Proceedings of the 5th annual symposium of the German Society for Paleo Nutrition held in 2017

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Abstract
We present the scientific abstracts of the 5th Annual Symposium of the German Society for Paleo Nutrition (Deutsche Gesellschaft für Paläoernährung) which took place on September 23rd 2017 in Giessen, Germany.

Besides an overview on existing interventional studies on Paleolithic diets, a focus of this year’s symposium was on secondary plant metabolites: presence, standardization by phytoneering and the role of phytomedicine for prevention and treatment of cancer. Further topics were the role of vitamin-D and omega-3 fatty acids, as well as molecular anthropology and personalized nutrition.

Keywords
Ancestral health, cancer, epigenetics, personalized nutrition, Paleolithic diet, physical activity, secondary plant metabolites

Cover Page Footnote
All authors contributed equally to this work.
The role of epigenetics for our understanding of nutrition

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For a long time, our genes have been blamed for all kinds of chronic diseases including e.g. metabolic syndrome and cancer. After the ‘Human Genome Project’ identified only approx. 25,000 genes, it is now commonly accepted that not the presence/absence of a specific gene per se, but its expression/silencing within a specific cell and at a specific time point plays the most important role for proper development of our body. Epigenetic signatures located on the DNA and the DNA-binding histone proteins play a critical role in the regulation of gene expression and represent a bridge between nutrition and health [1].

Feeding experiments on Agouti mice clearly demonstrated that ingredients of our daily food, esp. vitamins of the B-family and secondary plant metabolites, can modify epigenetic signatures resulting in aberrant gene expression [2]. Authors reported that genetically identical mice exhibit a different phenotype depending on the mother’s diet during pregnancy. While offspring from mice with normal diet become obese and diabetic and were prone to cancer during adulthood, mice receiving a diet supplemented with various B-vitamins gave birth to healthy littermates. This means that modified epigenetic signatures can even be inherited to children and possibly grandchildren and affect health in their later life. Some years later, it could be demonstrated that a folate-deficient diet of the paternal mice could also affect offspring health [3].

The two best-studied epigenetic mechanisms include methylation of DNA and modification of histones, such as methylation, acetylation and phosphorylation. While DNA methylation solely takes place at cytosine followed by guanine (CpG-island) and always results in gene silencing, histone modifications reveal both activating and repressing potential on gene expression depending on the type of modification and the number of amino acids affected. Vitamins of the B-family and secondary plant metabolites (e.g. carotinoids, glucosinolates, polyphenols and sulfides) are well-known to affect epigenetic signatures and, subsequently, modify gene expression. Most studies have been performed in cancer cells [4–6]. It could be demonstrated that metabolites did not directly affect epigenetic signatures, but act indirectly upon enzymes which transfer/remove chemical groups to/from DNA and histones, e.g. DNA methyltransferase (DNMT), histone acetyltransferase (HAT) and histone deacetylase (HDAC).

Summarized, there is now clear evidence that our daily food affects epigenetic signatures which are involved in regulating gene expression. In case that our germ cells are affected, aberrant epigenetic signatures will be transmitted to our children and even grandchildren and may influence their health in later life [7].

Secondary plant compounds: presence and effects

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Secondary plant metabolites are a large and diverse group of compounds that can be formed in all higher plants belonging to many botanical families. Based on their chemical structure
they can be classified into phenolic compounds (flavonoids, phenolic acids, lignans, avenanthramides), terpenoids (hemi-, mono-, sesqui-, di-, tri- and tetraterpenes), glucosinolates, sulfides, saponins, coumarins and alkaloids. All these compounds play in different ways a major role in the interaction of plants with their environment [8].

