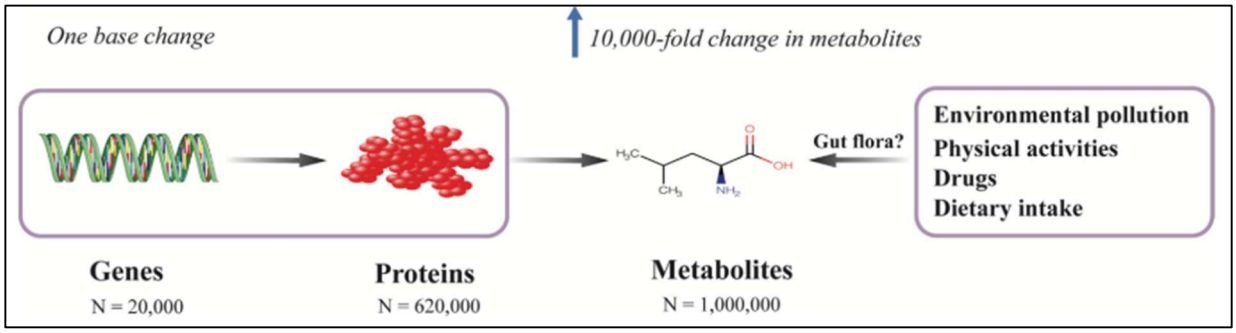

Figure 3 – From genome to metabolome, «the number of metabolites is estimated to be around one million, while the number of genes and proteins are about 20 000 and 620 000, respectively». Adapted from *Jin & Ma, 2021.*

2.1.2 Metabolic profile is a better predictor of obesity than genetic risk

A study showed that two groups of obese adults with the same BMI did not present the same metabolic alterations, one considered metabolically healthy compared to the other (Cirulli *et al.*, 2019). This study proved there was no association between BMI genetics and metabolome changes in humans (Cirulli *et al.*, 2019). Individuals with abnormal BMI and healthy metabolome are two to five times less risks to develop cardiovascular disease than those who have a normal BMI with an abnormal metabolome (Cirulli *et al.*, 2019). Another study distinguished metabolically healthy obese patients from metabolically unhealthy obese patients by reporting differences in acyl-carnitine and lysophosphatidylcholine profiles (Rangel-Huerta *et al.*, 2019). In fact, acyl-carnitine (C3:0), acyl-lysophosphatidylcholine (C16:1) were higher and acyl-lysophosphatidylcholines (C18:1; C18:2) were lower in unhealthy obese patients (Rangel-Huerta *et al.*, 2019). Metabolites concentrations could predict obesity with high sensitivity and specificity (res. 90%-80%) and thus detect unhealthy metabolome (Cirulli *et al.*, 2019). To the best of our knowledge, no previous study has been reported on dogs.

2.2 Obesity and changes in plasma metabolome

2.2.1 Lipid profile

Diverse findings were reported about fatty acid metabolism in obese people and dogs affecting different lipids classes, like polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) as well as lysophospholipids and sphingolipids, from different studies (Forster *et al.*, 2018; Rangel-Huerta *et al.*, 2019). Lipids alteration is the most common similarity detected in plasma from both obese humans and dogs (Forster *et al.*, 2018; Qu *et al.*, 2022). An increase in free fatty acids in plasma, including SFAs has been observed in adults (Xie *et al.*, 2012) but an untargeted metabolomic approach in obese children discovered a decreased of SFAs (Goodson *et al.*, 2018)**.** Among lipids the most represented in obese subjects are glycerol and glycerophospholipids (*e.g.*, triglycerides, phosphatidylcholines (PC)), sterols, sphingolipids, ceramides (Forster *et al.*, 2018) and other fatty acids like linoleic acid and stearidonic acid in both humans and dogs (Qu *et al.*, 2022; Zhang *et al.*, 2017).

Lysophospholipids are one class of the metabolites presenting alterations in both obese humans and dogs even in obese children (Kim *et al.*, 2010; Qu *et al.*, 2022; Rangel-Huerta *et al.*, 2019). Different studies reported lysophosphatidylcholine (lysoPC) C18:1 inversely correlated with the BMI (Kim *et al.*, 2010; Qu *et al.*, 2022; Rangel-Huerta *et al.*, 2019) as for lysoPC C16:0, decreased in obese people and dogs (Forster *et al.*, 2018; Zhang *et al.*, 2017). Moreover, significant differences in the plasma levels of PC species were also quantified by untargeted and targeted approaches between females and males (Rangel-Huerta *et al.*, 2019). For instance, lower concentrations of lysoPC C18:2, C20:4, C20:5 are lower in obese women in relation to obese men (Rangel-Huerta *et al.*, 2019). A systematic review of metabolic signature of human obesity reported that increase or decrease of lysoPCs in obese conditions seems to depend on each study (Rangel-Huerta *et al.*, 2019), for example, lysoPC C18:2 is not

significantly different between obese and lean men (J. Y. Kim *et al.*, 2010) whereas an inverse correlation with BMI was reported by *Rangel-Huerta et al.* (Rangel-Huerta *et al.*, 2019). In dogs, a comparative study between clinically healthy normal weight, overweight and obese companion dogs showed a decrease of this metabolite in overweight and obese dogs (Forster *et al.*, 2018).

