Types of Epigenetics Modifications	Model Used	Outcome during DR	Lesson learnt from studies	References
DNA Methylation	*Drosophila*	• No significant change in DNA methylation level.	• DNA methylation not the determining mechanism in contributing to lifespan extension in *Drosophila* during DR.	Lian et al., 2019
	Daphnia magna	• DNA methylation control gene expression changes related to methylation and acyl-CoA dehydrogenase actions.	• Understanding of how acyl-CoA dehydrogenase is regulated during CR in an invertebrate model.	Hearn et al., 2019
	Mice	• DR has been shown to protect against age-associated methylation status in mice liver. CR represses DNA methylation in the DNA of mice during early periods of CR, but the effects became less prominent as the mice aged. Notably, CR also suppress the mouse liver DNA against age-dependent increase in *c-myc* gene methylation • DR protects against age-associated changes in DNA methylation in female mouse liver, which in turn drives epigenetic reprogramming of lipid metabolic processes, thereby leading to a metabolic switch towards reduction in triglyceride level and short-chain triglyceride-associated fatty acids. The effects induced by DR in lipid metabolic process switch is more pronounced with age as a result of DNA methylation changes • CR is able to induce attenuation of DNA methylation at both the CG and CH (*non*-CG) sites in aged mice hippocampus, which in turn leads to enrichment of pathways that are neuroprotective as well as associated with benefits towards brain aging. • Aging has been shown to increase the level of HDAC2 in mouse hippocampus but CR attenuate this age-associated increase in both the CA3 and CA1-2 subregions. Moreover, the level of HDAC2 correlates with 5-methylcytidine DNA methylation levels in the nucleus of hippocampal cells. • DNA methyltransferase 3a (DNMT3a) is implicated in aged mice hippocampus, which may implicate cognitive functioning. Refuted by another group, who reported that DNMT3a2 is not differentially regulated with aging regardless of sexes in mice hippocampus. • CR has also demonstrated ability to suppress age-associated increase in both DNA methylation and hydroxymethylation in cerebellar Purkinje cells. • CR induce hypermethylation at CpG sites for transcriptional regulator CCCTC-binding factor of both estrogen receptor 1 and 2 (*ESR1* and *ESR2*), thereby leading to transcriptional activation of both estrogen receptor alpha and beta (*ERα* and *ERβ*) gene expression level. Also, CR is able to attenuate the obesity-associated increase in *DNMT1* DNA methylation, and has significantly reduce the development of mammary tumorigenesis. • Blood studies shown that unique DNA methylation signatures became more prominent with age and significantly contributes towards aging following remodeling of the genome. CR, on the other hand, has been shown to remodel these DNA methylation signatures and thereby contributes to longevity effects. • DR during maternal pregnancy has resulted in intrauterine growth restricted fetus (IUGR), which in turn has promoted the development of chronic diseases (such as glucose intolerance, increased fat deposition, as well as hypercholesterolemia) in adult male mice offspring. DR influences the placenta environment which causes hypomethylation at the genome-wide level. As a result, this finding leads to differentially expressed pathways which are associated with IUGR phenomenon, with male mice showing higher risk.	• DR is able to protect against age-associated changes in DNA methylation in liver, hippocampus, cerebellum and blood. • Subsequent gene expression changes as a result of DNA methylation protection against age-associated changes leads to metabolic switching processes, cognitive improvement and longevity. However, controversial findings arisen may highlight the complexity behind DNA methylation processes. • Crosstalk between DNA methylation and HDAC activities exist during DR to prevent age-associated changes in hippocampus. • DR demonstrates ability to influence DNA methylation of key genes that may be protective against mammary tumorigenesis.	Miyamura et al., 1993; Cole et al., 2017; Hahn et al., 2017 Heena P. Santry, MD MS, John C. Madore, BS, Courtney E. Collins, M. Didem Ayturk, George C. Velmahos, LD Britt, and Catarina I. Kiefe, 2017; Chouliaras et al., 2011; Hadad et al., 2016; Lardenoije et al., 2015; Rossi et al., 2017; Maegawa et al., 2017; Sziráki et al., 2018; Chouliaras et al., 2013.