Significant amounts of secondary plant compounds can be found in several plant-based foods particularly in whole-grain products, fruits and vegetables, but also in medicinal and aromatic plants which represent an important source for active pharmaceuticals. Among the group of medicinal and aromatic plants (MAP’s), essential oil plants have the longest tradition and a broad application in phytotherapy, cosmetic and food manufacture. Essential oils may contain up to 500 compounds characterized by small molecule size, high volatility and intensive flavour. They can be stored in peltate or capitate glandular trichomes primarily formed in the flowers, leaves, fruits or seeds [9]. Well known compounds that are present in important essential oils are menthol (in mint), carvacrol and thymol (in oregano), matricin and α-bisabolol (in chamomile), limonene (in citrus), citral (in lippia, lemongrass and lemon balm), citronellal (in lemon balm), and thujone (in common sage). Many of these compounds are known due to their antimicrobial, antifungal and anti-inflammatory effects.

In addition to mono and sesquiterpenes, also phenolic acids, flavonoids, di- and triterpenes (triterpene acids) can be synthesized in many MAP’s like black cohosh, lemon balm, rosemary and oregano [10–12]. It was found that antioxidant and radical scavenging activities are the main properties of compounds belonging to polyphenols, phenolic diterpenes or triterpenes [10,12]. The most important MAP’s in Germany are chamomile, pepper mint, lemon balm, valerian, parsley, marjoram and fennel seeds that are produced in large-scale cultivation. With smaller amounts several further MAP’s like calendula, leaf coriander, linseeds, leaf artichoke, basil, leaf digitalis, hollyhock, mallow, black cohosh and comfrey are produced under field conditions in Germany. Due to genetic determination and environmental influences on the synthesis of secondary plant metabolites, detailed investigations are necessary to understand how the quality of MAP’s can be improved by breeding and cultivation methods.

In ongoing research with medicinal used valerian (Valeriana officinalis L.) a better understanding of essential oil formation and DNA identification of this plant was achieved [13,14]. In a further study with German chamomile, an organ-specific expression of five terpene synthase enzymes that are involved in sesqui-terpene biosynthesis could be identified [15]. The current findings can be used in scientifically based plant breeding of medicinal and aromatic plants to increase their content of secondary compounds in future.

The Phytoneering Principle: Gold-Standard in Phyto Medicine

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The use of herbal drugs for the treatment of various diseases become more and more important. However, pharmaceutical activity, safety and quality of modern herbal drugs have to correspond to allopathic standards. Furthermore, modern herbal drugs have to comply with the demands of evidence-based medicine. In this case, herbal drugs may represent a suitable alternative to pharmaceuticals.
Applying ´Phytoneering´ the company Bionorica SE established the gold standard for both research and production of herbal drugs. The term ´Phytoneering´ stands for the exploration of active principles in herbal substances (Phyto) using innovative technologies (Engineering) in order to investigate, develop and produce highly active and safe herbal drugs.

From the development of suitable seeds to the production of the final pharmaceutical product, the whole process has to be standardized and validated. In addition, comprehensive preclinical and clinical studies are necessary in order to ensure effectiveness, safety and quality of herbal drugs for prevention and treatment of diseases.

To date, Bionorica SE collaborates with more than 500 scientists world-wide. In recent years, several groundbreaking clinical studies have been performed in compliance with the guidelines of ´Good Clinical Practice (GCP)´ and subsequently have been included as recommendations in national (German) clinical guidelines.

Bionorica SE is a family-owned enterprise representing the market leader in the field of herbal drugs in Germany, Russia, Ukraine, Kazakhstan, Belarus and Uzbekistan. Currently, the market includes 50 countries world-wide. The product portfolio focuses on drugs for the treatment of respiratory and urinary tract diseases, gynecological pain and analgetics. For further information, see: http://english.bionorica.de/en/company/research-and-developement.html.