In humans, metabolomics studies have made some progress with analyses in pregnant women and children, including results from the umbilical cord (Rangel-Huerta *et al.*, 2019). At birth, the higher presence of medium and long chain fatty acids, such as stearate, oleate or palmitate in the umbilical cord might predispose to the development of obesity at the age of 3 to 5 years (Rangel-Huerta *et al.*, 2019). Obese pregnant women showed higher concentration of Very-Low-Density Lipoprotein (VLDL) and lower High-Density Lipoprotein (HDL) and PUFAs (Rangel-Huerta *et al.*, 2019). No studies in canine pregnancy or puppies have been published.

2.2.2 Amino Acid and other obesity biomarkers profile

The importance of carnitine in the metabolism of fatty acids made it one of the major metabolites analyzed in human and dog research (Söder *et al.*, 2019). Numerous metabolites derived from carnitine or composed by carnitine (*e.g.*, acyl-carnitine) were detected in both obese humans and dogs (Söder *et al.*, 2019). Otherwise, L-carnitine, and three acyl-carnitines (propionyl-, butyryl- and hexanoyl-carnitine) were considered potential biomarkers in humans (J. Y. Kim *et al.*, 2010). In obese people, acyl-carnitines concentrations have been shown to increase, and it has been confirmed by a nontargeted metabolomics analyses in dogs with obesity induced by high-fat-diet (*e.g.*, adipoylcarnitines, oleoylcarnitines) (Qu *et al.*, 2022). However, others were depleted in the plasma (*e.g.*, adipoylcarnitines) (Qu *et al.*, 2022).

A systematic review showed a decrease of glycine and glutamine levels in plasma from obese people and increase of branched-chain amino-acid (BCAAs), confirmed by both untargeted and targeted approaches (Rangel-Huerta *et al.*, 2019). These results were also confirmed by untargeted analysis in obese children (Goodson *et al.*, 2018). According to a study by *Petrus et al., 2020*, which compared metabolites released from adipose tissue in obese and non-obese women, glutamine levels were found to be downregulated in obese women and were associated with adipose tissue inflammation (Petrus *et al.*, 2020). In dogs, a decrease of glutamine was detected after post-prandial period in both obese and lean dogs and do not seem particularly related to obese conditions (Söder *et al.*, 2019). On the other hand, glycine observed an inverse correlation between plasma glycine levels and BMI in adults but also in obese adolescents and obese children (Rangel-Huerta *et al.*, 2019).

Caffein pathway is also implicated in obesity pathology (Qu *et al.*, 2022). In fact, a nontargeted metabolomic approach on plasma from dogs with obesity induced by high-fat-diet identified significative higher concentrations of metabolites, such as 3-methylxanthine and theophylline (Qu *et al.*, 2022).

2.3 Urine and saliva metabolomes and obesity

2.3.1 Urine – metabolomic analysis in obesity research

The organic acids are concentrated in urine and result from the catabolism of amino acids, sugars and fatty acids, although there are variations depending on feed *status* (Qu *et al.*, 2022).

Taurine and citrate concentrations are decreased, while allantoin concentration is increased, in postprandial urine from obese dogs unlike lean dogs (Söder *et al.*, 2017). In fasting urine, there was no difference between lean and obese dogs (Söder *et al.*, 2017). This study showed that the use of food challenge in dogs revealed metabolites alterations in post-prandial urine that were not detectable on fasting conditions, as in humans (Söder *et al.*, 2017). However, this study did not find any association between body condition and urinary citrate concentration in fasting or postprandial samples (Söder *et al.*, 2017). In humans, citrate urine excretion could be a marker for glucose metabolism and insulin resistance and has been reported lower in obese people (Xie *et al.*, 2012). Furthermore, urine concentration of taurine is decreased in spontaneously obese dogs and in the plasma of obese mice (Qu *et al.*, 2022) and a negative association with BMI in people has been found (Söder *et al.*, 2017). This result was also confirmed in postprandial urine as well as in fasting urine in obese dogs compared to lean individuals (Söder *et al.*, 2017).

Another metabolite that caught researcher's attention was trigonelline or N-methylnicotinate. In obese humans, it has been reported to decrease presumably due to depletion of glutathione storage in liver (Calvani *et al.*, 2010). A study by *Söder et al.*, reported no significant alteration in the urine of obese dogs (Söder *et al.*, 2017). However, another study found a decrease of this metabolite in both plasma and urine samples (Qu *et al.*, 2022).

An increase of betaine and carnosine, both antioxidants, was also observed in a comparative study metabolome between lean and obese dogs (Forster *et al.*, 2018), but a study combining urinary metabolic signatures in obese humans found that the elevated carnosine levels were non-significant at the obese state (Elliott *et al.*, 2015).

Moreover, purine metabolism seems enhanced in obese people (Forster *et al.*, 2018), and dogs by the increase of methylxanthine metabolite while 1-methylhypoxanthine decreases in the urine from obese dogs (Forster *et al.*, 2018). Figure 4 illustrates some obesity-related metabolites interactions within the metabolic network enounced here (Xie *et al.*, 2012).