	Rat	• CR was found to increase DNA methylation of *ras* DNA in rat pancreatic acinar cells. Across generations, CR cells demonstrated reduced oncogene expression level and mutation, as well as showing a decrease in p53 tumor suppressor gene mutation, proliferation and transformation. • CR induces increase in DNA methylation in the promoter and intronic regions of kidney, which in turn repress pathways associated with age-associated diseases such as cancer and diabetes. • CR pregnant rat demonstrated altered fatty acid synthase (*Fasn*) gene expression changes in the liver and blood cholesterol levels, but exhibit no changes in DNA methylation level. As a result, such effects were not reflected in offspring. Only significant changes occurred in *Dnmt1* in the first generation of fetal liver, but no significant differences in second or third generation. However, the authors found no significant differences in DNA methylation potential (defined as the ratio of S-adenosylmethionine to S-adenosylhomocysteine) in prenatal restricted diet rat. • CR in pregnant rats and found that DNA methylation level do not differ significantly in female fetuses across three generations.	• DR influences DNA methylation levels in pancreatic acinar cells and kidney, which thereby result in protection against tumorigenesis, cancer and diabetes. • DNA methylation is not altered across three generation as a result of DR in the liver, despite showing changes in *Fasn* and *Dnmt1*.	Hass et al., 1993;; Kim et al., 2016; Nowacka-woszuk et al., 2017; Nowacka-Woszuk et al., 2018; Nowacka-Woszuk et al., 2019.
	Rhesus monkeys	• CR is able to attenuate age-associated DNA methylation drift using blood studies, which has been correlated with lifespan in these mammals.	• DR is able to protect against age-associated changes in DNA methylation in blood.	Maegawa et al., 2017
	Caco-2 human epithelial colorectal adenarcinoma cells and human umbilical vein endothelial cells	• Sirt1 mediates DNA methylation which in turn controls differentially expressed genes that were commonly reported to respond to DR.	• Implication of Sirt1 in influencing DNA methylation during DR, highlighting potential crosstalk mechanism.	Ions et al., 2013
	Normal WI-38 cells and immortalized WI-38/S cancer cells	• Glucose restriction induced DNA methylation changes and subsequent chromatin remodeling at both hTERT and p16 promoter regions. In turn, normal WI-38 cells exhibit improvement in longevity, whereas WI-38/S cancer cells exhibit growth inhibition and apoptosis.	• DR exhibit ability to influence DNA methylation that demonstrates differential effects in normal and cancer cells, providing potential for cancer treatment.	Li et al., 2019
	Human	• Postmenopausal women who are either overweight or obese possess significant DNA methylation differences at 35 loci prior to CR in their subcutaneous adipose tissue, but showed a reduction to only three significant DNA methylation differences following it. At these three loci, DNA methylation controls genes that were involved in body weight control, insulin secretion as well as genome imprinting. • Hypomethylation of both leptin and TNF-α promoter regions was reported following CR-induced weight loss in obese women blood samples or peripheral blood mononuclear cells. Other genes that might be controlled by DNA methylation modulation includes cluster determinant 36 (*CD36*), cluster determinant 14 (*CD14*), pyruvate dehydrogenase kinase 4(*PDK4*), as well as fatty acid desaturase 1 (*FADS1*). • Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine and obese men subjected to CR-induced weight loss demonstrated hypomethylation at the TNF-α promoter region in blood mononuclear cells. • Key changes in DNA methylation loci at the ATPase phospholipid transporting 10A (*ATP10A*), cluster determinant44 (*CD44*) and Wilms tumour 1 (*WT1*) genes were also being modulated in peripheral blood mononuclear cells from overweight or obese men following CR. • Overnight fasting and 36-hour fasting in young low birth weight and normal weight subjects demonstrated hypermethylation in both leptin (*LEP*) and adiponectin (*ADIPOQ*) genes from plasma samples, which positively correlates with total body fat composition • Obese nondiabetic patients who have been placed under a very-low caloric diet (VLCD) only exhibit hypomethylation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*) in their blood whereas those who underwent a Roux-en Y gastric bypass (RYGB) show