Brokkoli & Co in cancer therapy and prevention

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Nutrition and lifestyle are suggested to contribute to healthy aging and to reduce cancer risk and prolong life of cancer patients—this is proposed by actual research data and epidemiological studies [16]. Vegetables of the cruciferous family such as broccoli seem to play a special role [17]. Crucifers produce mustard oils to protect themselves from pests. Especially the isothiocyanate sulforaphane, which is enriched in broccoli, is well examined regarding it´s cancer inhibitory and chemotherapy-supporting effects. Our data demonstrate that sulforaphane even breaks the resistance of the highly aggressive cancer stem cells [18]. This small population of progenitor cells is found in pancreatic cancer and other tumor entities and is made responsible for tumor growth, therapy resistance and metastasis. Sulforaphane attacks cancer stem cells by inhibition of the master inflammatory factor NF-κB, which is upregulated and maintains cancer stem cell features [19]. It is becoming increasingly clear that inflammation is a key factor involved in cancer and other diseases. Meantime, we and other researchers found that not only sulforaphane but many bioactive plant agents are toxic for cancer stem cells by acting anti-inflammatory. One prominent example is salycinic acid, present in several fruits and vegetables. Originally, salycinic acid from willow bark served as starting material for the development of today´s aspirin, which we demonstrated to inhibit pancreatic cancer stem cells [20]. Thus, an anti-inflammatory nutrition seems to play a major role for cancer prevention and therapy. Recent results involving ours indicate that plant substances activate epigenetic signaling, which in turn mediates the protective effect [21,22]. Ongoing research examines the impact of the epigenome on cancer and how to translate this knowledge into the development of an epigenetic anti-cancer diet. Here, we present actual research data and provide information of how to integrate them in daily nutrition.
Increased levels of physical activity (PA) reduce the risk for various types of cancer in a dose-dependent manner [23]. Moreover, elevated PA and targeted therapeutic exercise programs are associated with a decreased progress and mortality, a reduction of frequently observed side effects (e.g. fatigue, incontinence, lymphedema, cognitive impairments) and an increase in quality of life in cancer patients [23]. However, recommended PA levels are met by less than one third of this growing population - especially during medical treatment. In turn physiological and psycho-social constitution further declines, leading to prolonged recovery rates. In general, targeted and supervised exercise programs are most successful in reducing therapy-associated side effects.

On the molecular and cellular level, several effects of PA are suspected to have a positive influence on cancer development, progress and a reduction of relevant side effects. Firstly, regular exercise has anti-inflammatory properties, thereby counteracting a hallmark of cancer and many side effects. It is hypothesized that this systemic anti-inflammatory effect of regular exercise is mainly driven by a reduction of fat mass [24], an induction of regulatory T-cells [25] and a release of specific cytokines [24]. Secondly, exercise has proven to mobilize and activate immune cells which are involved in the host-tumor defense, such as natural killer cells and cytotoxic T-cells [26,27]. Thirdly, it was speculated that exercise influences intra-tumoral angiogenesis [28]. Research of the past decade suggests, that some of the mentioned effects of PA are driven by exercise-induced epigenetic modifications [29,30]. However, most of these results are derived from animal models and need to be confirmed in prospective clinical trials.

In conclusion, PA and exercise can be seen as safe, effective and low-priced supportive therapy for cancer patients during and after medical treatment. More research is warranted in order to improve general and specific existing exercise recommendations.

A summary of intervention studies using a Paleolithic diet

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A Paleolithic diet is a diet trying to mimic human eating behavior during the Paleolithic era when human ancestors lived as hunter-gatherers. “Paleo diets” have gained popularity not only from a scientific perspective, but also as part of a healthy lifestyle based on the rationale that current environmental changes are too rapid for human physiology to adapt to. The aim of this investigation was to summarize available evidence from published studies investigating the effects of Paleolithic diets on physiological parameters.

During the last five years, the author has searched PubMed for “Paleolithic diet” regularly at least once per month; additional articles were found by searching the reference sections of papers on this subject and reading the Journal of Evolution and Health.