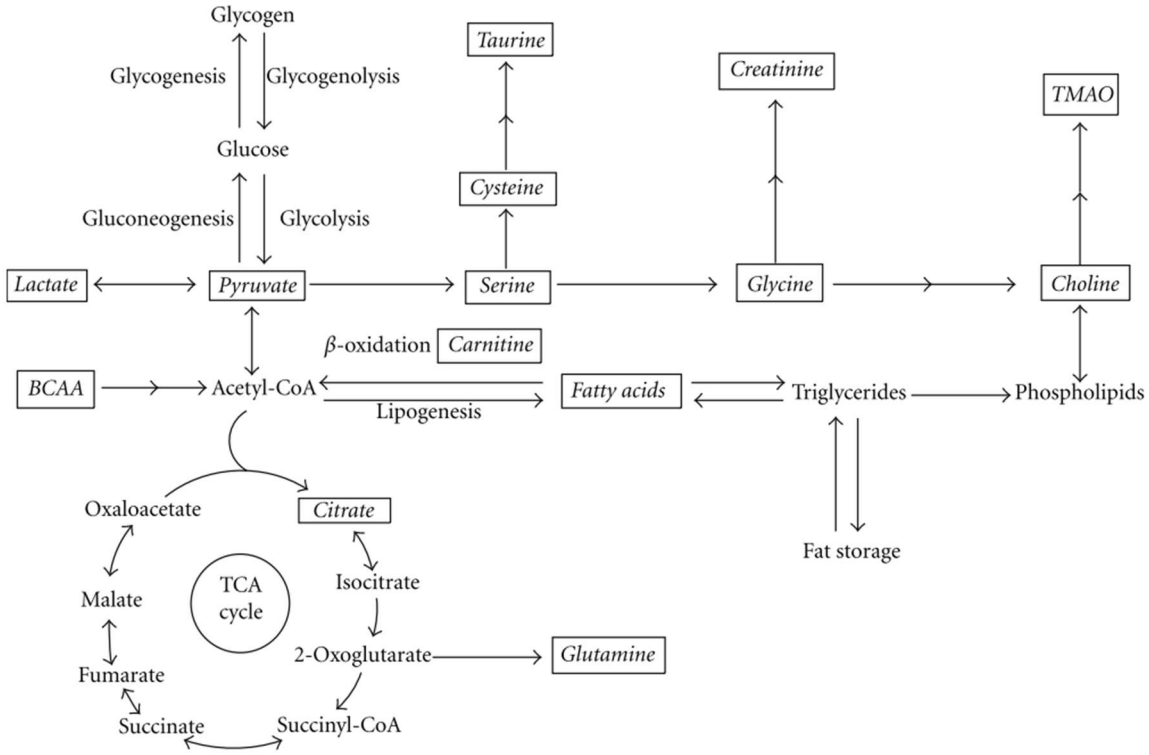

Figure 4 – Obesity-related metabolites interactions within the metabolic network. BCAAs, branched chain amino acids; TMAO, trimethylamine N-oxide; metabolites explicitly mentioned by *Xie and al*. have been written in boxes and *italics*. Adapted from *Xie and al. 2012*.

2.3.2 Saliva – a metabolomic analysis in obesity

Only a few publications are related to metabolome analysis from saliva in obese humans and dogs. The function of salivary metabolites remains unexplored although some pathways involved in obesity were detected (Figure 5) (Muñoz-Prieto *et al.*, 2021).

A study aiming to identify salivary and plasma biomarkers for obesity in children by non-targeted metabolomic analysis found that the most detected metabolites in saliva were peptides, and not lipids, like in plasma (Goodson *et al.*, 2018).

Muñoz-Prieto et al. is the first study illustrating metabolic changes in canine saliva from obese dogs by metabolic targeted approach (Muñoz-Prieto *et al.*, 2021). In dogs, five amino acids, alanine, serine, citrulline, glycine and lysine, and acylcarnitine are significantly increased (Muñoz-Prieto *et al.*, 2021). Serine, citrulline and acylcarnitine are also increased in humans (Muñoz-Prieto *et al.*, 2021). In the same study, di- and triglycerides as phosphatidylcholines showed an up and down regulation whereas ceramides, like Cer(34:0) and Cer(41:1), are upregulated (Muñoz-Prieto *et al.*, 2021). Glutathione S-transferase was also reported increased in obese dogs with ORMD (Lucena *et al.*, 2019). These metabolites interfered in ammonia recycling, alanine, glutathione, carnitine, glycine and serine metabolism, significantly altered in dog obesity (Figure 5).

Gardner et al. reviewed current knowledge about salivary metabolomics in humans but gave few details in relation to obesity (Gardner *et al.*, 2020). Phosphate, biogenic amines (putrescine and cadaverine) are increased in salivary sample from obese people (Gardner *et al.*, 2020) as they have been reported in obese children (Goodson *et al.*, 2018).

In fact, phosphate seems to be a potential biomarker of obesity in obese children (Goodson *et al.*, 2018). The precursors of putrescine and cadaverine (ornithine and lysine) were also reported to be increased (Goodson *et al.*, 2018).