alteration in methylation levels at *PPARGC1A*, transcription factor A (*TFAM*), interleukin-1 beta (*IL1-B*), interleukin-6 (*IL-6*) as well as tumor necrosis factor-α (*TNF-α*) promoter regions. Moreover, these RYGB patients' blood also have a corresponding hypermethylation in pyruvate dehydrogenase kinase isozyme-4 (*PDK4*), IL1-β, IL-6 and TNF-α after one year. • In the skeletal muscle, it has been reported that PPARGC1A, PDK4 and sorbin and SH3 domain containing 3 (*SORBS3*) DNA methylation levels were altered following RYGB. • DNA methylation level of many CpG sites of promoter regions, such as cholesteryl ester transfer protein (*CETP*), forkhead box P2 (*FOXP2*), HDAC4, DNMT3B, potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*), as well as *Hox*, were being altered following gastric bypass in adipose tissue. • Obese women placed under an energy-restricted Mediterranean diet have an increase in interleukin-6 (IL-6) DNA methylation, whereas those placed under bariatric surgery has a contradictory reduction at the same loci. • Humans impacted from the Dutch famine also exhibit DNA methylation changes during early gestation in whole blood, but not in mid or late gestation period.	• DNA methylation changes at key gene loci can therefore be used as an early indicator of response to weight-loss interventions. • Surgical employment to treat obesity may have a robust impact on DNA methylation of a myriad of genes, which may provide the impetus of for the study of DNA methylation association with obesity, as well as better understanding on the account for the improvement in metabolic health in these patients following surgery. However, consideration needs to be given for contradictory results. • DR may affect prenatal environment under specific window period via DNA methylation, and further studies on the subsequent functional outcome need to be conducted.	Mill et al., 2010; Cordero et al., 2011; Amaral et al., 2014; Campión et al., 2009; Milagro et al., 2011; Hjort et al., 2017; Barres et al., 2013; Day et al., 2017; Benton et al., 2015; Nicoletti et al., 2016; Chen et al., 2013; Tobi et al., 2015
Histone Modifications/ Remodeling	*Yeast*	• CR has reduced the level of N-terminal histone acetylation at histone H4 (NacH4), which in turn induce the upregulation of stress-response genes such as *Pnc1* which regulates Sir2 activity and is important for longevity effects. • Nutritional stress has enhanced the phosphorylation of H3T11, which also modulate the expression of stress-response genes that influence chronological lifespan. • Deletion of an ATP-dependent chromatin remodeling enzyme complex *ISW2* in yeast leads to effects that mirror CR. Yeast with *ISW2* deletion demonstrated increase in lifespan, upregulation of stress-response and genotoxic stresses. Effects distinct from TOR signaling suppression during CR.	• Implication of NacH4 and Sir2 activities during DR in longevity. • Implication of H3T11 phosphorylation and chronological lifespan during DR. • Implication of *ISW2* in lifespan determination during DR.	Molina-Serrano et al., 2016; Oh et al., 2018; Dang et al., 2015
	Drosophila	• During short-term fasting, activation of adipokinetic hormone (AKH) pathway will subsequently inhibit liver kinase B1 (LKB1) and thereby induce HDAC4 nuclear localization to alter *brummer* gene expression, thus modulating lipid storage. • During prolonged fasting, an AKH-independent signaling pathway will downregulate the activity of LKB1-salt inducible kinase 3 (SIK3) pathway which in turn induce lipolysis. • FOXO activity is being regulated by this axis. • CR activates dSir2 via HDAC Rpd3 to promote lifespan extension.	• Involvement of LKB1-SIK3-HDAC4 axis during fasting to modulate lipid homeostasis is highly dependent on the duration as well as the regulatory partners partnered. • Implication of sirtuin and HDAC to promote longevity.	Choi et al., 2015; Crunkhorn, 2011; Wang et al., 2011;Rogina and Helfand, 2004;
	Normal WI-38 cells and immortalized WI-38/S cancer cells	• Glucose restriction results in a decrease in HDAC1 activity around the transcriptional initiation site of human telomerase reverse transcriptase (hTERT) in both normal WI-38 and immortalized WI-38/S cells. an increase in HDAC1 activity was found around the transcriptional initiation site of p16 promoter region in normal WI-38 cells and a loss of HDAC1 binding at the p16 promoter in immortalized WI-38/S cells. The end result is favoring longevity in normal cells and apoptosis in precancerous cells.	• DR influences HDAC1 activity which holds potential in cancer treatment.	Li et al., 2019