Between 2006 and June 2016, a total of 22 publications from eight randomized-controlled trials (RCTs), eight one-armed interventions and one two-phase intervention were published,
describing mainly metabolic outcomes for a total of 413 patients, as reviewed by us recently [31]. Minimum and maximum study duration was 1 day and 2 years, respectively. In addition, the following studies had been found: (i) a single-arm intervention study in ten Australian aborigines from 1984 [32]; (ii) nine case reports on Paleolithic-type ketogenic diets, of which eight refer to patients with various chronic diseases treated by the Hungarian group of Clemens & Tóth (see [33] and references therein) and one refers to a self-described case of weight reduction using a combination of dietary intervention and exercise that was presented at our first symposium in 2013 [34]; (iii) a web-based randomized cross-over intervention in 35 subjects comparing a Paleolithic diet with a Dutch consensus diet (each lasting four weeks) with respect to psychological and somatic symptoms [35]; (iv) a single-arm multimodal intervention study in 15 patients with active inflammatory bowel disease [36]. In total, only two subjects were reported to experience side effects (abdominal pain in one case [37], dizziness and fatigue in another [35]). In the RCTs, metabolic health parameters improved more in the Paleolithic diet groups than in controls; a meta-analysis using four of these studies with 159 participants revealed significantly greater short-term improvements in waist circumference, triglycerides, blood pressure, HDL cholesterol and fasting blood glucose [38]. In patients with autoimmune diseases, multimodal interventions involving modified Paleolithic diet versions were able to achieve beneficial effects in the majority of subjects. A point of critique is the lack of control for energy intake in most studies which resulted in involuntary weight loss, probably due to higher satiating effects of Paleolithic nutrition.

However, even studies controlling for energy intake revealed greater improvements with a Paleolithic compared to control diets considered healthy by official authorities suggesting the absence of grains and/or dairy products as key factors driving beneficial metabolic changes. Now, we should begin collecting long-term follow up data of people eating Paleolithic type diets.

Scientific facts with regard to old and new functions of vitamin-D

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In Germany as well as in many other countries worldwide it has been recognized that there is a need for a higher intake of vitamin D in all age groups from infants to aged people, which is due to new perceptions with respect to physiological and pathophysiological functions of vitamin D, besides its classical effects on bone [39–41]. Thus, a low vitamin D status not only increases the risk of developing rickets in infancy or osteoporosis in adults, but is also of great importance for the intestinal absorption of calcium, specific and unspecific defense systems, the maturation of the immune system or the development of some forms of cancer [42–45]. Although the new functions are controversially discussed, there is increasing evidence from sound publications that vitamin D exerts non-skeletal effects.

On the basis of new knowledge, the German Society of Nutrition significantly increased the recommendations for the daily vitamin D intake from 200 IU to 800 IU for nearly all age groups in 2012 [46]. As the daily vitamin D supply from food comprises only about 50–100 IU, some 500–700 IU need to be obtained from other sources [47]. In addition, during the winter months little or no vitamin D can be synthesized in the skin; therefore, an improvement of the vitamin D status can hardly be achieved without the intake of vitamin D enriched food or supplements. However, vitamin D supplements are often not recommended by health
authorities because of their potential negative effects, although there is no scientific evidence for a risk of hypervitaminosis D at these levels of intake, not even for high risk groups. Vitamin D itself is inactive. Only if required, it will be converted into its active form 1,25(OH)2D (hormone) in the kidney [44]. This activation is largely independent of the dietary vitamin D intake and from synthesis in the skin. This is the reason why no negative effects can be observed even after intake of very high amounts. It is misleading for the general population that even in scientific publications there is often no distinction made between data obtained by the hormonal form of vitamin D, i.e. 1,25(OH)2D, which is never recommended or given for preventive reasons and the native vitamin D available as vitamin D2 or D3.

The human body has several control mechanisms preventing a strong increase in 25 OH D: (i) a local increase of vitamin D in the skin to higher levels already leads to a self-regulated degradation within the skin, (ii) the transport binding proteins (DBPs) have a high capacity to bind large amounts of vitamin D and metabolites with the highest affinity for vitamin D and 25 OH D and, most importantly, (iii) there is a strong endogenous control of the renal production of the active vitamin D metabolite, 1,25(OH)2D, which will only be synthesized, if the hormonal form is required.