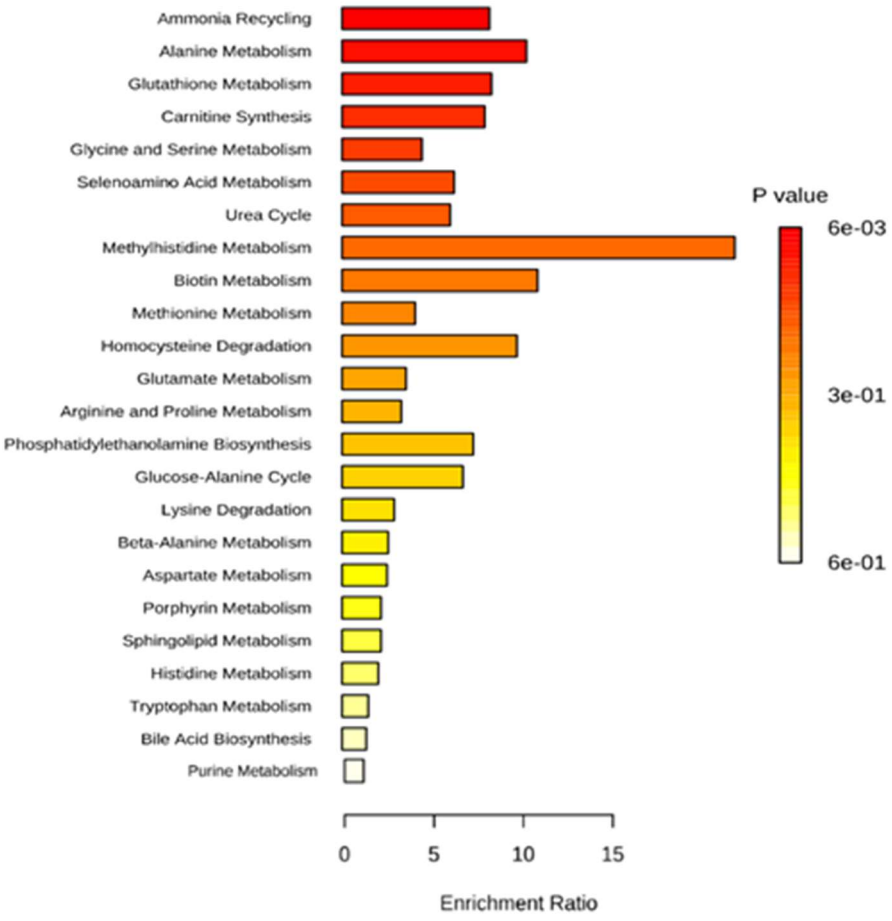

Figure 5 - «Metabolites set enrichment overview» illustrating metabolic pathways in relation with metabolites alterations in saliva from obese dogs. Ammonia recycling, alanine, glutathione, carnitine, glycine and serine metabolism are significantly altered in obese dogs. Adapted from *Muñoz-Prieto et al., 2021.*

2.4 Gut microbiota and obesity

2.4.1 Alterations of gut microbiota could influence metabolomic results

Gut microbiota is known to affect host energy homeostasis and modulates immune system by metabolizing nutrients from the diet and by producing various metabolites (*e.g.*, short-chain-fatty-acids, SCFAs) (Bermudez Sanchez *et al.*, 2021) which act locally or enter in the bloodstream through absorption into the enterohepatic circulation (Bermudez Sanchez *et al.*, 2021).

Only a few studies in dogs evaluated differences in fecal microbiota between lean and obese individuals (Forster *et al.*, 2018; Söder *et al.*, 2022). Firmicutes/Bacteroidetes ratio increases in both obese people and dogs (Söder *et al.*, 2022) with a predominance of the *Firmicutes phylum* (Forster *et al.*, 2018). Predominant fecal bacteria genera in dogs differed among studies. However, these results do not differ significantly between obese and lean individuals (Forster *et al.*, 2018; Söder *et al.*, 2022). But in dogs, as well as in humans, large inter-individual variations exist, and high microbiota diversity might be synonym of «healthy» gut microbiota (Fan & Pedersen, 2021). In fact, diversity of intestinal microbiota seems to be reduced in obese individuals (Apper *et al.*, 2022; Forster *et al.*, 2018). Further, microbiota richness is influenced by weight loss, diet change (Bermudez Sanchez *et al.*, 2021), environmental exposure during childhood and antimicrobial therapy (Fan & Pedersen, 2021; Osto & Lutz, 2015).

Specific metabolites associated with an obese phenotype can join microbiota analysis to identify potential biomarkers of obesity as microbiota end-products influence host metabolism (Fan & Pedersen, 2021).

2.4.2 Metabolomics, a useful tool to investigate microbial end-products

Important functions derived from gut microbiota in host physiology were already studied (Boulangé *et al.*, 2016). They are implicated for example in energy production by fatty acid metabolism, in digestion by biliary acid metabolism, in protective functions by maintaining an integrative intestinal epithelium barrier or by modulating the immune system (Bermudez Sanchez *et al.*, 2021). Through these microbial processes, gut microbiota and its end-products contribute to obesity (Bermudez Sanchez *et al.*, 2021).

A recent study published in Nature Reviews Microbiology reviewed literature on the intestinal microbiota of humans and enhanced a link between intestinal microbiota and fecal metabolome (Fan & Pedersen, 2021). In obese state, gut microbiota seems to promote a chronic low-grade inflammation by having an increase of pro-inflammatory bacteria (*e.g.*, *Bacteroides* spp.) and a decrease of anti-inflammatory bacteria (*e.g.*, *Faecalibacterium prausnitzii*) (Boulangé *et al.*, 2016). In fact, it was observed a decrease of *Blautia* gender bacteria in obese dogs (Alessandri *et al.*, 2020). This result could be also confirmed in humans, as butyrate producers' bacteria was higher in lean humans (Fan & Pedersen, 2021). Moreover, in dogs a decrease of *Eubacterium* gender bacteria, producer of SCFAs such as butyrate, has been identified (Alessandri *et al.*, 2020). Furthermore, gram negative bacterial membranes possess

lipopolysaccharides (LPS), released by dead bacteria or in case of endotoxemia for instance, which become a stimulator of host immunity (Bermudez Sanchez *et al.*, 2021).