	Mice	• Aging has been shown to increase the level of HDAC2 in mouse hippocampus but CR attenuate this age-associated increase in both the CA3 and CA1-2 subregions. • HDAC3 knockout mice exhibit increased bone marrow fat. Bone marrow fat has been reported to produce adiponectin during CR in controlling the metabolic activity of muscles residing nearby. • Fasted mice exhibit increased levels both HDAC3 and HDAC4 in the medial hypothalamus, as well as a reduction in the number of acetylated histones H3 and H4 cells in the ventrolateral subdivision of the ventromedial hypothalamus. • Overnight fasting in mice showed an increased in cyclic adenine monophosphate (cAMP) signaling which increased the binding of both HDAC4 and HDAC5 binding to glucose transporter protein 4 (GLUT4) promoter region, thus decreasing GLUT4 mRNA expression. • Inhibition of SIK2 (mouse SIK3 homologue) induced HDAC4 dephosphorylation to promote gluconeogenic gene transcription following hormonal glucagon injection. • Rapid phosphorylation of class IIa HDACs (HDAC4 and HDAC5) occurs during injection of glucagon hormone into mice, which in turn drives their translocation to the nucleus and promote binding to gluconeogenic enzymes glucose-6-phosphatase (G6Pase) promoter region within the liver. Moreover, once in the nucleus, both HDAC4 and HDAC5 will subsequently recruit a class I HDAC member, HDAC3, to induce the deacetylation and activation of FOXO family of transcription factors. In turn, this will drive the induction of transcription of gluconeogenesis gene. • Histone acetylation is greatly enhanced at the promoter region of mitochondrial activating genes in the skeletal muscle of mice fasted for 72-hour. Correspondingly, glucose tolerance, body weight and exercise endurance were improved in fasted mice. • Mice who were fasted for 16-hour and 24-hour exhibited H3K9me$_3$ changes, which in turn lead to robust transcriptomic changes that resulted in metabolic switching processes within the cerebellum.	• Crosstalk between DNA methylation and HDAC activities exist during DR to prevent age-associated changes in hippocampus. • HDAC3 may function a critical role in the skeletal muscle in modulating metabolism during DR. • Both HDAC3 and HDAC4 are implicated in the modulation of gene expression changes in the hypothalamus in response to fasting. • Implication of HDAC3, HDAC4 and HDAC5 during DR to maintain glucose homeostasis. • Histone acetylation is important in mitochondrial homeostasis in skeletal muscle of DR mice.	Chouliaras et al., 2013; McGee-Lawrence et al., 2016; Weems et al., 2012;Crunkhorn, 2011; Mihaylova et al., 2011;
	Rat	• An increase in Hdac1 gene was found in CR pregnant rat fetal liver in the first two generations of offspring, but a concomitant decrease was observed at the third generation. Moreover, a global histone H3 acetylation was observed in the fetal liver in both the first and second generation. • in utero undernutrition (50% CR) during the final week of gestational period in rat revealed a decrease in H3K14Ac and an increase in H3K9me$_3$ in the skeletal muscle. As a result, Glut4 expression in the skeletal muscle in the adult offspring is being repressed. • in utero undernutrition (50% CR) during gestational period leads to a decrease in H3K4me$_2$ and an increase in H3K4me$_3$ levels at the IGF-1 locus in the liver of rat offspring. These intrauterine growth-restricted (IUGR) offspring often demonstrated rapid catch-up growth and develop higher risks of metabolic syndrome development as well as obesity. Offspring who exhibited this phenomenon also have similar epigenetic modification as those who underwent in utero undernutrition at the IGF-1 locus in the liver, which may contribute partially to both an increment in liver and body weight.	• Prenatal CR can modulate histone modifications with possible transgenerational effects. • in utero undernutrition (50% CR) affects H3K14Ac and H3K9me$_2$ in the skeletal muscle to influence glucose homeostasis. • Mechanistic relationship between IUGR and histone methylation can help to predict the risks of future diseases development.	Nowacka-Woszuk et al., 2018; Raychaudhuri et al., 2008;Tosh et al., 2010