According to the recommendations of the EFSA (European Food Safety Agency) and the Institute of Medicine (USA), 4000 IU/day are physiologically safe, even during pregnancy and lactation. Neonates should receive not more than 1000 IU/day, young children up to 1 year of age not more than 2000 IU/day. The upper limit for older children, adolescents and adults is 4000 IU/day [47].

**Evolutionary aspects of omega-3 fatty acids**

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In 2011, Simopoulos suggested that human beings evolved on a diet that had a ratio of omega-6 to omega-3 fatty acids (FA) of about 1/1; today, Western diets have a ratio of 10/1 to 20-25/1 indicating that Western diets are deficient in omega-3 FA compared with the diet on which humans evolved and their genetic patterns were established [48]. At that time, studies with non-human primates and human newborns indicated that docosahexaenoic acid (DHA) is essential for normal functional development of the brain and retina, particularly in premature infants. DHA accounts for 40% of the membrane phospholipid FA in the brain. Both eicosapentaenoic acid (EPA) and DHA have an effect on membrane receptor function and even neurotransmitter generation and metabolism. The balance of omega-6 and omega-3 FA is important for homeostasis and normal development throughout the life cycle.

In 2017, new families of poly-unsaturated fatty acids (PUFA) derived lipid mediators, including resolvins derived from EPA and DHA, and protectins and maresins derived from DHA, are being increasingly investigated due to their active role in the “return to homeostasis” process and resolution of inflammation. Recent findings document a reduced risk of breast, prostate, colon and renal cancers [49]. Along with their lipid-lowering properties, omega-3 LC-PUFAs also exert cardio-protective functions, such as reducing platelet aggregation and inflammation [50,51] and controlling the presence of DHA in our body, especially in our liver and brain, which is crucial for optimal brain functionality explaining the influence on patients with major depressive and bipolar disorders [52], Alzheimer disease [53] and Parkinson’s disease [54].
The prenatal environment is now recognized as a key driver of non-communicable disease risk later in life. Our working group calls this time in the human uterus from the fertilization of the egg until birth the “second evolution” of man (i.e. the evolution of the individual being versus the first evolution of the human species). Within the developmental origins of health and disease (DOHaD) paradigm, studies are increasingly identifying links between maternal morbidity during pregnancy and disease later in life for offspring [55]. Nutrient restriction, metabolic disorders during gestation, such as diabetes or obesity, and maternal immune activation provoked by infection have been linked to adverse health outcomes for offspring later in life. These factors frequently co-occur and the potential for compounding effects of multiple morbidities on DOHaD-related outcomes has still to be evaluated [56].

As to be expected, omega-3 FA play a role in this important early period of life, too. Several studies indicate that the intake of α-linolenic acid (ALA) can alter the concentration of both ω-6 and ω-3 fatty acids in both mother and offspring with consequences on postnatal brain development [57–59]. Omega-3 supplementation is associated with a lower risk of spontaneous preterm delivery in smokers and low birth weight is also less frequent in smokers receiving omega-3 supplementation [60].

In addition, the maternal ALA availability during gestation and lactation induces epigenetic changes like alterations in the Fads2 DNA methylation in both maternal and offspring livers, at the end of the lactation period [61]. A recent study showed that toddler methylation status was related to immediate memory performance, whereas maternal methylation status was related to delayed memory performance. Thus, prenatal experience and maternal Fads2 status have a pervasive, long-lasting influence on the brain development of the offspring [62]. Other results suggest that PUFA-deficiency during the early neurodevelopmental period in mice could model the prodromal state of schizophrenia through changes in the epigenetic regulation of nuclear receptor genes [63].