Another study showed that fecal decrease of fatty acid in obese dogs could be consistent with a chronic inflammation state and oxidative stress (Forster *et al.*, 2018). In obese humans it was reported a large amount of volatile fatty acids (VFAs) decreased after weight loss when compared to lean individuals (Fan & Pedersen, 2021). But one study of fecal bacteria in obese dogs after a caloric diet restriction noticed there was no difference in VFAs between both obese and lean dogs and no diet influence (Vecchiato *et al.*, 2023). However, despite these differences, changes in lipid metabolism may affect the host's metabolism and can be detected by metabolomic techniques (Osto & Lutz, 2015). It also has been written that gut microbiota promoted triglyceride deposition in adipocytes by downregulating intestinal expression of fasting induced adipose factor (Boulangé *et al.*, 2016). A decrease in abundance of fecal vitamin D metabolites has also been reported in obese and overweight dogs (Forster *et al.*, 2018).

Another study of untargeted fecal metabolome in obese dogs after weight loss, has reported a decrease of trigonelline, purine, L-methionine, coumestrol metabolites (Bermudez Sanchez et al., 2021). Trigonelline derived from gut microbiota, has also been detected in urine and plasma samples (Zhang *et al.*, 2017).

Although there are many human studies in obesity (Fan & Pedersen, 2021), studies in dogs are still limited (Qu *et al.*, 2022). A non-exhaustive list of possible or confirmed common obesity biomarkers has been proposed on Table 3.

Table 3 – Concise summary of several potential biomarkers for diagnosing obesity in both humans and dogs. The presence of a metabolite in analytical fluids (plasma, urine, saliva, feces) is depicted by a cross for obese (OB) humans and dogs. A circle denotes no reported metabolite in this context. A line refers controverse founded between obesity and the metabolite orientation reported in studies; increased metabolites are represented in standard font, while decreased metabolites are indicated in *italics*.

Pathways	Metabolites class or metabolites	Fluid analyzes	Present in OB humans	Present in OB dogs	References
Lipid, glucid and bile acids metabolism	SCFAs (acetate, *butyrate propionate*)	Plasma, feces	X	X	(Alessandri *et al.*, 2020; Apper *et al.*, 2020; Fan & Pedersen, 2021;)
Lipid metabolism	Glycerophospholipids, sterols, sphingolipids, ceramides	Plasma	X	X	(Forster *et al.*, 2018)
Lipid metabolism	Linoleic acid C (18:2)	Plasma	X	X	(Qu *et al.*, 2022 ; Zhang *et al.*, 2017)
Lipid metabolism	Stearidonic acid C (18:0)	Plasma, saliva	X	X	(Qu *et al.*, 2022)
Lipid metabolism	Oleic C (18:1), palmitic C (16:0) acids	Plasma	X	O	(Rangel-Huerta *et al.*, 2019)

Lipid metabolism	LysoPCs : *lysoPC C (18:1) and C (16:0)* lysoPC C (18:2)	Plasma, saliva	X	X	(Forster *et al.*, 2018; Kim *et al.*, 2010; Muñoz-Prieto *et al.*, 2021; Qu *et al.*, 2022; Rangel-Huerta *et al.*, 2019; Zhang *et al.*, 2017)
Lipid metabolism	*Acyl-lysophosphatidylcholines (C18:1); (C18:2)*	Plasma	X	X	(Rangel-Huerta *et al.*, 2019)
Lipid, caffein metabolism	*L-carnitine*	Plasma	X	X	(Rangel-Huerta *et al.*, 2019; Söder *et al.*, 2019)
Lipid metabolism	Acyl-carnitine (C3:0), acyl-lysophosphatidylcholine (C16:1)	Plasma	X	O	(Rangel-Huerta *et al.*, 2019)
Lipid metabolism	Acylcarnitines (Propionyl-, butyryl- and hexanoyl-carnitine)	Plasma	X	O	(Kim *et al.*, 2010)
Lipid metabolism	Other carnitines (*oleoylcarnitine*, adipoylcarnitine)	Plasma saliva, urine	X	X	(Muñoz-Prieto *et al.*, 2021 ; Qu *et al.*, 2022)
Lipid metabolism	Acyl-carnitines C (3:0), C (5:0)	Saliva, urine	O	X	(Gardner *et al.*, 2020)
Protein metabolism	BCAAs (leucine, isoleucine, valine)	Plasma	X	X	(Apper *et al.*, 2020; Goodson *et al.*, 2018; Rangel-Huerta *et al.*, 2019)
Amino acid, bile salts, glucose, and urea metabolism	*Glycine* glycine	*Plasma*, saliva	X	X	(Goodson *et al.*, 2018; Muñoz-Prieto *et al.*, 2021; Rangel-Huerta *et al.*, 2019)
Amino acid, glucose, and urea metabolism	*Glutamine*	Plasma	X	O	(Petrus *et al.*, 2020; Söder *et al.*, 2019)
Caffein metabolism	3-methylxanthine	Plasma	X	O	(Qu *et al.*, 2022)
Caffein metabolism	Theophylline	Plasma	X	O	(Qu *et al.*, 2022)
Uric acid metabolism	Allantoin	Urine	O	X	(Söder *et al.*, 2017)
Glucose metabolism	*Citrate*	Urine	X	O	(Söder *et al.*, 2017; Xie *et al.*, 2012)
Lipid metabolism	*Taurine*	Plasma, urine	X	X	(Qu *et al.*, 2022; Söder *et al.*, 2017)
Niacin metabolism	*Trigonelline*	Plasma, urine, feces	X	X	(Calvani *et al.*, 2010; Qu *et al.*, 2022; Söder *et al.*, 2017)
Glucose metabolism	*Alanine, lysine*	Saliva	O	X	(Gardner *et al.*, 2020; Muñoz-Prieto *et al.*, 2021)
Lipid and protein metabolism	Serine, citrulline	Saliva	X	X	(Gardner *et al.*, 2020 ; Muñoz-Prieto *et al.*, 2021)
Lysine and uric acid metabolisms	Putrescine, cadaverine, phosphate	Saliva	X	O	(Goodson *et al.*, 2018)

3. Discussion

The literature concerning to the parallelism between humans and dog's obesity and overweight is sparse and it is important to enhance studies' limitations with respect to the biases noted above. Several important factors, such as age, sample size, sex, breed and background diet differ among literature (Forster *et al.*, 2018).