microRNAs	*C. elegans*	• DR induces the expression of both miR-71 and miR-228. miR-228 is then able to repress both defective pharynx development (PHA-4) (an ortholog of human FOXA3 transcription factor) and skinhead (SKN-1) transcription factor, whereas miR-71 is able to repress only PHA-4. Promotion of lifespan. • Dicer has been shown to decline with age and defective Dicer results in decreased lifespan and stress tolerance. • IF has been shown to upregulate RISC components, such as Argonaute and GW-182, as well as DRSH-1. The upregulation of these miRNAs processing machinery in turn helps to modulate the expression of target genes, such as DAF-16, the insulin/IGF-1 signaling player, which plays an important role in IF-induced longevity	• Implication of miR-71 and miR-228 during DR in longevity. • Implication of Dicer in lifespan during DR. • Implication of RISC in lifespan during DR.	Smith-Vikos et al., 2014; Mori et al., 2012; Kogure et al., 2017
	Mice	• During aging, there is an increase in miR-181a-1, miR-30e and miR-34a in mice brain, resulting in a decrease in expression of Bcl-2 gene involved in apoptosis. CR is able to counteract the age-dependent increase in these miRNAs, and correspondingly increase the expression of Bcl-2, decreasing apoptosis and contributes to neuroprotection through improvement in neuronal survival. • In the liver, CR is able to increase both global and mitochondrial-specific miRNAs. Notably, the most abundant altered miRNAs during CR, miR-122, are critical for the corresponding activation of mitochondrial translation and induce mitochondrial unfolded protein response, thereby improving mitochondrial proteotasis. • miR-125a-5p was upregulated during CR in female mice liver, preventing an age-associated decrease. Consequently, the downstream target genes (signal transducer and activator of transcription 3 (Stat3), caspase 2 (Casp2) and StAR-related lipid transfer domain protein 13 (Star d13)) were downregulated and may contribute to delay in aging. • Circulating serum miRNAs levels in young, old and CR mice are distinct. CR is able antagonize the age-related alterations in these miRNAs, which in turn influence differential biological pathways that are normally implicated in aging, such as cellular metabolic pathways, Wnt signaling pathway, as well as apoptosis. • Mice possessing Dicer knockout are hypersensitive to oxidative stress in adipose tissue. • Coordinated transition between fed-fast period is mediated by RISC association between miRNAs and transcriptome in the liver, which governs a plethora of homeostatic processes (such as metabolic and mitochondrial homeostasis). This biological oscillation has been shown to be dysregulated during aging, and the subsequent inability of this oscillator may result in metabolic derangements and ultimately diseases development	• Implication of in miR-181a-1, miR-30e and miR-34a in apoptosis during DR. • Implication of miR-122 during DR to maintain mitochondrial homeostasis. • Implication of miR-125a-5p during DR in influencing aging. • Implication of existing and novel miRNAs in circulation during DR in countering aging. • Implication of Dicer in oxidative stress in adipose tissue during DR. • Implication of RISC in metabolic homeostasis during feeding and DR.	Khanna et al., 2011; Zhang et al., 2019; Makwana et al., 2017; Dhahbi et al., 2013; Mori et al., 2012; Maniyadath et al., 2019
	Rat	• miR-98-3p was altered in the cerebral cortex of CR rat, which is able to alter both HDAC and HAT activities. • CR is able to normalize and decrease the expression of miR-200a, which has been tied to mammary tumor progression.	• Crosstalk between microRNAs and HDAC/HAT activities during DR. • Implication of miR-200a during mammary tumorigenesis and DR possessing potential for cancer treatment.	Wood et al., 2015; Devlin et al., 2016
	Rhesus monkeys	• miR-125a-5p was downregulated during CR and these miRNAs correlates positively with adiposity and negatively with insulin sensitivity. Other CR induced change in circulating miRNAs are correlated with bodyweight, adiposity as well as insulin responses. • miR-451, miR-144, miR-18a and miR-15a were being upregulated whereas miR-181a and miR-181b were being downregulated in old rhesus monkey skeletal muscle. Following CR, the levels of miR-181a is rescued, and CR prevented the age-associated increase of miR-451 and miR-144 levels. The miRNAs expression in CR monkeys mimic those of the young phenotypes.	• Implication of miR-125a-5p in adiposity and insulin homeostasis. • Implication of miR-451, miR-144 and miR-181a in aging.	Schneider et al., 2017; Mercken et al., 2013