In conclusion, there is a growing body of evidence that omega-3 FA play an important role as well in the first as in the second evolution of man. In consequence, the lack of omega-3 FA in modern Western diets is at the origin of multiple diseases and could easily be prevented by substitution of omega-3 FA, when supplied in sufficient amounts.

Molecular anthropology:
The three main ancestral components in modern-day Europeans

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Recent paleogenetics studies have provided direct insights into the biological relatedness and ancestry of prehistoric Europeans associated with particular archaeological cultural complexes. Accompanying progress in genomic data analysis at population level has massively altered the way in which we approach the wealth of new and contextual information from the diverse neighboring disciplines such as genetics, anthropology, archaeology, and linguistics. In this talk I will provide a chronological overview on how the genetic makeup of modern-day Europeans was shaped over the past 10,000 years.

Indigenous hunter-gatherers of the Mesolithic and those of the Paleolithic prior to the Last Glacial Maximum best represent the basic genetic substratum of Europe. At the same time, a closely related but distinctive population of hunter-gatherers with an affinity to Paleolithic
Siberians and Native Americans inhabited Eastern Europe. In stark contrast, the Early Neolithic period in Europe (~8,000-7,000 years ago) was characterized by closely related groups of early farmers that are genetically differentiated from hunter-gatherers and resemble pre- and early farming groups from the Fertile Crescent in the Near East. This supports the view that the spread of farming practices was facilitated not just by the spread of ideas and techniques alone but carried by people, who also brought new genetic ancestry to Europe.

During the Middle Neolithic in Europe (~7,000-5000 years ago) we observe a resurgence of hunter-gatherer ancestry throughout much of Europe, while the contemporaneous steppe herders of the Eurasian steppes shared ancestry with both the preceding eastern European hunter-gatherers and a fourth component from south of the Caucasus, which in turn is different from early farmers in the Levant.

With the emerging Bronze Age this blend of ‘steppe-related ancestry’ expands westward reaching Central Europe ~4,500 years ago. Individuals associated with the Late Neolithic Corded Ware complex traced ~75% of their ancestry to the steppe herders, which persisted in subsequent Bronze Age Europeans and is ubiquitous in Europeans today. The arrival of ‘steppe ancestry’ documents a second major expansion into Europe and the third main ancestry component.

The magnitude of the second expansion also has bearings on the spread of Indo-European language groups, supporting an older (but vital) alternative to the language-farming dispersal hypothesis. Intriguingly, the recent findings of plague-related agents in Bronze Age individuals from Central and Eastern Europe extend the scope of explanations to also include paleo-epidemiological scenarios.

**Personalized nutrition**

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Like other sectors, food markets demonstrate the thrive for diversification and individualization. Currently this is mainly based on food/taste preferences and enjoyment as visible in sectors such as coffee, chocolate or beverages. The highest level of personalization is currently achieved by internet offers to compose your “own” food item such as a breakfast cereal (mymüsli) or chocolate.

Health is considered as a key market driver. When taken into the food and nutrition sector, the key question is of course of how health-promotion can be achieved at the level of the individual and the foods consumed. What can be predicted is that a wide range of web-based health services will become available and those will also employ numerous electronic devices that allow assessment of food intake and measurements of a variety of lifestyle parameters (exercise, sleep, leisure time) and health indicators (blood pressure and glucose, metabolite profiles etc). Whether genetics should/will be included is to be seen, but is very likely. Based on these parameters individualized dietary recommendations but also menu plans can be generated and those customized menus may be preordered in a restaurant or for home-delivery. Electronic devises will also be available as shopping guides. It can be foreseen that the entire supply of foods becomes personalized as consumers outsource this to a health and food service provider. Health insurances may be part of such services and measures of compliance may be used in adjusting individual health insurance plans. Such a

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system of course challenges some fundamental principles of liberal societies and it remains to be seen how societies cope with this. Personalization can not only be the highest level of possible services but will clearly also be the highest level of personal responsibility. For further information on individual projects, see http://www.food4me.org/.

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