Concerning to the age factor, in dogs, every study dealt with adults, and only two studies approached children in humans (Goodson *et al.*, 2018; Ranger-Huerta *et al.*, 2019). Despite efforts, a low number of dogs were included and enrolling healthy obese dogs seemed to be difficult (Söder *et al.*, 2019).

Gender differences might also be considered (Rangel-Huerta *et al.*, 2019). For instance, significant differences were reported between obese males and females, namely, higher concentrations of creatine, palmitic acid, linoleic acid and lower concentrations of several lysoPCs and uric acid were more detected in females than in males (Rangel-Huerta *et al.*, 2019). In humans, sex-based differences have been founded by quantifying individual metabolites although gender metabolite profile did not differ (Gardner *et al.*, 2020). In dogs, the majority were castrated or entire males except two studies with both males and females (Apper *et al.*, 2020; Bermudez Sanchez *et al.*, 2021). Thus, neuter *status* could be another bias. It might wondered whether the hormonal influence could modify metabolite profiles (Bermudez Sanchez *et al.*, 2021). Further, some studies worked with numerous different races especially more than ten and we could wonder if metabolite variations depend on inter-race differences or individual scale (Forster *et al.*, 2018).

In relation to the methods for quantifying the amount of fat mass (outlined part 1), imaging could also be used through computed tomography and magnetic resonance imaging (Qu *et al.*, 2022).

In relation to analytical fluids, saliva samples were scarce in dogs compared to humans (Goodson *et al.*, 2018; Lucena *et al.*, 2019). However, saliva is an interesting fluid to analyze metabolites as it is collected by a non-invasive method (Muñoz-Prieto *et al.*, 2021).

Regarding metabolomics results, plasma is the most studied fluid and consequently seemed to be the most altered in obesity studies (Forster *et al.*, 2018; Ranger-Huerta *et al.*, 2019). Among studies which have been analyzed, lipids are the most affected (Forster *et al.*, 2018). It is believed that lysoPCs may contribute to metabolic dysregulation associated with obesity through their involvement in inflammation, insulin resistance and lipid metabolism (Muñoz-Prieto *et al.*, 2021). The exact mechanisms underlying the relationship between lysoPCs and obesity are still being investigated (Rangel-Huerta *et al.*, 2019). LysoPCs like C16:0 and C18:1 could be proposed as potential biomarkers for obesity as their decrease in obese state have been reported in different studies (Forster *et al.*, 2018, Rangel-Huerta *et al.*, 2019). Interestingly, lysoPC C18:1 has been found upregulated in saliva from obese dogs (Muñoz-Prieto *et al.*, 2021). However, conflicting findings between studies require further research to determine which other specific lysoPCs could be used as potential biomarkers for diagnosing obesity (Rangel-Huerta *et al.*, 2019). Further, it is known that phospholipids are essential components of cell membranes by their

structural role and their signal transduction function (Muñoz-Prieto *et al.*, 2021). Hemolytic blood has been detected more in obese dogs rather than lean dogs (Forster *et al.*, 2018). Additionally, obesity is related to changes in lysoPCs and choline concentrations and could repercuss in red blood cell's structure making them more friable (Forster *et al.*, 2018).

Carnitine is an amino acid derived from lysine and methionine and is essential for the fatty acid β-oxidation within the mitochondria (Xie *et al.*, 2012). Due to the excessive accumulation of adipose tissue and increased metabolic stress, it has been reported that the excessive demand and consumption of carnitine for β-oxidation could lead to a depletion of carnitine levels (Söder *et al.*, 2019). It was therefore recognized that carnitine pathway is one of the main concerns in human and dog obesity studies (Rangel-Huerta *et al.*, 2019).

Regarding obesity, studies have suggested an association between taurine and lipid metabolism, as dietary supplementation of taurine in obese mice decreases body weight and serum lipid concentrations (Tsuboyama-kasaoka *et al.*, 2015). In fact, taurine is involved in fat digestion by participating in bile formation as a bile acid conjugator (Söder *et al.*, 2017). A decrease of such amino acid in urine from obese subjects could be explain by a less efficient lipid turnover or a decrease synthesis of taurine, but the exact mechanism beyond this reduction is not clearly known and need further research (Söder *et al.*, 2017). Additionally, taurine has an antioxidant role, as it allows a better absorption of zinc, for example (Söder *et al.*, 2017). Although the composition of the test diet may have an influence in urinary results, *Söder et al.* proved that fasting condition did not modify such observation, making taurine a biomarker of obesity in dogs (Söder *et al.*, 2017).

Citrate is an intermediate in the tricarboxylic acid cycle (Xie *et al.*, 2012). Urinary excretion of citrate is influenced by acid-base *status* (Xie *et al.*, 2012). Obesity alters glucose metabolism, and the presence of insulin resistance leads to citrate accumulation, triggering metabolic acidosis and therefore a reduction in excretion of citrate (Xie *et al.*, 2012). In purine metabolism, uric acid is converted to allantoin (Maiuolo *et al.*, 2016). In humans, a lack of uricase enzyme makes allantoin synthesis impossible unlike in many mammals (Maiuolo *et al.*, 2016). Therefore, allantoin will not be treated as a biomarker of obesity (Maiuolo *et al.*, 2016).

Glycine is an amino acid precursor of creatine in the liver, a compound of bile salts, making them more soluble (Alves *et al.*, 2019). Otherwise, a targeted approach in humans has detected a positive correlation between BMI and creatine-related metabolites (*e.g.*, carnitine, choline and glycerophosphocholine) (Rangel-Huerta *et al.*, 2019). As choline is a precursor of glycine, it could be assumed that a decrease of glycine might be due to a decrease of choline formation (Rangel-Huerta *et al.*, 2019). Lower concentrations of glycine have been associated with obesity and it is defined as a potential plasma biomarker of obesity in humans (Alves *et al.*, 2019). *Alves et al., 2019* hypothesyses that the reduction of plasmatic glycine concentration could be due to a decrease of gut absorption or biosynthesis, or to an increase of its catabolism or its urine excretion (Alves *et al.*, 2019). Its interaction in lipid metabolism (bile salts synthesis), glucose metabolism (glycine decreases hepatic glucose

production by activating NMDA receptor), its regulation of methyldonors function (glycine is indirectly linked with trigonelline synthesis by regulating S-adenosylmethionine (SAM) production), and glutathione (GSH) synthesis (impact on oxidative stress) make glycine a contributor of obesity pathogenesis and consequent comorbidities (Alves *et al.*, 2019). In dogs, no source was reported here.

Alves et al., 2019 suggests that Glycine is a limiting factor for GSH synthesis (Alves *et al.*, 2019). GSH is a tripeptide composed by glutamic acid, cysteine and glycine (Picklo *et al.*, 2015). GSH has antioxidant properties and has been proved to be a positive indicator of humans' health (Picklo *et al.*, 2015). GSH is linked to obesity as its implication in inflammation state and oxidative damage is associated with an increase of BMI or BCS (Picklo *et al.*, 2015). It has been observed an increase of glycine in saliva fluid from obese dogs and glutathione metabolism is one of the most altered pathways in the obesity state (Muñoz-Prieto *et al.*, 2021). Moreover, glutathione-S-transferase (GST), an enzyme implicated in the metabolism of glutathione, is increased in obese dogs with ORMD (Lucena *et al.*, 2019). It has a detoxify function (Picklo *et al.*, 2015). In fact, PUFAs like linoleic acid C (18:2) or arachidonic acid C (20:4) produce cytotoxic aldehydes which are substrates for GSH conjugation and are detoxified by GSTs (Picklo *et al.*, 2015). Interestingly, linoleic acid is reported to be increased in both plasma and saliva from humans and dogs (Zhang *et al.*, 2017; Qu *et al.*, 2022). In cases of obesity, GST appears to be downregulated, leading to high levels of cytotoxic aldehyde molecules (Picklo *et al.*, 2015).

Theophylline and 3-methyllxanthine were reported higher in obese dogs (Qu *et al.*, 2022). In humans, no alteration was reported here but anti-obesity effects have been suggested by dietary caffein, which might decrease fat accumulation in adipocytes and inhibit insulin-stimulated glucose uptake (Qu *et al.*, 2022).

Trigonelline is a gut flora-derived metabolite or a by-product of the conversion of SAM to S-methylhomocysteine (Mizuno *et al.*, 2014). This molecule has anti-obesogenic effects and can attenuate adipocyte differentiation and lipid accumulation by being involved in choline metabolism (Ilavenil *et al.*, 2014) and reduces cardiovascular risk in humans by inhibiting synthesis of trimethylamine (TMA), (Fan & Pedersen, 2021). TMA is synthesized by gut microbiota into L-carnitine or lecithin metabolites (Richard *et al.*, 2020). It is oxidized in trimethylamine N-Oxide (TMAO) in the liver and has been reported to have increased in obese people (Fan & Pedersen, 2021). Indeed, TMAO is known to be a biomarker of atherosclerosis (Fan & Pedersen, 2021). However, the comorbidity of arteriosclerosis is rare in dogs and does not seem to be obesity induced (Lucena *et al.*, 2019). BCAAs, like isoleucine, valine and leucine are linked to some acyl-carnitines metabolism, as C3 acyl-carnitines are a by-product of isoleucine and valine catabolism and C5 acyl-carnitines are intermediates in mitochondrial isoleucine and leucine catabolism (Xie *et al.*, 2012). Interestingly, acylcarnitine C (3:0) and C (5:0) has been reported up-regulated in saliva from chronic obese dogs (Gardner *et al.*, 2020). In relation to analytical fluids, saliva samples were scarce in dogs compared to humans (Goodson *et al.*, 2018; Lucena *et al.*, 2019).

Citrulline is a non-proteinogenic amino acid with antioxidant properties and modulates protein and lipid metabolism. In relation to lipid metabolism, this molecule seemed to reduce overweight in obese rats by decreasing glyceroneoglucogenesis and increasing liberation of non-esterified fatty acids (Joffin *et al.*, 2016). Citrulline also enables nitric oxide to be excreted by acting as an intermediate in the urea cycle and as a precursor of arginine (Joffin *et al.*, 2016). The increase of citrulline in the saliva of obese dogs and humans requires further studies to determine its exact implication in the pathology of obesity.

The mechanisms behind the metabolic alterations observed in the obese subjects are far from being elucidated (Qu *et al.*, 2022). This is the case of phosphate it is increased in saliva from obese children and is a potential biomarker of obesity (Goodson *et al.*, 2018). Its up regulation could be due to local salivary gland modifications (Hartman & Groppo, 2013) and no alteration was observed in plasma from obese children (Goodson *et al.*, 2018). However, a decrease of phosphate is reported in plasma from obese humans and has a role in the pathogenesis of metabolic syndrome (Stoian *et al.*, 2013). It would be interesting to perform targeted analysis in dogs to confirm phosphate, glycine, and glutathione as biomarkers for obesity.

Furthermore, it is important to consider the local microbiota (Gardner *et al.*, 2020). Indeed, biogenic amines, detected in both adults and children, reflect oral bacterial products from protein catabolism and may be not disease-specific (Gardner *et al.*, 2020). In 2004 and 2006, it has been discovered that gut microbiota regulates harvest energy from diet and energy storage and transferrable microbiota from obese mice to lean mice induced obesity in the lean animals, respectively (Fan & Pedersen, 2021). High-polysaccharide diet induced higher concentrations of SCFAs in fecal, blood or urine samples (Fan & Pedersen, 2021). Diet influences in metabolome profile of obese dogs and humans has not been reported here and constitutes another way to identify possible biomarkers of obesity (Y. J. Kim *et al.*, 2019). In relation to SCFAs, butyrate as well as propionate are qualified «anti-obesogenic» through their stimulation of anorexigenic hormones and leptin synthesis (Fan & Pedersen, 2021). Further, butyrate is also considered as an inflammatory reducer by being one of the main energy supplies of colonocytes (Apper *et al.*, 2020). A decrease of butyrate production modifies the structure of the intestinal epithelium, leading to increased intestinal permeability and the passage of molecules (*e.g.*, lipopolysaccharides) to the bloodstream (Apper *et al.*, 2020). Additionally, an increase of butyrate concentration increases β-oxidation and oxygen consumption in the gut by activating the peroxisome proliferator-activated receptor γ (PPARγ) (Fan & Pedersen, 2021). It contributes to homeostasis of anaerobic conditions in the gut (Fan & Pedersen, 2021). A decrease of butyrate in obese people and dogs could thus explained dysbiosis (Fan & Pedersen, 2021). Vitamin D stimulates the innate immune system and regulates gut microbiota, and a deficit of its metabolites may induce dysbiosis and greater susceptibility to gut injury (Forster *et al.*, 2018). Further research must be developed to understand the exact mechanisms linking gut microbiota and vitamin D alterations with obesity (Boulangé *et al.*, 2016), especially as obesity could be a cause or a consequence of microbiota end-products (Fan & Pedersen, 2021). Thus, the field of metabolomics is indirectly linked to the analysis of the gut microbiota which plays a crucial role in various aspects of human and dog health, including metabolism (Apper *et al.*, 2020; Boulangé *et al.*, 2016). By

analyzing the metabolites produced but the gut microbiota, researchers can gain insights into how it contributes to metabolic changes associated with obesity (Fan & Pedersen, 2021). However, further studies are needed to investigate similitudes between samples such as saliva and urine or fecal metabolites in relation to plasma concentration (Forster *et al.*, 2018).

Further, in the light of what has been done in humans (Rangel-Huerta *et al.*, 2019), it would be interesting to conduct a meta-analysis study of the most recurrent obesity-related metabolic alterations in dogs (González-Arostegui *et al.*, 2022; Muñoz-Prieto *et al.*, 2021). Another recommendation would be to carry out a "standard metabolomics protocol" for future canine obesity studies. This could be based on principles like the nine-point BCS scale, breed selection or larger cohorts in order to minimize biases, reduce inter-individual variation and obtain a higher Evidence Based Medicine (EBM) grade.

4. Conclusion

Obesity is a wide multifactorial and complex condition. Genetics alone is not sufficient to induce obesity since besides an eventual predisposition, enabling external factors are necessary.

Understanding the diagnosis and management of this condition has led to a deeper exploration of obesity by metabolomics studies, aiming to identify specific markers. Among the metabolites, lipids are the most altered. It has been observed that, at the molecular level, an obese metabolome can be diagnosed even before any detectable weight alteration in humans, which opens opportunities of research in dogs as well, eventually as a spontaneous model of disease for translational purposes. However, more comprehensive studies and standard protocols are necessary to advance the field and identify common biomarkers between humans and dogs, even if the similarities observed between humans and dogs are encouraging for utilizing dogs as translational study models.

An integrative approach combining various «-omics» fields (*e.g.* genomic and proteomic) is essential to understand the complex metabolic network. Metabolomics, by identifying obesity biomarkers could also be considered as a quantitative method of obesity diagnosis and follow up, but further research is required to fully understand the metabolomic changes associated with obesity and overweight in humans and dogs. Metabolomics is also used to analyze obesity in many other species such as cats and horses (Wallis & Raffan, 2020) but also in other fields like endocrinology.

It is accepted that the metabolomic profile in obese patients, humans, and dogs, is partially overlapped and, in some clinical contexts, follow the same variation pattern. The epidemiologic obesity tendence presses early diagnostic reliable and quantifiable tools and this important medical need is not yet satisfied. Veterinary medicine can be the ideal research field since animals share many genetic and environmental predisposition factors with humans alongside to metabolomics. In future work, it is important to design research in order to find biomarkers and its usefulness on diagnostic, follow up and prognostic.

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